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12/13/91

Reviewed by: Timothy F. McMahon, Ph.D. 2/13/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/13/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Acute oral-rats (81-1) Tox. Chem. No.: 271N

MRID Number: 415639-08

Test Material: CGA 163935 technical

Synonyms/CAS No:

Study number: 5645-88

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
9525 Town Park Drive
Houston, Texas 77036

Title of Report: Acute Oral Toxicity Study in Rats
EPA Guidelines No. 81-1

Author: Janice O. Kuhn, Ph.D.

Report Issued: November 22, 1988

Conclusions: The acute oral LD50 for CGA 163935 technical was 4613 mg/kg in male rats and 4212 mg/kg in female rats. The acute oral LD50 for male and female rats was 4458 mg/kg.

Toxicity Category III

Classification: core-guideline

This study fulfills the guideline (81-1) requirements for an "Acute oral toxicity study in rats."

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I. MATERIALS

A. Test Material: CGA 163935 technical; Description: dark brown liquid

B. Test Animals: HSD:(SD)BR rats; Source: Harlan Sprague-Dawley, Inc., Houston, Texas; Age: young adult; Weight: males, 206-292g; females, 187-229g.

II. METHODS

Fifteen male rats were used for oral administration of CGA 163935 at doses of 4000, 4500, and 5050 mg/kg, while 15 female rats were used for oral administration of CGA 163935 at doses of 3500, 4000, and 5050 mg/kg. Food (Purina Formulab Chow #5008) and tap water were available ad libitum throughout the study with the exception of a 16 hr fast prior to dosing.

The test material was administered undiluted to rats based upon fasted body weights. Animals were observed one-half hour, 3 hours, and 6 hours after dosing and then daily thereafter up to 14 days. Body weights were recorded just prior to dosing (Day 0), and then on days 7 and 14 after dosing. Rats were observed for mortality and pharmacologic and/or toxicologic signs at least three times on day 0, and once daily thereafter. Gross necropsy was performed on each animal at 14 days or at the time of discovery after death.

III. RESULTS

Body weight fluctuated only slightly during the post-dosing period. No deaths occurred at 3500 mg/kg in female rats (male rats not treated at this dose). At 4000 mg/kg, 2 of 5 female rats died by day 1 post-dosing, and 1 female rat died by day 2 post-dosing. No deaths occurred in male rats at 4000 mg/kg. At 4500 mg/kg, 1 of 5 male rats died by day 2 post-dosing (female rats not treated at this dose). At 5050 mg/kg, 1 male rat was dead by 6 hours post-dosing, 2 more by day 1, and the remaining 2 by day 2. In female rats at 5050 mg/kg, 1 was dead by day 1, 3 more died by day 2, and the remaining rat survived to day 14.

Based on these results, the LD50 was calculated (using the method of Litchfield and Wilcoxon) to be: males, 4613 mg/kg (with 95% confidence limits of 4447-4785 mg/kg; females, 4212 mg/kg (with 95% confidence limits of 3454-5136 mg/kg).

Gross necropsy findings related to administration of the test substance included the following: Female rats demonstrated no significant abnormalities at 3500 mg/kg. At 4000 mg/kg, no significant abnormalities were observed in male rats, while in 3 female rats at this dose, abnormalities consisted of a tan slurry in the stomach, red-brown mucoid material in the small intestine, and a green paste in the cecum. Two female rats showed no significant abnormalities at this dose. At 4500 mg/kg, 1 male rat displayed black and brown paste in the stomach and red-yellow mucoid material in the small intestine, while the remaining 4 showed no abnormalities.

Necropsy findings at 5050 mg/kg consisted of those previously observed, as well as diarrhea, salivation, and polyuria.

Pharmacologic and/or toxicologic signs observed during this study consisted of piloerection, salivation, epistaxis, ataxia, nasal discharge, and hematuria. With the exception of piloerection, all pharmacologic and/or toxicologic signs were of slight to moderate severity and of low incidence and duration.

IV. CONCLUSIONS

The acute oral LD50 for CGA 163935 technical was 4613 mg/kg in male rats and 4212 mg/kg in female rats. The acute oral LD50 for male and female rats was 4458 mg/kg.

Toxicity Category III

V. CLASSIFICATION

Core-guideline

This study fulfills the guideline (81-1) requirements for an "Acute oral toxicity study in rats."

00827[illegible]

Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 14 Dec 90
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James N. Rowe, Ph. D. James N. Rowe 12/14/90
Section III, Tox. Branch II(H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity (§81-1)

TOX. CHEM NO.: 271 N

MRID NO.: 415639-09

TEST MATERIAL: CGA-163935 2E-A FL-900351 ARS-8640

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 6937-90

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Acute Oral Toxicity Study in Rats

AUTHOR(S): Janice O. Kuhn. Ph. D.

REPORT ISSUED: MAY 21, 1990

CONCLUSION: The acute (14 day) oral LD_{50} for CGA-163935 administered neat was 4605 mg/kg (95 % confidence limits are 3942 to 5379 mg/kg) for males and 4449 mg/kg (95 % confidence limits are 4032 to 4909 mg/kg) for females in young adult Harlan Sprague-Dawley rats. This study was performed at three dose levels: 4010, 4520, and 5050 mg/kg.

Toxicity Category: III

LD_{50} (95 % confidence limit) = 4514 mg/kg (4140 - 4923 mg/kg) overall for males and females combined.

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§81-1) for an Acute Oral Toxicity study.

MATERIALS:

1. Test compound: CGA-163935 2E-A FL-900351. Description: dark-brown liquid. Batch # GP-051401. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: Rat. Strain: Harlan Sprague Dawley (HSD) BR. Age: not reported. Weight: at time of test males were 175-253 g, and females were 192-236 g. Source: Harlan Sprague Dawley, Inc., Houston, Texas.

METHODS:

Rats were fasted overnight before dosing. Test material was administered orally by gavage as neat material. Animals were observed 0.5, 3, and 6 hours after dosing and daily for a total of 14 days. Rats were weighed on days 0, 7, and 14. Gross necropsy was performed on all animals that died on study and on all survivors which were sacrificed on day 14. Doses and lethality are presented in the table under results.

RESULTS:

Clinical Signs.
Frequent clinical signs noted were piloerection in all or most males at 0.5 hrs through day 3 along with decreased activity at 6 hrs and 1 day after treatment. All or most females experienced piloerection from 0.5 hrs through day 5 post-treatment and deceased activity (4-5 females) from 3 hrs through 4 days post treatment. Additionally, there were deaths of each sex in each dose group, which are shown in Table 1. Body weights and gross necropsy findings are presented in Table 2.

