CASWELL FILE

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

007121

APR 11 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 241-GRT. Event™ Grass Growth Regulator. Registration Application for a Terrestrial Noncrop Use Pattern.

Tox. Chem. No. 3F and 3I
Project No. 9-0388

TO: Robert Taylor, PM Team #25
Fungicide, Herbicide Branch
Registration Division (H7505C)

FROM: Pamela M. Hurley Ph.D., Toxicologist Pamela M. Hurley 3/1/89
Section I, Toxicology Branch I
Insecticide, Rodenticide Support
Health Effects Division (TS-769c)

THRU: Edwin R. Budd, Section Head
Section I, Toxicology Branch I
Insecticide, Rodenticide Support
Health Effects Division (TS-769c)

Budd 4/11/89

Record No(s). 228,567

Background and Request:

American Cyanamid Company has submitted an application for registration of Event™, a grass growth regulator for use on tall fescues, perennial ryegrasses, bluegrasses, and bahiagrasses. This formulation contains two active ingredients, imazethapyr and imazapyr, at the following concentrations: 17.26% and 0.64%, respectively. Imazapyr is already registered as an active ingredient and imazethapyr has completed the Toxicology Branch review process for registration in another product and will be registered shortly.

Event™ controls seedhead production by tall fescue and controls or suppresses seedhead production by perennial ryegrass, bluegrass, and bahiagrass. It may be used on limited care-low maintenance areas, such as roadsides, airports, fairgrounds, and golf course roughs, and limited wear areas such as industrial, institutional, and cemetery grounds. It will be applied at a rate of 8 to 10 fluid ounces per acre.

128829

Response:

The Toxicology Branch (TB-I) cannot support registration of this product until deficiencies in toxicity testing requirements are fulfilled. The following paragraphs discuss the requirements in detail.

The following toxicity studies are recommended to be submitted in support of the proposed registration. Those recommendations that have been satisfied are indicated:

	Required	Satisfied
Technical Products (both)		
Acute oral LD_{50} - rat	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
21-Day dermal	Yes	Yes
Teratology - 2 species	Yes	Yes
Gene mutation	Yes	Yes
Chromosomal aberration	Yes	No[1]
Other genotoxic effects	Yes	No[1]
Formulation (EventTM)		
Acute oral LD_{50} - rat	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
21-Day dermal	Yes	No[2]

1. Studies for chromosomal aberrations and for other genotoxic effects have not been conducted on imazapyr. Since this technical product is present in the formulation in such a low concentration, and since this is a use in which there is low exposure of the formulation to the general population, the lack of these two studies will not delay registration. However, these two studies are still required and they should be submitted as soon as possible.

2. A 21-day dermal study will be required on the formulation because there is some dermal exposure to the general population, particularly from the golf courses. Completion of this study is required prior to registration.

3. Clearance of the inerts for this product will be addressed by the Registration Division.

4. The label is acceptable as written.

Imazapyr/
Pursuit toxicology review

Page _____ is not included in this copy.

Pages 3 through 7 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

X A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed By: Pamela Hurley Pamela M. Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral - rat (81-1) TOX. CHEM. NO.: 3I + 3F

ACCESSION NUMBER/MRID NO.: 407634-02

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr formulation

REPORT NUMBER: A87-3

SPONSOR: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TITLE OF REPORT: Acute Oral, Acute Dermal, Eye Irritation, and Skin Irritation Studies With Event Formulation

AUTHOR(S): C.A. Lowe

REPORT ISSUED: ~~7/15/88~~ 1/16/87

IDENTIFYING VOLUME: Volume 5

CONCLUSION: Male and female rats were fed by gavage one oral dose of Event formulation at a level of 5000 mg/kg. The acute oral LD_{50}'s of the formulation in rats were greater than 5000 mg/kg for either males or females or both combined.

Toxicity Category: IV

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Names: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: liquid
Batch #(s), Other #(s): AC 5329-101-C, Tox. Sample No: 86-195
Purity: 16.1% Imazethapyr, 0.61% Imazapyr
Source: American Cyanamid
Vehicle (if applicable): N/A
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female albino rats, strain CHRCD
Age: 7-10 weeks
Weight(s): 167-173 g (M), 193-199 g (F)
Source(s): Charles River

3. Procedure: Five male and five female rats were fasted for 18 hours prior to dosing. The animals were all dosed with 5000 mg/kg of the test material as received by the Sponsor in a volume of 4.8 ml/kg. All animals were observed daily for clinical signs of toxicity up to a period of 14 days. Gross necropsies were conducted on all the animals, survivors and decedents alike. Body weights were recorded weekly.

