7-23-92

DOC930130

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DATA EVALUATION REPORT

CGA-163935

Study Type: A Dermal Radiotracer Absorption Study in Rats

Prepared for:

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Contract Number 68D10075
Work Assignment Number 1-126
Clement Number 93-116
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DATA EVALUATION REPORT

STUDY TYPE: Dermal absorption study

TEST MATERIAL: CGA-163935

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

MRID Number: 422381-05

STUDY NUMBER: WIL-82041

SPONSOR: Agricultural Division, CIBA-GEIGY Corporation, Post Office Box 18300, Greensboro, NC

TESTING FACILITY: WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH

TITLE OF REPORT: A Dermal Radiotracer Absorption Study in Rats with ^{14}C-CGA-163935

AUTHOR: Charles L. Hauswald, M.S.

STUDY INITIATION: December 5, 1989

EXPERIMENTAL TERMINATION DATE: April 3, 1990

STUDY COMPLETION: March 3, 1992

CONCLUSIONS: A single dermal dose of 0.01, 0.1, and 1.0 mg/cm^2 ^{14}C-CGA-163935 administered to male rats was rapidly absorbed, distributed and eliminated. The amount absorbed increased with duration of exposure. Using the direct procedure to calculate skin absorption, the average ^{14}C-CGA-163935 absorbed within 24 hours was 64.9, 63.86, and 30.85% of the applied dose for the low-, mid-, and high-dose groups, respectively. Using the indirect procedure to calculate skin absorption, the average ^{14}C-CGA-163935 absorbed within 24 hours was 56.83, 66.74, and 33.84% of the applied dose for the low-, mid-, and high-dose groups, respectively. The direct procedure is the more realistic procedure to use.

CLASSIFICATION: Acceptable. Although this study does not fulfill all the requirements suggested in Dr. R. Zendzian's draft "Procedure for Studying Dermal Absorption," the deficiencies are not major and do not affect the interpretation of the data (see Reviewers' Comments, Section C).

A. MATERIALS and METHODS

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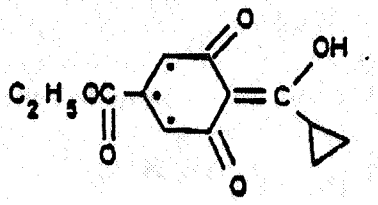
Test Material

Test material: CGA-163935
Synonyms: Cimectarb
Chemical name and structure: 4-(cycloproplyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane-carboxylic acid ethylester



*Denotes position of radiolabel

Test Animals

Species: Rat
Strain: Charles River CD®
Source: Charles River Breeding Laboratories, Inc., Portage, MI
Sex: Male
Source: Charles River Breeding Laboratories, Portage, MI
Receipt dates: 12/14/89, 12/28/89, and 01/04/90
Numbers: 16 Rats/dose (4 rats/subgroup)
Housing: Individual wire mesh cages (before treatment); individual metabolism cages (after treatment)
Identification: Ear tag
Acclimation: Five days
Age: 5.5-7 weeks
Weight: 202-235 g (at randomization)
Feeding: Feed (Purina® Certified Rodent Chow® #5002) and water provided ad libirum.
Selection: Computer generated randomization procedure by weight

Test Article Analyses for Purity and Stability

Three suspensions of the test material, ^{14}C-CGA-163935, were prepared by the Chemical Synthesis Group, Metabolism Department, CIBA-GEIGY Corporation. The first suspension (Sponsor reference GAN-XVII-74) had a specific activity of 20.7 µCi/mg. A radiopurity of 96.9% and a chemical purity of >96.6% were determined by the Sponsor. The first suspension was used to dose the low-dose (Group I). The second suspension (Sponsor reference of GAN-XVII-74) had a specific activity 20.7 µCi/mg. A radiopurity of 97.4% and a chemical purity of >96.6% were determined by the Sponsor. The second suspension was used to dose the mid-dose (Group II). The third suspension (Sponsor reference GAN-XIX-13) had a specific activity of 2.5 µCi/mg. A radiopurity of 97.3% and a chemical purity of 97.1% were determined by the Sponsor. The third suspension was used to dose the high-dose (Group III).