Table 1. Doses and Deaths

Dose	Males	Females
mg/kg	deaths/dosed	deaths/dosed
4010	1 / 5	1 / 5
4520	3 / 5	3 / 5
5060	3 / 5	4 / 5
LD_{50}(+95%CL)=	4605 mg/kg (3942-5379)	4449 mg/kg (4032-4909)

Table 2. Rat Acute Oral Toxicity

Animal Number / Dose (mg/Kg)		Body Weights (g) Day 0	Day 7	Final	Time of Death Day	Gross Necropsy F
1-M	4010	253	298	335	14	NOA
2-M	4010	235	---	214	2	Signs of saliva discharge; brow throughout gast tract; gastroir distended with
3-M	4010	238	276	323	14	NOA
4-M	4010	243	290	322	14	NOA
5-M	4010	246	288	316	14	NOA
6-F	4010	234	237	259	14	NOA
7-F	4010	213	231	235	14	NOA
8-F	4010	222	---	204	03	Signs of saliv discharge; brov throughout gas tract; gastroi distended with
9-F	4010	236	265	284	14	NOA
10-F	4010	236	249	275	14	NOA
1-M	4520	242	277	304	14	NOA
2-M	4520	245	---	162	07	Signs of nasal polyuria; brow in gastrointes testes drawn i cavity.
3-M	4520	235	---	209	02	Signs of saliv discharge and drawn into abd light brown mu gas throughout tract.

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Table 2 Continued

Animal Number Dose (mg/kg)	Body Weights (g) Day 0	Day 7	Final	Time of Death Day	Gross Necropsy
4M 4520	241	---	213	02	Signs of saliva discharge and p drawn into abdo light brown muc gas throughout tract.
5M 4520	243	284	321	14	NOA
6F 4520	208	---	190	02	Brown mucoid ma gastrointestina
7F 4520	192	199	211	14	NOA
8F 4520	211	---	194	02	Brown mucoid ma gastrointestina
9F 4520	200	---	185	01	Signs of nasal lacrimation; wh throughout gast tract.
10F 4520	221	230	248	14	NOA
1M 5060	253	---	239	02	Signs of saliva discharge; abdc gas; stomach fi occupied entire cavity; testes abdominal cavit
2M 5060	175	230	257	14	NOA
3M 5060	250	---	229	02	Signs of saliva discharge; abdc gas; stomach fi and occupied er cavity; testes abdominal cavit
4M 5060	239	268	303	14	NOA

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Table 2 Continued

Animal Number	Dose (mg/kg)	Body Weights (g) Day 0	Day 7	Final	Time of Death Day	Gross Necropsy
5M	5060	237	---	221	01	Signs of saliv discharge and brown material gastrointestin
6F	5060	218	---	196	02	Signs of saliv discharge and mucoid materia throughout gas tract.
7F	5060	220	---	204	02	Signs of red r brown mucoid m throughout gas tract.
8F	5060	195	212	215	14	NOA
9F	5060	209	---	190	02	Brown mucoid m throughout gas tract.
10F	5060	223	---	201	03	Signs of red r brown mucoid m throughout gas tract.

[1]No Observable Abnormalities (NOA)

008271

Reviewed by: Timothy F. McMahon, Ph.D. 10.15.90
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 10/15/90
Section I, Toxicology Branch II (HFAS) (H7509C)

DATA EVALUATION REPORT

Study Type: Acute Dermal-rats (81-2) Tox. Chem. No.: 271N

MRID Number: 415639-10

Test Material: CGA 163935 technical

Synonyms/CAS No:

Study Number(s): 871407

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing Facility: CIBA-GEIGY Limited
Tierfarm
Sisseln, Switzerland

Title of Report: Acute Dermal Toxicity Study in Rats
EPA Guidelines No. 81-2

Author: Dr. H.R. Hartmann

Report Issued: October 16, 1987

Conclusions: The acute dermal LD50 for CGA 163935 technical was greater than 4000 mg/kg body weight in male and female rats.

Toxicity Category III

Classification of data: Core guideline

This study satisfies the guideline (81-2) requirements for an "Acute Dermal Toxicity Study in Rats."

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I. MATERIALS

A. Test Material: CGA 163935 technical; Purity: 96.6%; Batch no.: P.705002
Description: liquid

B. Test Animals: SPF hybrid albino rats; Source: CIBA-GEIGY Ltd., Tierfurm, Sisseln, Switzerland. Weight: 212-254 g; Age: 7-8 weeks

II. METHODS

Five male and five female rats were acclimated at least 5 days to experimental conditions prior to the study. Food (NAFAG No. 890) and water were provided ad libitum throughout the study. Twenty four hours prior to dosing, the fur was clipped from an area on the back of the rat with an electric clipper.

A dose of 4000 mg/kg of test material was evenly dispersed on the skin of both male and female rats. The treated area was covered with a gauze-lined semiocclusive dressing, which was held in place by an adhesive elastic bandage fastened around the trunk.

After an exposure period of 24 hours, the dressing was removed and excess test material was removed with lukewarm water. Animals were observed for mortality and clinical signs of toxicity daily. Body weights were recorded on days 1, 7, and 14 after treatment.

III. RESULTS

No mortality was observed in this study. No dermal toxicity nor gross pathologic abnormalities were observed at necropsy. Clinical signs of dyspnea, ruffled fur, abnormal body position, and reduced spontaneous activity were reported as slight in all treated animals. No significant changes occurred in body weights during this study.

IV. CONCLUSIONS

The acute dermal LD50 for CGA 163935 technical was greater than 4000 mg/kg in male and female rats.

Toxicity Category III

V. CLASSIFICATION

Core Guideline

This study fulfills the guideline (81-2) requirements for an "Acute Dermal Toxicity Study in Rats."

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Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 14 Dec 90
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James Rowe, Ph. D. James Rowe 12/14/90
Section III, Tox. Branch II(H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity (§81-2)

TOX. CHEM NO.: 271-N

MRID NO.: 415639-11

TEST MATERIAL: CGA-163935 2E-A FL-900351 ARS-8640

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 6938-90

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Acute Dermal Toxicity Study in Rabbits

AUTHOR(S): Janice O. Kuhn. Ph. D.

REPORT ISSUED: May 10, 1990

CONCLUSION: This study sought to determine the acute dermal toxicity of CGA-163935 2E-A FL 900351 in rabbits using a single dose (limit dose) of the test article at 2020 gm/kg.

Toxicity Category: III

LD_{50}(95 % confidence limit) > 2020 mg/kg

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§81-2) for an Acute Dermal Toxicity study.