B. RESULTS: There were no signs of toxicity in any of the treated animals. In addition, no treatment-related gross lesions were observed at necropsy. The acute oral LD_{50}'s were >5000 mg/kg for either males or females, or both combined. A signed good laboratory practice statement was provided.

C. DISCUSSION: This was a limit test. It is classified as Core Guideline.

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Reviewed By: Pamela Hurley Pamela M. Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C) 4/1/89

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity - rabbit (81-2)

TOX. CHEM. NO.: 31 + 3F

ACCESSION NUMBER/MRID NO.: 407634-02

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr formulation

REPORT NUMBER: A87-3

SPONSOR: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TITLE OF REPORT: Acute Oral, Acute Dermal, Eye Irritation, and Skin Irritation Studies With Event Formulation

AUTHOR(S): C.A. Lowe

REPORT ISSUED: ~~7/15/88~~ 1/16/87

IDENTIFYING VOLUME: Volume 5

CONCLUSION: Male and female rabbits were tested with 2000 mg/kg of Event formulation via the dermal route. The LD_{50}'s were greater than 2000 mg/kg for either males or females.

Toxicity Category: III

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Names: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: liquid
Batch #(s), Other #(s): AC 5329-101-C, Tox. Sample No: 86-195
Purity: 16.1% Imazethapyr, 0.61% Imazapyr
Source: American Cyanamid
Vehicle (if applicable): N/A
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female albino rabbits, NZW strain
Age: 12-14 weeks
Weight(s): 2.20-3.01 kg
Source(s): Skippac Farms

3. Procedure: Five male and five female animals were used for the study. The animals were not fasted prior to dosing with the test material. They were shaved 24 hours prior to dosing. The test material was applied neat to the dorsal skin in an area which encompassed approximately 10% of the body surface, at a dose level of 2000 mg/kg in a volume of 1.92 ml/kg. The test material was covered with an impervious plastic cuff for 24 hours. At the end of the 24 hour exposure period, the cuff was removed, the application site was wiped with a moistened gauze pad and the animals were fitted with fiber collars to prevent ingestation of any remaining test material. The animals were weighed weekly and observed daily for clinical signs of toxicity for 14 days after exposure to the test material. Gross necropsies were conducted on all animals.

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B. RESULTS: No clinical signs of toxicity were observed during the treatment and observation periods. One male rabbit was found dead on day 12 of the study. The report stated that the animal appeared to have died from an incurrent respiratory infection. The gross necropsy findings included the following: kidney - pale; lungs - consolidation, adhesions, and fluid in the pleural cavity. No other gross lesions were observed in any of the other animals. The LD_{50}'s were greater than 2000 mg/kg for either males or females.

Quality Assurance Measures: A signed Good Laboratory Practice Statement was provided.

C. DISCUSSION: This was a limit test. It is classified as Core Guideline.

Reviewed By: Pamela Hurley Pamela M. Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C)
Budd 4/11/89

DATA EVALUATION REPORT

STUDY TYPE: Acute inhalation - rat (81-3)

TOX. CHEM. NO.: 3I and 3F

ACCESSION NUMBER/MRID NO.: 408657-02

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr, ACP 1911X

REPORT NUMBER: 87-5952A

SPONSOR: American Cyanamid Company

TESTING FACILITY: Biosearch Incorporated, Philadelphia, PA

TITLE OF REPORT: Acute Inhalation Study With ACP 1911X (Event)

AUTHOR(S): R. J. Hershman

REPORT ISSUED: ~~7/15/88~~ 2/2/88

IDENTIFYING VOLUME: Volume 6

CONCLUSION: Ten rats of each sex were exposed to one dose of the formulation, Event via the inhalation route. The acute LC_{50} for 4 hours was calculated to be greater than 3.24 mg/l of air based on Imazapyr and 3.07 mg/l of air based on Imazethapyr. The report stated that these were the maximum analytical concentrations which could be attained.

