00827o
00827o

Reviewed by: Deborah L. McCall, Review Section, Toxicology Branch II. (H7509C) / HED DLMcCall 10/3/90

Secondary reviewer: James Rowe, Ph.D, Review Section, Toxicology Branch II, (H7509C) / HED James Rowe 10/3/90

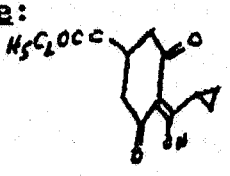
DATA EVALUATION REPORT

STUDY TYPE: Repeat dose dermal toxicity: 21 day study in rabbits (§82-2)

EPA Identification Nos.: EPA MRID (Accession) No. - 415639-22
EPA ID No. -
Caswell No. - 271N
HED Project No. - 0-1874

Test Material: CGA 163935 Technical (FL 872026)
Synonyms: none

Chemical Structure:



MW 252.27

Sponsor: Agricultural Division, CIBA-GEIGY Corporation, P.O. Box 18300, Greensboro, North Carolina

Testing Facility: Division of Toxicology/Pathology CIBA-GEIGY Corporation, Summit, New Jersey

Title of Report: 21-Day Dermal Toxicity Study in Rabbits (MIN 882084)

Author(s): K.R. Huber, G. Batastini and A.T. Arthur

Report Issued: June 26, 1989

Conclusions:

At doses of 100 and 1000 mg/kg, CGA 163935 appeared to have compound-related effects on various biochemistry parameters (decreases in total bilirubin, increase in mean albumin/globin ratios, and increase in mean phosphorus) and in microscopic observations of the skin (hyperkeratosis and subacute lymphocytic infiltrates).

The Dermal and Systemic No Observed Effect Level (NOEL) = 10 mg/kg in both sexes.

Core Classification: Core Minimum

This study satisfies the guideline requirements (§82-2) for a 21 day repeat dose dermal toxicity in rabbits.

1. MATERIALS, METHODS AND RESULTS

00827

A. Test compound: Purity: 96.6%
Description: dark brown liquid
Lot No.: FL 872026
Contaminant: not provided

1. Vehicle(s): Dehydrated alcohol, USP. Supplied by Toxicology/Pathology Administration and Technical Operations.

[Reviewer note: No other information was provided on the vehicle - selection justification, batch/lot number, percent ingredient, or manufacturer.]

B. Test animals: Species: Rabbit
Strain: New Zealand white
Source: H.A.R.E., Inc. Hewitt, New Jersey
Age: 14-17 weeks
Weight: 2.38 - 3.04 kg

1. Animal Assignment: Animals were acclimated to laboratory conditions for 21 days, identified by Monel ear tags, and assigned randomly (Beckman TOXSYS®) to the following test groups:

Test Group	Dose Concentration (mg/ml)	Dose Level (mg/kg)	No. of Animals Males	Females
Control[a]	- -	0	5	5
Control[b]	- -	0	5	5
Low	6.7	10	5	5
Mid	67.0	100	5	5
High	667.0	1000	5	5

[a] = Untreated controls, animals were wrapped in dressing and tape.
[b] = Vehicle controls, animals received dehydrated alcohol, USP under occlusive wrap.

C. General Observations: Cage-side examinations were conducted once daily for abnormal behavior, appearance and excreta. In addition, each rabbit was examined prior to dosing for erythema, edema, and other dermal changes, and approximately 30 minutes after each dosing period (including washing).

1. Preparation of Animal Skin: The fur on the dorsal area of the trunk was clipped prior to dosing and as needed thereafter. Twenty-two or 23 doses of test material or vehicle were administered to each animal over a 22-24 day period. The vehicle (dehydrated alcohol) and the test solution was applied at a dose volume of 1.5 mL/kg; to approximately 10 percent (240 sq cm) of the total body surface. A gauze dressing was applied over the test site and secured with adhesive wrapping (Vetrap, 3M or Zonas Porus, Johnson and Johnson). Following dosing the animals were fitted with an Elizabethan collar. Approximately 6 hours after the application, the dressings were removed and the application site was gently washed with tap water and/or alcohol and then

patted dry with a clean paper towel. Once a week the doses were adjusted to the most recent body weight.

Clinical signs were "not remarkable" for the duration of the study. Dermal macroscopic observations included flaking, fissuring, atonia and thickened skin.

2. Body Weights: Animals were weighed prior to dosing, and weekly thereafter.

A statistically significant decrease in the mean body weight was seen on test days 7 and 14 for the low dose (10 mg/kg) females. This effect appears to be of little toxicological significance, considering no dose-related effects were seen in mean body weights of the males or any of the higher doses (see table 2).