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MATERIALS:

1. Test compound: CGA-163935 2E-A FL-900351. Description: dark-brown liquid. Batch #: GP-051401. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: Rabbit. Strain: New Zealand White. Age: Young adult. Weight: at time of test males were 2.800-3.175 kg, and females were 2.075-2.600 kg.
Source: Ray Nichols Rabbitry, Lumberton, Texas.

METHODS:

The dorsal surface of the rabbits was clipped on the day prior to treatment. The test article was applied undiluted at 2020 mg/kg (1.89 ml/kg) under surgical gauze (10 cm x 10 cm two layers thick). The animals were then wrapped with a semipermeable dressing to retard evaporation and to prevent ingestion of the test article. The animals were exposed to the test article for 24 hrs. Animals were observed 0.5, 3, and 6 hours after dosing (day zero) and then daily for 14 days. Rabbits were weighed on days 0, 7, and 14.

RESULTS:

Doses and lethality are presented in Table I.
Gross necropsy was performed on all animals, which were sacrificed on day 14 (Table II). Clinical signs of toxicity are shown in Table III.

Table I. Mortality

Dose mg/kg	Males deaths/dosed	Females deaths/dosed
2020 mg/kg	0 / 5	0 / 5
LD_{50}(+95%CL)	> 2020 mg/kg	> 2020 mg/kg

Table II. Gross Determinations

Animal Number	Body Weights (kg) Day 0	Day 7	Final	Time of Death, Day	Gross Necropsy Findings
8956M	2.95	3.05	3.30	14	NOA[1]
8960M	3.02	3.32	3.48	14	NOA
8962M	3.08	3.20	3.38	14	NOA
8964M	2.80	2.58	2.90	14	NOA
8966M	3.18	3.20	3.40	14	NOA
8933F	2.08	2.12	2.38	14	NOA
8965F	2.60	2.75	2.98	14	NOA
9021F	2.45	2.60	2.88	14	NOA
9023F	2.50	2.70	2.92	14	NOA
9025F	2.55	2.72	2.98	14	NOA

[1]No Observable Abnormalities (NOA)

Table III. Clinical Signs of Toxicity

Reaction and Severity	Time after Treatment: Hours .5	3	6	Days 1	2	3	4	5	6	7	8	9	10	11	12
Males															
Decreased Defecation	0	0	0	0	0	0	0	1[1]	1	0	0	0	0	1	1
Females															
No Defecation	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Decreased Defecation	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

[1]Numbers indicate surviving animals exhibiting reaction.

Reviewed by: Timothy F. McMahon, Ph.D. 10/3/90
Section I, Toxicology Branch II (HFAS) (H7509C) 10/15/90
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D.
Section I, Toxicology Branch II (HFAS) (H7509C)

DATA EVALUATION REPORT

Study Type: Acute inhalation - rats (81-3) Tox. Chem. No.: 271N

MRID Number: 415639-12

Test Material: CGA 163935 technical

Synonyms/CAS No.:

Study Number: 871409

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing facility: CIBA-GEIGY Limited
Stein, Switzerland

Title of report: Acute Inhalation Toxicity Study in Rats
EPA Guidelines No. 81-3

Author: Dr. H.R. Hartmann

Report Issued: March 15, 1988

Conclusions:

The acute LC50 for CGA 163935 under the conditions of this study was > 5.3 mg/L in both male and female rats.

Toxicity Category IV

Classification:

Core Guideline

This study fulfills the guideline (81-3) requirements for an "Acute Inhalation Toxicity Study in Rats."

I. MATERIALS

A. Test Material: CGA 1863935 technical; purity: 96.6%; Batch no.:P705002; description: liquid

B. Test Animals: (Tif:RAI f(SPF)) albino rats. Source: CIBA-GEIGY Ltd., Animal Production, 4332 Stein/Switzerland; Weight: 191-238g; Age: Young adult.

II. METHODS

A. Atmosphere Generation

A 30% (w/w) solution of CGA 163935 in absolute ethanol was prepared in order to generate a liquid aerosol. The aerosol was generated utilizing a pneumatic nebulizer with a small aspirating reservoir and an attached bulk fluid container. The aerosol was diluted with filtered humidified air to yield a total flow of 32 L/min. Coarse particles were removed by way of a glass cyclone.

B. Exposure

Rats were placed in individual Macrolon animal holders positioned radially around the exposure chamber and were exposed for 4 hours to the test atmosphere. Atmospheric concentrations of the aerosol were determined gravimetrically 5 times during exposure (comment: times of measurement were not specified). Particle size analysis was conducted twice during exposure using an eight-stage cascade impactor. Control animals were exposed under identical conditions to the ethanol vehicle atmosphere.

C. Observations

Rats were examined for clinical signs and mortality during exposure at 1, 2, and 4 hours, 2 hours after exposure, and then daily thereafter up to day 14 (day of terminal sacrifice). Body weights were recorded immediately prior to exposure, and on days 7 and 14 post-exposure. Gross pathological examination was performed on all animals on day 14.

III. RESULTS

A. Atmosphere Generation

The mean atmospheric concentration of test substance measured was approximately 5.3 mg/L. Particle size distribution of the test atmosphere contained approximately 25% particles with aerodynamic diamter < 1.0 um. (NOTE: This value was estimated from data supplied in figure 2, page 21 of registrant's report).

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PARTICLE SIZE CHARACTERIZATION

Mean Particle Concentration (mg/L)		% Particles		
	MMAD (µm)	<1µm	<3µm	<7µm
5.3	2.1	25.0	64.8	89.2

from page 16 of registrant report

B. Animal Observations

No mortality was observed during the exposure or post-exposure period in male or female rats. Clinical signs of ruffled fur, dyspnea, hunched posture, and reduced spontaneous activity were noted in both control and test article exposed rats of both sexes. However, only ruffled fur and dyspnea appeared to be related to test article, and the severity of these signs was slight. No sex differences were observed in the duration or severity of clinical signs. Body weights were not significantly affected during the course of the study. At necropsy, no observable abnormalities were noted in control or test article exposed rats of either sex.

IV. CONCLUSIONS

The acute LC50 for CGA 163935 under the conditions of this study was > 5.3 mg/L in both male and female rats.

Toxicity Category IV

V. CLASSIFICATION

Core Guideline

This study fulfills the guideline (81-3) requirements for an "Acute Inhalation Toxicity Study in Rats."