Toxicity Category: III

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: Yellow-brown liquid
Batch #(s), Other #(s): Lot # AC 5811-11
Purity: 16.48% imazethapyr, 0.61% imazapyr
Source: American Cyanamid Company, Agricultural Research Division
Vehicle (if applicable): N/A
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female outbred Sprague-Dawley rat
Age: Not given
Weight(s): 212-288 grams
Source(s): Buckshire Corp., Perkasie, PA

3. Procedure: Ten male and 10 female rats were used for the study. The test article was dosed as supplied, in aerosol form. Each rat was individually held in a restraining cage. One dose level was administered for 4 hours. The aerosol was generated by an eight jet Collison nebulizer. The nominal average concentration of the aerosol over the four hour exposure period was calculated by differential weighing of the flask from which the aerosol was generated. Particle size of the aerosol was determined using an Andersen Sampler cascade impactor at the rate of 1 cubic foot per minute. The report stated that "the amount of aerosol impacting on each plate of the Andersen Sampler was determined by differential weighing. From these values the mass median aerodynamic diameter of the aerosol was calculated. The respirable concentration..... was also calculated from the mass collected on the appropriate plates divided by the total air volume sampled. In addition, the nominal gravimetric concentration was

determined from the total mass collected by the Andersen Sampler."

The procedures also stated that "gravimetric determination of the chamber concentration was measured by sampling air from the breathing zone through a glass fiber filter...The airborne concentration of nonvolatile materials was determined by the weight gain of the filter divided by the volume of air sampled.

Four sets of tandem bubbler samples were taken during the exposure period. Analytical analyses were determined from these measurements.

The animals were observed for 14 days following exposure. They were observed frequently during the day of exposure, and twice per day during weekdays. On weekends and holidays, they were observed once per day. Individual bodyweights were recorded on the day before exposure, the day of exposure, weekly thereafter, and prior to sacrifice. At termination of the study, the animals were euthanized and gross necropsies were conducted on all animals.

The following organs were weighed at necropsy: lungs, liver, heart, kidneys, and gonads; and the following organs were removed and fixed in formalin for possible future microscopic examination: lungs, trachea, bronchi, tissues from the nasal passages, liver, kidneys, heart, thyroid, adrenals, gonads, spleen, stomach, small and large intestines, bone marrow, brain and any tissue which appeared abnormal.

B. RESULTS: No mortalities were observed during the study. One female exhibited a weight loss at 7 days, however, gained weight between days 7 and 14 of the observation period. All of the other animals gained weight. All animals exhibited muzzle staining during exposure and on day 1. By day 2, this had disappeared. All animals appeared normal throughout the rest of the observation period. At necropsy, no gross abnormalities were observed in any of the animals. Organ weights and body/organ weight ratios were reported, however, only one dose level was tested and no controls were run, so these data are limited as far as interpretation is concerned.

The nominal average concentration was calculated to be 15.60 mg/l; the mean analytical concentrations were calculated to be 3.07 mg/l (based on imazethapyr) and 3.24 mg/l (based on imazapyr); the mean gravimetric concentration, determined by the Andersen Sampler, was calculated to be 2.47 mg/l; the mean respirable concentration was calculated to be 1.04 mg/l

and the mean gravimetric concentration (filter) was calculated to be 1.103 mg/l. The mean particle size (mass median aerodynamic diameter (MMAD)) was calculated to be 1.81 micrometers. The particle size distribution chart indicated that the majority of the particles were within 0.7-4.7 micrometers, with a heavy concentration between 1.1 and 2.1 micrometers. The acute LC_{50} for 4 hours was calculated to be greater than 3.24 mg/l of air based on imazapyr and 3.07 mg/l of air based on imazethapyr. The report stated that these were the maximum analytical concentrations which could be attained.

C. DISCUSSION: This was a limit test. The only question concerning this study is that the report stated that the concentrations attained were the maximum attainable. However, there was no further explanation or discussion of this statement, except in one of the Appendices, which stated that 5 mg/L was the target concentration for each active ingredient in the formulation. The particle sizes attained are generally in the respirable range (in rats, a size of one micrometer can enter the alveoli and 1-2 micrometers can enter the trachea and bronchi). The study is classified as Core Guideline.

Reviewed By: Pamela Hurley Pamela M. Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation - rabbit (81-4)

TOX. CHEM. NO.: 3I + 3F

ACCESSION NUMBER/MRID NO.: 407634-02

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr formulation

REPORT NUMBER: A87-3

SPONSOR: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TITLE OF REPORT: Acute Oral, Acute Dermal, Eye Irritation, and Skin Irritation Studies With Event Formulation

AUTHOR(S): C.A. Lowe

REPORT ISSUED: ~~7/15/88~~ 1/16/87

IDENTIFYING VOLUME: Volume 5

CONCLUSION: Event was tested for potential to induce eye irritation in six male rabbits. The PIS score was zero and the test material was considered to be non-irritating.