3. Food and Water Consumption: Food consumption was measured at predose and weekly thereafter. Animals received food (Purina Certified Rabbit Chow® # 5325) and tap water ad libitum via an automatic delivery system.

The low dose females (10 mg/kg) had a statistical significant decrease in mean food consumption during the second and third weeks of the study (study days 7 & 14). This decrease corresponds with the loss seen in the body weights of the same group. However, a dose response relationship was not observed in all groups. Therefore, this reduction in food consumption appears to be of little toxicological significance. (See table 3.)

4. Ophthalmoscopic examination: Examinations were performed on all animals on pretest day -7 and test day 20 by Donald M. Schiavo, Ph.D., staff ophthalmologist. Dr. Schiavo's report was included in the study report.

5. Statistics: A copy of the statistical methods used for the data analysis is attached (Appendix A).

6. Quality Assurance: A quality assurance statement and a statement of compliance with FIFRA Good Laboratory Practice Standards were signed and dated June 26, 1989.

D. Hematology and Clinical Chemistry: Blood was collected from the auricular artery at predose, test days 13-14, and at termination (days 22-23) from all (fasted) animals for hematology and clinical chemistry analyses. The CHECKED (X) parameters were examined:

Table 1

INCIDENCE OF DERMAL OBSERVATIONS

	MALES					FEMALES		
Dose Level (mg/kg)	0[a]	0[b]	10	100	1000	0[a]	0[b]	10
OBSERVATIONS:								
Macroscopic								
Erythema								
Grade 1	0/5	1/5	3/5	2/5	1/5	0/5	0/5	0/5
Grade 2	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5
Fissuring	0/5	1/5	3/5	2/5	3/5	0/5	2/5	1/5
Flaking	0/5	1/5	2/5	3/5	4/5	0/5	3/5	1/5
Atonia	0/5	0/5	2/5	3/5	0/5	0/5	0/5	1/5
Thickened Skin	0/5	0/5	2/5	2/5	1/5	0/5	0/5	0/5
Microscopic								
Acanthosis	0/5	5/5	5/5	5/5	5/5	0/5	5/5	5/5
Hyperkeratosis	0/5	1/5	0/5	2/5	4/5	0/5	0/5	0/5
Scab	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
Subacute Lymphocytic Infiltration	0/5	1/5	0/5	2/5	1/5	0/5	0/5	0/5

[a] : untreated controls;
[b] : alcohol controls; treated as controls for the statistical analysis
** : P <= .01, Two-tailed Dunnett test.

(Dermal observations were scored according to the Draize method. Data
7.3 and Appendices 8.1.3, 8.3)

Table 2

GROUP MEAN BODY WEIGHT (kg)

	MALES				FEMALES		
Days on Study	0	7	14	21	0	7	
Dose level (mg/kg)							
0[a]	2.63±.11	2.65±.09	2.75±.11	2.80±.09	2.75±.07	2.76±.07	
0[b]	2.53±.06	2.57±.07	2.62±.09	2.69±.08	2.81±.06	2.90±.05	
10	2.82±.05	2.77±.03	2.84±.04	2.87±.04	2.6[illegible]±.07	2.63±.09*	
100	2.56±.05	2.53±.07	2.62±.07	2.67±.09	2.87±.08	2.92±.07	
1000	2.64±.08	2.67±.08	2.75±.08	2.79±.09	2.68±.06	2.81±.06	

(Based on 5/sex/group)

[a] : untreated controls;
[b] : alcohol controls; treated as controls for the statistical analysis
* : .01 < P <= .05, Two-tailed Dunnett test;
** : P <= .01, Two-tailed Dunnett test;
± : Standard error.
(Data was extreacted from Table 7.4 and Appendix 8.1.4)

5

Table 3

GROUP MEAN FOOD CONSUMPTION (G/WEEK)

	MALES				FEMALE	
Days on Study	0	7	14	21	0	7
Dose level (mg/kg)						
0[a]	1228.6 ±76.1	1259.6 ±84.4	1265.4 ±76.4	1238.2 ±92.6	1352.6 ±77.7	1336.0 ±83.3
0[b]	1131.2 ±91.4	1213.0 ±114.3	1233.0 ±97.8	1150.8 ±76.6	1475.0 ±82.7	1560.6 ±43.0
10	1359.4 ±40.3	1182.2 ±54.5	1229.8 ±90.3	1187.6 ±59.6	1292.8 ±48.2	1252.6 ±55.2*1
100	1137.4 ±111.0	1058.8 ±104.6	1124.4 ±95.0	1115.8 ±108.7	1371.4 ±55.8	1391.8 ±61.8
1000	1262.4 ±60.4	1266.0 ±79.9	1453.2 ±47.7	1265.4 ±101.9	1313.4 ±77.3	1412.2 ±52.5

(Based on 5/sex/group)

[a] : untreated controls;
[b] : alcohol controls; treated as controls for the statistical analysis
* : .01 < P <= .05, Two-tailed Dunnett test;
** : P <= .01, Two-tailed Dunnett test;
± : Standard error.
(Data was extracted from table 7.4 and appendix 8.1.4.)