00827

Reviewed by: Dan W. Hanke, Ph. D. Dan W. Hanke 14 Dec 90
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James N. Rowe, Ph. D. James N. Rowe 12/14/90
Section III, Tox. Branch II(H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity (§81-3)

TOX. CHEM NO: 271 N

MRID NO.: 415639-13

TEST MATERIAL: CGA-163935 2E-A FL-900351 ARS-8640

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 6940-90

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Acute Inhalation Toxicity Study in Rats

AUTHOR(S): Mark S. Hobert

REPORT ISSUED: June 11, 1990

CONCLUSION: This acute inhalation study was performed on 5 male and 5 female albino rats (all of which survived) at a single maximum average concentration of 0.912 g/L, which did not reach the nominal concentration of 2.14 g/L.

Toxicity Category: III

LC_{50} > 0.912 mg/L (mean concentration; range = 1.27 mg/L at 1 hr to 0.588 mg/L at 4.0 hrs) when the test article is administered as an undiluted aerosol, which is the maximum attainable concentration with 25 % of the particles < 1.1 um.

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§81-3) for an Acute Inhalation Toxicity study.

MATERIALS:

1. Test compound: CGA-163935 2E-A FL-900351 ARS-8640. Description: dark-brown liquid. Batch # GP-051401. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: Rat. Strain: Harlan Sprague Dawley (HSD) BR. Age: young adult. Weight When Tested: males (298-347 g), females (201-241 g). Source: Harlan Sprague Dawley, Inc., Houston, Texas.

METHODS:

The concentration of the aerosolized test article in the inhalation chamber was sampled every hour for four hours and analyzed by gas chromatography. The respective hourly concentrations of test article were 1.27, 0.835, 0.96, 0.588 mg/L with an average of 0.912 mg/L, which did not reach the nominal concentration of 2.14 mg/L. After four hours 50% of aerosolized test article contained particles of a median aerodynamic diameter of ≤ 1.476 um with 95 % of the particles ≤ 11.93 um as determined by an Andersen cascade impactor. Sixteen per cent of the mass median diameters were ≤ 0.41 um. These median aerodynamic diameters generally meet the Inhalation SEP recommendation of 1-2 um or less. Test material was administered as an aerosol generated from the undiluted liquid test article for a period of four hours to five male and to five female rats. During the exposure period, the animals were individually housed in stainless steel cages within a 200 L New York University design, stainless steel, dynamic flow inhalation chamber. The animals were returned to their stock laboratory cages at the termination of the exposure period. Animals were observed for mortality and pharmacologic and/or toxicologic signs on the day of exposure (day of exposure considered Day 0) at 0.5, 1.0, 2.5, 4.5, 6.0 hrs and at least once daily thereafter for 14 days.

RESULTS:

Doses and lethality are shown in Table I. Gross necropsy data are in Table II. Clinical signs of toxicity are reported in Table III. Surviving rats showed the clinical signs of toxicity essentially only during the day of dosing, and the signs present did not exceed a moderate degree of severity.

Table I. Mortality

Dose mg/L	Males deaths/dosed	Females deaths/dosed
0.912	0 / 5	0 / 5
LC_{50}	> 0.912 mg/L	> 0.912 mg/L

Table II. Gross Determinations

Animal Number	Body Weights (g) Day 0	Day 7	Final	Time of Death, Day	Gross Necropsy Findings
1M	347	348	370	14	NOA[1]
2M	346	347	358	14	NOA
3M	314	328	349	14	NOA
4M	298	312	343	14	NOA
5M	321	321	330	14	NOA
6F	202	205	212	14	NOA
7F	241	253	260	14	NOA
8F	225	219	231	14	NOA
9F	201	211	219	14	NOA
10F	218	220	229	14	NOA

[1]No Observable Abnormalities (NOA)

Table III. Clinical Signs of Toxicity

Reaction and Severity	Time after Treatment: Hours 0.5	1.0	2.5	4.5	6.0	Days 1	2	3	4	5	6	7	8
Males													
Piloerection (v-m)	2[1]	2	2	5	5	5	0	0	0	0	0	0	0
Activity decrease (s-m)	2	2	2	5	5	0	0	0	0	0	0	0	0
Ptosis (s-m)	2	2	2	0	0	0	0	0	0	0	0	0	0
Lacrimation (v)	2	2	2	3	0	0	0	0	0	0	0	0	0
Nasal discharge (v-m)	0	2	2	5	5	0	0	0	0	0	0	0	0
Polyuria (v-s)	0	0	0	5	4	0	0	0	0	0	0	0	0
Salivation (s)	0	0	0	1	0	0	0	0	0	0	0	0	0
Respiratory gurgle (s)	0	0	0	0	2	4	4	0	0	0	0	0	0
Females													
Piloerection (v-m)	2	2	2	5	5	5	0	0	0	0	0	0	0
Activity decrease (s-m)	2	2	2	5	5	0	0	0	0	0	0	0	0
Ptosis (s-m)	2	2	2	0	0	0	0	0	0	0	0	0	0
Lacrimation (v-s)	2	2	2	2	0	0	0	0	0	0	0	0	0
Nasal discharge (v-m)	0	2	2	5	4	0	0	0	0	0	0	0	0
Polyuria (v-s)	0	0	0	5	5	0	0	0	0	0	0	0	0
Salivation (v-s)	0	0	0	3	0	0	0	0	0	0	0	0	0
Respiratory gurgle (s)	0	0	0	0	3	2	2	0	0	0	0	0	0

[1]Numbers indicate surviving animals exhibiting reaction.

Key: v - very slight; s - slight; m - moderate; e - extreme

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Reviewed by: Timothy F. McMahon, Ph.D. 2/11/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/15/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Primary eye irritation-rabbits (81-4)

Tox. Chem. No.: 271N

MRID Number: 415639-14

Test Material: CGA 163935 technical

Synonyms/CAS No:

Study number: 5646-88

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
9525 Town Park Drive
Houston, Texas 77036

Title of Report: Primary Eye Irritation Study in Rabbits
EPA Guidelines No. 81-4

Author: Janice O. Kuhn, Ph.D.

Report Issued: October 14, 1988

Conclusions: CGA 163935 was minimally irritating to the eyes of male and female albino rabbits under the conditions of this study.

Toxicity Category III

Classification: core-guideline

This study fulfills the guideline (81-4) requirements for a "Primary Eye Irritation Study in Rabbits."

I. MATERIALS

A. Test Material: CGA 163935 technical; Description: dark brown liquid

B. Test Animals: New Zealand White Rabbits; Source: Jojo's Rabbbitry of Seguin, Segion, Texas; Age: young adult

II. METHODS

Six male and three female rabbits were quarantined for at least one week prior to the study. Food (Purina Rabbit Chow) and tap water (automatic watering system) were provided ad libitum throughout the study. Both eyes of each rabbit were examined 24 hours prior to treatment with 0.2% fluorescein sodium ophthalmic solution, and again immediately prior to treatment but without the fluorescein sodium ophthalmic solution. Only animals without eye defects or irritation were selected for testing.