Toxicity Category: IV

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Names: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: liquid
Batch #(s), Other #(s): AC 5329-101-C, Tox. Sample No: 86-195
Purity: 16.1% Imazethapyr, 0.61% Imazapyr
Source: American Cyanamid
Vehicle (if applicable): N/A
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): New Zealand White rabbits, male

Age: Not given
Weight(s): Not given
Source(s): Skippac Farms, Skippac, PA

3. Procedure: 0.1 ml of the test material was instilled into the conjunctival sac of the right eye of each of six rabbits. The left eye of each rabbit served as a control. Following application of the test substance, the lids of each eye were held shut for 5 seconds. At the end of a 24 hour exposure period, the treated eyes were rinsed with tap water and examined for irritation with the aid of ultraviolet light and fluorescin. The animals were examined immediately after dosing and at 1, 24, 48, and 72 hours post dosing. Scoring was done according to the Draize method. There was no indication that the animals were examined prior to dosing in order to ensure that there were no eye conditions that would influence the outcome of the test.

B. RESULTS: The scores for all the parameters for the cornea, iris and conjunctivae were all zero for all the rabbits at

all the observation periods. The test material is considered to be non-irritating (PIS=0).

Quality Assurance Measures: A signed Good Laboratory Practice Statement was provided.

C. DISCUSSION: This study is acceptable as presented. It is classified as Core Guideline. It should be noted that the animals should always be examined for eye conditions prior to dosing. Otherwise, a false positive result may occur.

Reviewed By: Pamela Hurley Pamela M Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation - rabbit (81-5)

TOX. CHEM. NO.: 31 + 3F

ACCESSION NUMBER/MRID NO.: 407634-02

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr formulation

REPORT NUMBER: A87-3

SPONSOR: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TESTING FACILITY: American Cyanamid Company, Agricultural Research Division, Princeton, NJ

TITLE OF REPORT: Acute Oral, Acute Dermal, Eye Irritation, and Skin Irritation Studies With Event Formulation

AUTHOR(S): C.A. Lowe

REPORT ISSUED: ~~7/15/88~~ 1/16/87

IDENTIFYING VOLUME: Volume 5

CONCLUSION: Event was tested for primary skin irritation potential on six male rabbits. The Primary Irritation score was zero and the test material was classified as non-irritating.

Toxicity Category: IV

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Names: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: liquid
Batch #(s), Other #(s): AC 5329-101-C, Tox. Sample No: 86-195
Purity: 16.1% Imazethapyr, 0.61% Imazapyr
Source: American Cyanamid
Vehicle (if applicable): N/A
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): New Zealand White rabbits, male
Age: Not given
Weight(s): Not given
Source(s): Skippac Farms, Skippac, PA

3. Procedure: Six male rabbits were used for the study. They were shaved 24 hours prior to the start of the study. 0.5 ml of the test material was applied to a 1" square intact site on the dorsal surface of each animal. A corresponding site on the opposite side of the midline served as the control. The site was covered with a gauze pad and plastic wrap for 4 hours. The occlusive dressing was then removed and the test sites were wiped with a gauze pad containing tap water. Each site was then scored for irritation according to the Draize method at 1, 4, 24, 48, and 72 hours post-dosing.

B. RESULTS: All of the erythema and edema readings for all the rabbits at all the observation time periods were zero (PIS=0). The test material was classified as non-irritating.

Quality Assurance Measures: A signed Good Laboratory Practice Statement was provided.

C. DISCUSSION: This study is acceptable as presented. It is classified as Core Guideline.

Reviewed By: Pamela Hurley Pamela M. Hurley 3/1/89
Section I, Tox. Branch, IRS (TS-769C)
Secondary Reviewer: Edwin Budd
Section I, Tox. Branch, IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization (81-6) - Guinea Pig

TOX. CHEM. NO.: 3F + 31

ACCESSION NUMBER/MRID NO.: 408657-03

TEST MATERIAL: Event

SYNONYMS: Imazethapyr/Imazapyr Formulation

REPORT NUMBER: 87-595I A

SPONSOR: American Cyanamid Company

TESTING FACILITY: Biosearch Incorporated, Philadelphia, PA

TITLE OF REPORT: Dermal Sensitization Study With ACP 1911X (EVENT)

AUTHOR(S): C. Reilly

REPORT ISSUED: ~~7/15/88~~ 1/12/88

IDENTIFYING VOLUME: Volume 7

CONCLUSION: Event was tested for potential to induce dermal sensitization in ten guinea pigs using a derivation of the Buehler closed patch test. It was not a sensitizer under the conditions of the study. The positive control gave a positive response.