1→
0

6

1. Hematology:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Erythrocyte count*
X Leukocyte count*
X Platelet*
X Leukocyte differential count*
X Coagulation: thromboplastin time (PT)*
X Reticulocyte count* (RETIC)
X Heinz body determinations*
X Red cell morphology
Mean corpuscular HGB concentration (MCHC)
Mean corpuscular volume (MCV)

* = performed only for animals in group 1, 2, 5.

There were no dose-related effects in any of the hematology parameters.

2. Clinical Chemistry:

Electrolytes

X Calcium*
X Phosphorus*
X Chloride*
X Sodium*
X Potassium*

Enzymes

X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Alkaline phosphatase (ALP)
X Gamma glutamyltransferase (GGT)

Other

X Albumin*
X Blood creatinine*
X Blood urea nitrogen*
X Glucose*
X Total bilirubin*
X Total protein*
X Albumin/globulin ratio

* Recommended by Subdivision F (October 1982) Guidelines

No adverse dose-related effects were noted in the clinical chemistry parameters. Several statistically significant trends were noted in the 100 and 1000 mg/kg groups (see Table 4). A decrease in mean total bilirubin was observed in the both sexes at the 100 and 1000 mg/kg groups. An increase in the mean albumin/globin ratio was observed in

Table 4 - GROUP MEAN BIOCHEMISTRY

MALES

Parameter	Total Bilirubin (mg/dl)		Albumin/Globulin ratio[c] (mg/dl)	
	Day		Day	
Dose Level (mg/kg)	13	21	13	21
0[a]	0.122 ±0.021	0.154 ±0.010	4.2	4.7
0[b]	0.148 ±0.027	0.194 ±0.011	4.8	4.4
10	0.132 ±0.028	0.116 ±0.014	4.4	4.8
100	0.128 ±0.026	0.138 ±0.011*	5.0	7.0*
1000	0.162 ±0.038	0.118±0.015**	4.8	6.4
			FEMALES	
0[a]	0.170 ±0.021	0.162 ±0.007	5.0	5.5
0[b]	0.166 ±0.023	0.156 ±0.019	4.3	5.1
10	0.154 ±0.021	0.152 ±0.016	5.1	5.4
100	0.204 ±0.016	0.114 ±0.008	5.1	6.7
1000	0.168 ±0.020	0.124 ±0.022	4.8	7.0

[a] : untreated controls;
[b] : alcohol controls; treated as controls for the statistical analysis
[c]: standard errors were not presented;
* : .01 < P <= .05, Two-tailed Dunnett test on ranked data;
** : P <= .01, Two-tailed Dunnett test on ranked data;

± : Standard error.

(Data was extracted from table 7.6 and appendix 8.1.6.)

the 100 and 1000 mg/kg groups. These changes appear to be dose-related when compared with the vehicle controls. An increase in mean phosphorus was noted in the high dose females (1000 mg/kg). The reviewer agrees with the study authors that these changes are of minimal toxicological significance, due to the lack of a relationship with total protein or albumin.

E. Urinalysis: Urinalysis was not performed.

F. Sacrifice and Pathology: All animals were subjected to gross pathological examination at sacrifice and the CHECKED (X) tissues were collected and preserved in 10% neutral buffered formalin. The brain, adrenals, heart, kidneys, liver (including the empty gallbladder), ovaries or testes, and pituitary were weighed.

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta	X Brain
X Salivary glands	X Heart	X Peripheral nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary
X Rectum	X Spleen	X Eyes (optic nerve)
X Colon	X Thymus	
X Cecum		
X Duodenum		
X Jejunum	Urogenital	Glandular
X Ileum	X Kidney*	X Adrenals
X Liver*	X Urinary bladder	X Lacrimal gland
X Gallbladder	X Testes	X Mammary gland
X Pancreas	X Epididymis	X Thyroids
	X Prostate	X Parathyroids
Respiratory	Seminal vesicle	X Harderian glands
X Trachea	X Ovaries	
X Lung	X Uterus	
	X Vagina	Other
		X Bone (sternum & femur)
		X Skeletal muscle
		X Skin (treated & untreated)*
		X All gross lesions & masses*

*Recommended by Subdivision F (October 1982) Guidelines

G. Organ Weights: Terminal body weights and selected absolute and relative organ weights are presented in Table 5. In the 10 mg/kg females, significant decreases in absolute heart weight ($P < .001$), and significant increases of the 100 mg/kg dose in the absolute pituitary ($P < .05$) and in the pituitary-to-brain weight ratio ($P < .001$). Additionally, a trend was noted in the absolute kidney weights in the females in that all doses were lower than controls. The reviewer agrees with the study authors conclusion that these changes should be considered incidental, due to a lack of a dose response relationship.