For testing, undiluted test material (0.1ml) was placed into the conjunctival sac of the right eye of all 9 rabbits, with the left eye of each rabbit serving as control. In three of the six treated male rabbits, irrigation of the treated eye with room temperature deionized water was performed for 1 minute beginning 30 seconds after administration of the test substance. The treated eye of all rabbits were examined for ocular reaction at 1, 24, 48, and 72 hours after treatment (NOTE: method used to score irritation not reported). Corneas of all treated eyes were re-examined immediately after the 24 hour observation with 0.2% fluorescein ophthalmic solution. Any positive staining at 24 hours was followed by re-examination with fluorescein sodium ophthalmic solution at each consecutive observation time until staining was negative.

III. RESULTS

Reaction of the cornea and iris to administration of the test substance was negative in treated eyes of all rabbits. Conjunctival redness, chemosis, and discharge was minimal in all rabbits. All ocular reaction was negative in treated eyes of all rabbits by 72 hours. Scores for ocular irritation in washed eyes were similar to those in unwashed eyes.

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OCULAR RESPONSES TO CGA 163935

Response	1hr	24hr	48hr	72hr
Corneal Opacity	0/9	0/9	0/9	0/9
Iritis	0/9	0/9	0/9	0/9
Conjunctiva				
redness	9/9	7/9	0/9	0/9
chemosis	9/9	1/9	1/9	0/9
discharge	6/9	1/9	0/9	0/9

IV. CONCLUSIONS

CGA 163935 was minimally irritating to the eyes of male and female rabbits under the conditions of this study.

Toxicity Category III

V. CLASSIFICATION

Core-guideline

This study fulfills the guideline (81-4) requirements for a "Primary Eye Irritation Study In Rabbits."

Reviewed by: Dan W. Hanke, Ph. D.
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James N. Rowe, Ph. D.
Section III, Tox. Branch II(H7509C)

Dan W Hanke 14 Dec 90
James N. Rowe 12/14/90

DATA EVALUATION RECORD

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

TOX. CHEM NO: 271-N

MRID NO.: 415639-15

TEST MATERIAL: CGA-163935 2E FL 882137

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 5765-88

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Primary Eye Irritation Study in Rabbits

AUTHOR(S): Janice O. Kuhn, Ph.D.

REPORT ISSUED: December 21, 1988

CONCLUSION: The objective of this study was to determine the eye irritation potential of the test material. The test article was rated as severely irritating in nonwashed eyes and moderately irritating in washed eyes with maximum average irritation scores of 29.0 at 72 hrs post treatment (moderately irritating) and 17.7 at one hr post treatment (moderately irritating) respectively (range 0-110).

Toxicity Category: I

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§81-4) for a Primary Eye Irritation study.

MATERIALS.

1. Test compound: Description: dark-brown liquid. Batch #: 318-81-1 FL 882137. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: Rabbit. Strain: New Zealand White. Age: Young Adult. Weight: Not Applicable.
Source: Ray Nichols Rabbitry, Lumberton, Texas.

METHODS.

Rabbits' eyes were examined twice prior to selecting animals for dosing, and only animals without eye defects or irritation were selected for testing. The left eye served as a control. Undiluted test material (0.10 ml) was placed into the conjunctival sac of the right eye of each animal. Three of the nine treated eyes were washed with room temperature deionized water for 1 min beginning 30 sec after treatment. Animals were observed and graded for ocular reaction at 1, 24, 48, and 72 hrs, and then at 4, 7, 11, 14, 17, and 21 days after treatment.

RESULTS.

During the first 72 hrs the test article ranged from non-irritating to the iris in washed eyes to minimally irritating in nonwashed eyes. Corneal effects during the first 72 hrs ranged from minimally to moderately irritating in nonwashed eyes to nonirritating in washed eyes. The conjunctival responses during the first 72 hrs ranged from minimally to mildly irritating in nonwashed eyes and from minimally to mildly irritation in washed eyes also. Corneal opacity and/or invasion of the cornea by blood vessels was present in one nonwashed eye on Day 21. Corneal opacity and/or invasion of the cornea by blood vessels was not present in any washed eyes on Day 21. Summary data are presented below in Table I.

Table I. Eye Irritation Scores and Score Summary

Time after Treatment	Nonwash Rabbit Number						Nonwash Average Score	Wash Rabbit	
	1-M	2-M	3-M	4-F	5-F	6-F		7-F	8-F
Hour 1	14	16	23	16	16	16	16.8	16	16
Hour 24	31	26	33	17	29	21	26.2	12	17
Hour 48	23	33	33	27	22	26	27.3	10	8
Hour 72	12	31	51	13	41	26	29.0	6	4
Day 4	6	25	39	4	37	10	20.2	4	4
Day 7	2	11	47	2	11	4	12.8	2	2
Day 11	0	2	4	0	4	2	2.0	0	0
Day 14	0	2	2	0	2	2	1.3	0	0
Day 17	0	0	4	0	0	2	1.0	0	0
Day 21	0	0	0	0	0	0	0.0	0	0

Nonwash Maximum Average Score = 29.0 (severely irritating)
Wash Maximum Average Score = 17.7 (Moderately Irritating)

008271

Reviewed by: Timothy F. McMahon, Ph.D. 2/13/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 2/13/91
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Primary dermal irritation-rabbits (81-5)

Tox. Chem. No.: 271N

MRID Number: 415639-16

Test Material: CGA 163935 technical

Synonyms/CAS No:

Study number: 5647-88

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing Facility: Stillmeadow, Inc.
Biological Testing Laboratory
9525 Town Park Drive
Houston, Texas 77036

Title of Report: Primary Dermal Irritation Study in Rabbits
EPA Guidelines No. 81-5

Author: Janice O. Kuhn, Ph.D.

Report Issued: October 28, 1988

Conclusions: CGA 163935 was slightly irritating to the skin of male and female albino rabbits under the conditions of this study.

Toxicity Category IV

Classification: core-guideline

This study fulfills the guideline (81-5) requirements for a "Primary Dermal Irritation Study in Rabbits."

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I. MATERIALS

A. Test Material: CGA 163935 technical; Description: dark brown liquid

B. Test Animals: New Zealand White Rabbits; Source: Ray Nichols' Rabbbitry, Lumberton, Texas; Age: young adult

II. METHODS

Three male and three female rabbits were quarantined for at least one week prior to the study. Food (Purina Rabbit Chow) and tap water (automatic watering system) were provided ad libitum throughout the study. One day prior to testing, an area of at least 8 x 8 cm was exposed on the dorsal area of the trunk by clipping the area free of hair. Only animals with no evidence of pre-existing skin irritation or defects in the exposure area were selected for testing.