Classification: Core Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: [(+)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (imazethapyr)

and

[2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-H-imidazol-2-yl]-3-pyridinecarboxylic acid]] (imazapyr)

Description: Yellow-brown liquid
Batch #(s), Other #(s): Lot # AC 5811-11
Purity: 16.76% Imazethapyr and 0.62% Imazapyr
Source: American Cyanamid Company
Vehicle (if applicable): None
Positive Control(s) (if applicable): 1-chloro-2,4-dinitrobenzene

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male Hartley guinea pigs
Age: not given
Weight(s): 400-600 g
Source(s): Buckshire, Corp., Perkasie, PA

3. Procedure: Ten animals were used per test group, naive control, positive control and treated group. Prior to initiation of the study, a screening for irritation potential of the test formulation was conducted. Four animals were selected for the screen and one each was dosed with 0.4 ml of one of the following solutions, the test article as supplied, or a 75%, 50%, or 25% dilution (w/v) in sterile saline. Each animal was exposed to the test material for 6 hours. Since no irritation was noted in any of the animals at either 24 or 48 hours, the test material as supplied was used in the main study.

A group of 10 guinea pigs was used for the main study. Each animal was clipped free of hair over an area of approximately 4 X 6 cm on their backs. Maintenance clipping was carried out throughout the duration of the induction phase and just prior to the challenge phase. The induction phase was conducted as follows: 0.4 ml of the test material was applied to a 1 inch square

gauze pad which was in turn applied to an intact skin site and wrapped with plastic wrap and tape. The patch was removed after a 6 hour exposure period and the site was examined for irritation at 24 and 48 hours, using the Draize method of scoring for irritation reactions. This sequence was repeated 3 times/week for 3 weeks (a total of 9 applications to the same test site). The animals were then rested for a 2 week period.

The challenge application was applied to a clipped area on the right flank for a contact period of 6 hours. The site was examined for dermal response at 24 and 48 hours, again using the Draize method for scoring for irritation.

Ten animals were used for the positive control group. 1-Chloro-2,4-dinitrobenzene was freshly prepared for each of the 10 applications as a 0.1% w/v suspension in a 50% ethanol:0.9% saline solution. The testing procedure was identical to the one described in the above paragraphs.

Ten animals were used for the naive control group. These animals were treated identically as the treated group except that they were not treated with the test chemical during the induction phase. They were, however, challenged with the test article during the challenge phase.

All animals were daily observed for clinical signs of toxicity throughout the study period and they were weighed 4 days prior to initiation of the study, weekly thereafter and at termination.

B. RESULTS: Clinical signs - treated group: one animal exhibited feces containing blood on days 13 and 14 and appeared thin on day 15. Loss of body weight was observed in one animal at week 1, in 4 animals at week 2 and in 1 animal at termination (this was one of the animals which lost weight during week 2). The report stated that the loss of body weight in the animals was probably due to a transient low level infection. Other than the above observations, no other clinical signs were observed. Naive control group: one animal was found dead on day 22. No other clinical signs were observed. Positive control group: One animal exhibited soft stools on day 9. Loss of body weight was observed in 2 animals at week 2. Again, the authors state that this was probably due to a transient low level infection. No other clinical signs were observed for this group.

Sensitization study - No effects were noted in the group treated with the test article. All the erythema and edema scores were zero for both the induction and challenge phases. The positive control test chemical was shown to be a primary skin irritant, a fatiguing agent* and a skin sensitizer in albino guinea pigs. During the induction phase, this chemical showed some irritation in the test animals in the form of erythema. Edema did not appear until the challenge phase. None of the naive controls showed any irritation.

*The report states that a fatiguing agent "consists of a subtle change in which the skin no longer exhibits its original refractoriness or resistance to the continued or repeated action of an agent". This happens in some cases when a substance which does not produce primary irritation may elicit severe skin reactions after a number of exposures. It is separate from sensitization because after 10-14 days, the skin recovers its original resistance to injury by the substance and does not produce a sensitization reaction.

Quality Assurance Measures: A signed quality assurance statement was provided.

C. DISCUSSION: This test is a derivation of the Buehler closed patch test. It is acceptable as presented. It is classified as Core Guideline.