Table 5

Terminal Body Weights and Selected Absolute & Relative

Parameter	MALES							
	0[a]	0[b]	10	100	1000	0[a]	0[b]	1
Body Wgt (g)	2818.80	2671.20	2915.80	2779.40	2791.20	2977.60	3127.60	28
Heart[c]	6.626 ±0.217	6.118 ±0.217	6.740 ±0.263	5.998 ±0.056	6.578 ±0.216	6.478* ±0.109	7.418 ±0.209	6. ±0
Kidney[c]	17.598 ±0.675	17.170 ±1.445	17.842 ±0.932	16.816 ±1.331	17.232 ±0.788	17.158 ±0.472	18.156 ±0.875	16 ±0
Liver[c]	82.678 ±3.23	77.670 ±6.64	76.014 ±3.71	74.572 ±7.37	77.596 ±2.79	77.450 ±7.65	87.962 ±5.92	77 ±4
Pituitary[c]	0.036 ±0.007	0.034 ±0.009	0.030 ±0.004	0.030 ±0.003	0.022 ±0.002	0.028 ±0.002	0.032 ±0.004	0. ±0
Pituitary[d]	0.382 ±0.091	0.360 ±0.108	0.305 ±0.049	0.312 ±0.030	0.237 ±0.027	0.296 ±0.023	0.331 ±0.039	0. ±0

(Based on 5/sex/group)

[a] : untreated controls;
[b] : alcohol controls; treated as controls for the statistical analysis;
[c] : absolute values;
[d] : relative-to-brain weight ratio;
* : .01 < P <= .05, Two-tailed Dunnett test;
** : P <= .01, Two-tailed Dunnett test;
± : Standard error.

(Data was extracted from table 7.7 and appendix 8.1.7.)

H. Gross Pathology: Lesions (dark, red or tan) of the treated skin were observed in all dose groups including the vehicle group (1/10 in the vehicle control, 2/10 in 10 mg/kg, 3/10 in 100 mg/kg, and 3/10 in the 1000 mg/kg group). These findings should be considered dose-related, e.g. control verses high dose incidence (males or females). Additionally, several gross nonneoplastic lesions were noted in the liver, kidneys and lungs. However, when compared to the vehicle controls these findings appear to be of little toxicological significance.

I. Microscopic Pathology: Microscopic observations included subacute lymphocytic infiltration in all high dose females, acanthosis in all treated animals (including alcohol treated controls) and hyperkeratosis in 100 and 1000 mg/kg dose animals (Table 1). Hyperkeratosis (both sexes) and lymphocytic infiltrates (females) appear to be "compound-related" at the 100 and 1000 mg/kg dose. Although, a synergistic effect can not be ruled out due to the dermal effects of the alcohol. Microscopic evaluations of the kidney, liver, and lungs did not indicate any direct relationship with CGA 163935. Scabs were noted in 1 male and 1 female of the 100 mg/kg dose and 2 females in the 1000 mg/kg dose group. The skin lesions of the vehicle control group and the 10 mg/kg were similar. These skin lesions do not appear to be systemic effects, but rather local effects due to the treatment with dehydrated alcohol.

2. DISCUSSION:

In all test groups (including the vehicle control) there appeared to be a dose-related increase in microscopic observations of the treated skin including hyperkeratosis and subacute lymphocytic inflammation. A statistically significant decrease was noted in the mean body weights and the mean food consumption of the 10 mg/kg females. Several statistically significant trends were observed in the 100 and 1000 mg/kg groups in the biochemistry parameters (increase in albumin/globin ratio, decrease in bilirubin in 100 and 1000 mg/kg and an increase in phosphorus in 1000 mg/kg). The skin lesions of the vehicle control group were similar to the 10 mg/kg dose group, therefore this dose represents the NOEL.

3. CONCLUSIONS:

At doses of 100 and 1000 mg/kg, CGA 163935 appeared to have compound-related effects on various biochemistry parameters (decreases in total bilirubin, increase in mean albumin/globin ratios, and increase in mean phosphorus) and in microscopic observations of the skin (hyperkeratosis and subacute lymphocytic infiltrates).

The Dermal and Systemic No Observed Effect Level (NOEL) = 10 mg/kg in both sexes.

STUDY CLARIFICATION: The registrant should provide justification for the selection of dehydrated alcohol as the vehicle. The registrant needs to respond to this data gap.

//