The test site of each rabbit was treated with 0.5ml undiluted test material by introducing the material beneath a 2.5 x 2.5cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. An orthopedic stockinette was then loosely wrapped around the trunk of each rabbit to retard evaporation and prevent ingestion of test material. Wrappings were held in place by non-irritating adhesive tape. Animals remained in their cages during exposure.

After four hours, wrappings and patches were removed and test sites washed gently with room temperature water and a wet cloth to remove residual test material. Test sites were evaluated for erythema and eschar formation, edema, and other dermal effects at 1, 24, 48, and 72 hours after washing, and also on day 7. For each observation time, erythema and edema scores were added and divided by the number of animals observed to determine an irritation score. Maximun irritation score was used to describe the dermal irritation of the test substance.

III. RESULTS

Very slight erythema and edema was reported in response to dermal application of CGA 163935. Brown staining of the test site hairs was also observed through 72 hours post-treatment. Incidence of response is listed below.

DERMAL RESPONSE TO CGA 163935

Response	1hr	24hr	48hr	72hr
Erythema	6/6	6/6	4/6	3/6
Edema	5/6	3/6	2/6	0/6

IV. CONCLUSIONS

CGA 163935 was slightly irritating to the skin of male and female rabbits under the conditions of this study.

Toxicity Category IV

V. CLASSIFICATION

Core-guideline

This study fulfills the guideline (81-5) requirements for a "Primary Dermal Irritation Study In Rabbits."

00827[illegible]

Reviewed by: Dan W. Hanke, Ph. D. Dan W Hanke 14 Dec 88
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James N. Rowe, Ph. D. James N Rowe 12/14/88
Section III, Tox. Branch II(H7509C)

DATA EVALUATION RECORD

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

TOX. CHEM NO: 271 N.

MRID NO.: 415639-17

TEST MATERIAL: CGA-163935 2E FL 882137

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 5766-88

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Primary Dermal Irritation Study in Rabbits

AUTHOR(S): Janice O. Kuhn, Ph. D.

REPORT ISSUED: December 13, 1988

CONCLUSION: Erythema was present at each observation time through day 14. Edema was present at each observation time through day 10. An irritation score of 3.3 out of a possible 8.0 was obtained at the 72 hr observation and was used to give the test article a descriptive rating of moderate irritant.

Toxicity Category: III

A signed quality assurance statement was present.

Core Classification: Minimum

This study satisfies the guideline requirements (§81-5) for a Primary Dermal Irritation study.

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MATERIALS.

1. Test compound: CGA-163935 2E. Description: dark-brown liquid. Batch # 318-81-1 FL 882137. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: rabbit. Strain: New Zealand. Age: young adults: three males and three females. Weight: not applicable. Source: Ray Nichols Rabbitry, Lumberton, Texas.

METHODS.

Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. There was one intact skin test site per animal. Each test site was treated with 0.5 ml of actual undiluted test material by introducing the test material beneath a surgical gauze patch measuring 2.5 x 2.5 cm and two single layers thick. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) to retard evaporation of the test article and to prevent possible ingestion of the test article. Four hours after treatment the wrappings and patches were removed. The test sites were wiped with a clean wet cloth to remove as much residual test material as possible. The test sites were observed for erythema and eschar formation, edema formation, and any other dermal defects or irritation at 1, 24, 48, and 72 hrs and on days 7, 10, 14, and 17 after wiping (day 0 is considered the day of treatment).

RESULTS.

The signs of dermal irritation are shown in Table 1. By one hour the degree of essentially well-defined and remained as such for 48 hrs in all six rabbits. Th formation over the first 48 hrs ranged from slight during the first 24 hrs to ve through 48 hrs.

Table 1. Signs of Dermal Irritation

	Erythema Formation						Edema Formation						Other Obs		
	Rabbit Number						Rabbit Number						Rabbit		
Observation Time	1M	2M	3M	4F	5F	6F	1M	2M	3M	4F	5F	6F	1M	2M	3M
1 Hour	1	2	2	2	2	2	1	2	2	2	2	2	X	X	
24 Hours	2	2	2	2	2	2	2	2	2	1	2	2	X	X	
48 Hours	2	2	2	2	2	2	1	2	1	1	2	2	X	X	
72 Hours	2	2	1	1	2	2	1	3	1	1	2	2	X	X	
Day 7	1	2	1	1	2	2	1	2	1	0	1	1	S	0	
Day 10	0	1	1	0	1	1	0	1	0	0	0	0	S	0	
Day 14	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
Day 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Maximum Irritation Score = 3.8
Descriptive Rating: Moderate Irritant

M - male; F - female
S - sloughing of skin of various thicknesses
X - brown staining of test site hairs

Legend to Table 1.

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum Possible	4

Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure	4
Maximum Possible	4

Descriptive Rating	Maximum Irritation Score	Remarks
Practically not an Irritant	0.0 - 0.4	
Slight Irritant	0.5 - 3.0	
Moderate Irritant	3.1 - 5.0	
Severe Irritant	5.1 - 7.0	severe erythema or edema without tissue destruction
Corrosive	7.1 - 8.0	tissue destruction into the dermis and/or scarring

008271

Reviewed by: Timothy F. McMahon, Ph.D.
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis H. Ioannou, Ph.D.
Section I, Toxicology Branch II (HFAS) (H7509C)

Data Evaluation Report

Study type: Dermal Sensitization-guinea pigs (81-6)

Tox. Chem. No.: 271N

MRID Number: 415639-18

Test Material: CGA 163935 technical

Synonyms/CAS No:

Study number: 871408

Sponsor: Agricultural Division
CIBA-GEIGY Corporation
Post Office Box 18300
Greensboro, N.C. 27419

Testing Facility: CIBA-GEIGY Limited
Toxicology GU 2.5
4002 Baste
Switzerland

Title of Report: Dermal Sensitization Study in Guinea Pigs
EPA Guidelines No. 81-6

Author: Dr. Phil Mauer

Report Issued: December 3, 1987

Conclusions: CGA 163935 was devoid of any skin sensitizing (contact allergenic) potency in albino guinea pigs.

Toxicity Category IV

Classification: core-supplementary

This study does not fulfill the guideline (81-6) requirements for a "Dermal Sensitization Study in Guinea Pigs."

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I. MATERIALS

A. Test Material: CGA 163935 technical; Description: liquid
Batch no.: P705002; purity: 96.6%

B. Test Animals: Pirbright guinea pigs, white strain (Tif:DHP); Source: Animal Production, CIBA-GEIGY, 4332 Stein/Switzerland; Weight: 305-456g; Age: approximately 10 weeks.

Negative Control: physiological saline

Positive Control: p-phenylenediamine (not run concurrently; data in first amendment of registrant report).

II. METHODS

A. General: Guinea pigs were quarantined for one week prior to initiation of the study. Food (NAFAG no. 846) and water (source not identified) were available ad libitum throughout the study. Body weights were recorded at the beginning and end of the study, and animals were housed individually during the study. Both intracutaneous and epicutaneous challenge was performed. The optimization test was used as the procedure for intracutaneous sensitization.

B. Induction: Injections (0.1ml) of a freshly prepared 0.1% solution of CGA 163935 technical were administered to 10 male and 10 female guinea pigs into the shaved skin of the right flank and back. These injections were performed once every second day (excluding weekends) for a total of 10 injections. Negative control guinea pigs (10 males and 10 females) received 0.1% physiological saline according to the same schedule. After the first injection, subsequent injections in all groups were given into the skin of the back. During the second and third week of induction, test material was incorporated into a 1:1 mixture of vehicle and complete Bacto adjuvant (NOTE: it is assumed that the concentration of test material was still 0.1% in this mixture). Reactions were recorded 24 hours after each injection during the first week.

C. Challenge:
1)intracutaneous: Fourteen days after the last sensitizing injection, a freshly prepared 0.1% solution of CGA 163935 technical in saline was administered into the skin of the left flank. Reaction was recorded 24 hours following injection. Dermal sensitization was judged to be positive in test article treated guinea pigs based upon comparison of the response after intracutaneous challenge to the skin irritation "threshold" for each animal. "Threshold" is defined as the mean volume ± one standard deviation of the induction reactions observed in each individual animal during the first week of induction.

2)epicutaneous: Ten days after intracutaneous challenge injection, a subirritant dose (3% in vaseline) of the test compound was applied under an occlusive dressing which was left in place for 24 hours. A 3% concentration of test substance was defined in preliminary experiments as a subirritant concentration. Control animals received vaseline alone. Reaction to epicutaneous challenge was evaluated 24 and 48 hours after removing the dressings, and were graded according to the Draize scale.

III. RESULTS

No positive skin reaction was observed in control or test article treated guinea pigs after intracutaneous or epicutaneous challenge with CGA 163935 technical.

IV. CONCLUSIONS

CGA 163935 was devoid of any skin sensitizing (contact allergenic) potency in albino guinea pigs.

Toxicity Category IV

V. CLASSIFICATION

Core-supplementary

This study does not fulfill the guideline (81-6) requirements for a "Dermal Sensitization Study in Guinea Pigs."

Clarification of animals used for challenge reaction after epicutaneous administration of CGA 163935 is needed. It appears that a different set of animals was used to evaluate sensitization at 48 hours compared to those animals used at 24 hours (pages 18 and 19 of registrant report).

00827

Reviewed by: Dan W. Hanke, Ph. D. Dan W. Hanke 14 Dec 90
Section III, Tox. Branch II(H7509C)
Secondary reviewer: James N. Rowe, Ph. D. James N. Rowe 12/14/90
Section III, Tox. Branch II(H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization (§81-6)

TOX. CHEM NO: 271 N

MRID NO.: 415639- 19

TEST MATERIAL: CGA-163935 2E FL 882137

SYNONYMS: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dicxo-cyclohexane carboxylic acid ethylester.

STUDY NUMBER: 6939-90

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: Stillmeadow, Inc. 9525 Town Park Drive Houston, TX 77036

TITLE OF REPORT: Dermal Sensitization Study in Guinea Pigs

AUTHOR(S): Janice O. Kuhn, Ph. D.

REPORT ISSUED: May 22, 1990

CONCLUSION: The proponents of this study claim a sensitizing reaction was produced in the guinea pigs by the positive control 2,4-dinitrochlorobenzene; whereas the test article did not produce a sensitizing reaction. However, this study appears unacceptable for the following reasons. The response in the positive controls is questionable. The response at the virgin site (RR) at day 36 after 24 and 48 hrs for both erythema and edema is minimal in the positive control, even though no response was observed with the test article. The minimal control response does not suggest an adequate response to a positive control particularly after 10 induction applications. Secondly, for the test article treated animals it is unclear why such a severe response is noted at the 2nd induction site, since it is a naive site and the concentration of test article was lowered from 10 % to 1 %. Such a dramatic response at a naive site during induction is suggestive of a sensitization response, however a definitive conclusion is not possible from this study. Furthermore, it is also unclear why the 10 % concentration was not used for the challenge dose at the virgin site, since no

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irritation was noted after a single application; the excessive (severe ?) irritation noted in the report on page 12 line 9 developed apparently on day 15. Finally, eschar formation should be noted underneath erythema in the legend to Table 1 and is not consistently associated with severe erythema (grade 4) during the induction period and not at all at challenge.

Sensitization potential cannot be determined from this study.

Core Classification: Invalid

A signed quality assurance statement was present.

This study does not satisfy the guideline requirements (§81-6) for a Dermal Sensitization study.

MATERIALS.

1. Test compound: CGA-163935 2E. Description: dark-brown liquid. Batch # 318-81-1 FL 882137. Purity: the 2E formulation (prepared from the technical grade test article) used in this study is 23 % test article (whereas the technical grade is 97 % ± 0.8 area per cent, n=12). The purities and chemical structure of the test article formulations are reported in volume 34 of 46 of the submission, which is MRID # 416042-06.

2. Test animals: Species: guinea pig. Strain: Hartley-Albino. Age: young adult. Weight: 305-385 g when tested. Source: Harlan Sprague Dawley, Inc., Houston, Texas.

METHODS.

Two male guinea pigs were used in a range-finding experiment lasting 48 hrs to establish the treatment dose at 0.50 ml of a 10.0 % v/v solution, that was the highest non-irritating level of the test article. Ten males were selected for each of two treatment groups. One treatment group was a positive control, that received 0.50 ml of a 0.06 % w/v solution of 2,4-dinitrochlorobenzene in ethanol. The second group was treated initially with 0.50 ml of a 10 % v/v solution of the test article in ethanol. The animals were prepared on the day before treatment, and thereafter as necessary, by clipping the back of the trunk free of hair to expose a longitudinal area at least 8 x 10 cm. The animals were treated on days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, and 36. Animals were weighed on days 0 and 35. Each treated animal was placed in a restrainer for approximately six hrs, after which time the test materials and bandages were removed and the animals returned to their cages. The same test site was used for each animal for each treatment for the positive control. However, the test article concentration used in group II proved to produce severe irritation and the concentration was lowered to 1 %. Then a fresh dose site was chosen on the left rear for subsequent testing of group II animals. On day 36 all animals were treated in a manner identical to the previous treatments with the addition of a second test site placed laterally on the right rear quadrant of the exposure area. The animals were observed for skin reactions approximately 24 hrs after each treatment for each test site. In addition observations for skin reactions were made approximately 48 hrs after treatment 1, 72 hrs after treatment 10, and 48 hrs after the challenge treatment on day 36. If there is a marked increase in positive skin reactions for the virgin test site after the day 36 treatment (challenge treatment), the material tested is considered a sensitizer.

RESULTS.

The average skin reaction sores for each group for day one and day 36 a
in Table 1. A sensitizing reaction in guinea pigs was produced by 2,4-
dinitrochlorobenzene at a concentration of 0.06 % w/v in ethanol. The
not produce a sensitizing reaction in these guinea pigs at a concentrat
ethanol. The actual skin reactions are detailed in Table 2, and the av
scores are shown in Table 3.

Table 1. Skin Reaction Scores

Treatment	Day of Study	Group	Original Test Site	Virgin
Initial	1	Positive Control	0.0	
Challenge	36	Positive Control	4.0	
Initial	1	Test Article	0.0	
Challenge	36	Test Article	1.4	

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Table 2. Skin reactions; Group I - Positive Control

	Hours after day of treatment															
	LF												LF		RI	
	Day 1		3	6	8	10	13	15	17	20	22		36		3(	
Animal Number	24	48	24	24	24	24	24	24	24	24	24	72	24	48	24	
Erythema																
1-M	0	0	0	0	1	2	2	2	2	3	3	3	2	2	1	
2-M	0	0	0	0	1	2	2	2	2	3	3	3	2	2	2	
3-M	0	0	0	1	2	2	2	2	2	3	3	3	2	1	1	
4-M	0	0	0	0	1	1	2	2	3	2	3	3	2	2	2	
5-M	0	0	0	1	2	2	2	2	2	2	3	3	2	2	1	
6-M	0	0	0	1	2	2	2	2	2	3	3	3	2	2	1	
7-M	0	0	0	0	1	2	2	2	2	2	2	3	2	2	1	
8-M	0	0	0	1	2	2	2	2	2	3	3	3	2	2	1	
9-M	0	0	0	1	2	2	2	3	3	3	3	3	2	2	2	
10-M	0	0	0	0	1	1	2	2	2	3	3	3	2	1	1	
Edema																
1-M	0	0	0	0	1	1	2	2	2	3	3	3	2	2	1	
2-M	0	0	0	0	1	1	1	1	2	3	3	3	3	2	1	
3-M	0	0	0	0	1	2	2	2	2	2	3	3	2	2	1	
4-M	0	0	0	0	1	2	2	2	2	2	2	3	2	2	1	
5-M	0	0	0	0	1	1	1	1	1	2	2	2	2	2	1	
6-M	0	0	0	0	1	1	1	1	2	2	2	2	2	2	1	
7-M	0	0	0	0	1	1	1	1	1	2	2	2	2	2	1	
8-M	0	0	0	1	1	1	2	2	2	3	3	3	2	2	1	
9-M	0	0	0	1	1	2	2	2	2	2	3	3	2	2	1	
10-M	0	0	0	0	1	1	1	2	2	2	3	3	2	2	1	

LF - left front test site
RR - right rear test site
M - male
underlined numbers indicate eschar formation

Table 2 Continued; Group II - Test

	Hours after day of treatment														
						LF			LR				LR		
	Day 1		3	6	8	10	13	15	17	20	22		36		
Animal Number	24	48	24	24	24	24	24	24	24	24	24	72	24	48	24
Erythema															
11-M	0	0	0	1	2	2	3	4	4	3	3	3	2	1	0
12-M	0	0	0	1	2	2	3	3	4	4	3	3	0	0	0
13-M	0	0	0	0	2	2	3	3	3	4	3	3	1	0	0
14-M	0	0	0	2	2	3	3	3	3	4	4	4	1	1	0
15-M	0	0	0	1	2	2	3	3	3	3	3	3	0	0	0
16-M	0	0	0	2	2	2	3	3	4	4	3	3	1	0	0
17-M	0	0	0	0	2	1	2	3	3	3	3	3	0	0	0
18-M	0	0	0	2	2	2	3	3	3	3	3	3	1	0	0
19-M	0	0	0	2	2	2	3	3	3	3	3	3	1	0	0
20-M	0	0	0	1	3	3	3	3	3	3	3	4	1	0	0
Edema															
11-M	0	0	0	1	2	2	2	4	4	3	3	3	3	2	0
12-M	0	0	0	1	2	2	2	3	3	3	3	3	0	0	0
13-M	0	0	0	0	1	1	2	3	3	3	3	3	1	1	0
14-M	0	0	0	1	2	2	3	3	3	4	3	3	1	1	0
15-M	0	0	0	0	1	1	2	3	3	3	3	3	0	0	0
16-M	0	0	0	1	2	2	2	3	3	3	3	3	1	1	0
17-M	0	0	0	0	1	1	2	3	3	2	2	3	0	0	0
18-M	0	0	0	1	2	2	2	3	3	3	3	3	1	1	0
19-M	0	0	0	1	2	2	2	3	3	3	3	3	1	1	0
20-M	0	0	0	1	3	2	2	3	3	3	3	3	1	1	0

LF - left front test site
LR - left rear test site
RR - right rear test site
M - male
underlined numbers indicate eschar formation

Legend to Table 2.

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible	4

Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema(raised more than 1 mm and extending beyond the are of exposure)	4
Maximum possible	4

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Table 3. Average Skin Reaction Scores

	Hours after day of treatment													
	LF												LF	
	Day 1		3	6	8	10	13	15	17	20	22		36	
Group	24	48	24	24	24	24	24	24	24	24	24	72	24	48
I (Positive Control)	0	0 (0)	0	0.7	2.5	3.1	3.5	3.7	4.0	5.0	5.5 (5.6)	5.7	4.1 (4.0)	3.8

	LF							LR				LR	
	Day 1		3	6	8	10	13	15	17	20	22		36	
	24	48	24	24	24	24	24	24	24	24	24	72	24	48
II (Test)	0	0 (0)	0	1.9	3.9	3.8	5.0	6.2	6.4	6.4	6.0 (6.1)	6.2	1.7 (1.4)	1.0

LF - left front test site
LR - left rear
RR - right rear test site

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