Preparation of Dosing Suspensions/Analysis of Dosing Suspensions

The test material was administered as a suspension in blank 2E formulant (Sponsor lot number FL-892627) and deionized water. The suspensions were prepared by the Sponsor and were shipped to WIL Research where they were stored at refrigerated temperatures. For the low-, mid-, and high-dose formulations, the concentrations of ^{14}C-CGA-16395 were 2.0, 17, and 67.5 mg/ml, respectively.

Prior to dosing, triplicate aliquots of the suspension for the low- and mid-dose groups and duplicate aliquots of the suspension for the high-dose group were diluted with methanol. Duplicate subsamples of each dilution were analyzed for ^{14}C. After dosing was completed, additional aliquots were diluted and analyzed. The values obtained before and after dosing were averaged for calculation of the amounts of ^{14}C-CGA-163935 applied to the animals.

Dosage Groups/Application of Test Material/Treatment Regimen

Twenty-four hours prior to treatment, the anterior dorsal hair of each rat was shaved with an electric shaver to avoid damaging the skin. The shaved area of skin was washed with acetone to remove oily secretions. Three groups (16/group) of rats were treated with single dermal doses of ^{14}C-CGA-163935 in a formulation of blank 2E formulant and deionized water. The low-, mid-, and high-dose groups were treated with an average of 0.1, 1, and 10 mg/rat, respectively. The targeted doses were 0.01, 0.1, and 1.0 mg/cm^2 for the low-, mid-, and high-dose groups, respectively. The dosing area was enclosed with a nonocclusive covering or "protective appliance," which consisted of a piece of Stomahesive, filter paper, and an aluminum bridge. The Stomahesive adhered to the skin with Skin-bond® cement forming a "well" surrounding the area of skin to be treated. The treated area was then covered with the filter paper elevated by the foil bridge to prevent contact with the applied test material. The application site within the "well" was 10 cm^2. The test material was spread evenly over the test site. The amount applied was quantitated by washing the pipet with methanol and determining the amount of ^{14}C in the pipet wash.

The treatment regimen is indicated below:

Group	Dosage Level (mg/rat)	Subgroup (4 animals/ subgroup)	Time of Exposure (hours)
I	0.1	1	2.0
		2	4.0
		3	10.0
		4	24.0
II	1.0	1	2.0
		2	4.0
		3	10.0
		4	24.0

Group	Dosage Level (mg/rat)	Subgroup (4 animals/ subgroup)	Time of Exposure (hours)
III	10.0	1	2.0
		2	4.0
		3	10.0
		4	24.0

The treated animals were placed individually in metabolism cages. At 2, 4, 10, or 24 hours postdosing, four animals per dose were euthanized. A single urine and feces collection was made from the time of dosing to the time of euthanization. Cage washings were also collected after sacrifice. The exposed skin and residual compound were collected separately. The bridge was washed with ethanol and the paper cover was extracted with methanol. A blood sample was taken. The application site was washed vigorously three times to remove recoverable ^{14}C with sterile gauze squares. The first two washes were with Dove® liquid detergent in deionized water and the third wash was with deionized water. The skin of the application site and the skin underneath the Stomahesive were dissected from the carcass separately. Urine in the bladder was removed and added to the urine collection.

Liquids (extracts and digests) were analyzed for ^{14}C by direct count in a liquid scintillation system. Solid materials were oxidized in a Harvey BMO (Biological Materials Oxidizer). Feces were freeze dried and homogenized. Skin samples were digested at room temperature in Soluene 350. Gauzes from skin washings were extracted with methanol. All analyses of ^{14}C involved measurement by liquid scintillation techniques.

Calculation of Quantity Absorbed

The amount of ^{14}C-CGA-163935 absorbed was determined using two procedures, direct and indirect. The direct procedure calculated the amount of ^{14}C-CGA-163935 absorbed and involved summing the amounts of ^{14}C in urine, feces, cage wash, carcass, and blood. The indirect procedure involved calculation of unabsorbed ^{14}C-CGA-163935, which was the sum of ^{14}C washed from the protective appliance (bridge wash, Stomahesive wash, paper extract, and paper digest) and the amounts washed from the skin, and the amount associated with the skin. The amount absorbed was equal to the applied dose (100%) minus the measured unabsorbed ^{14}C-CGA-163935.

B. RESULTS/CONCLUSIONS

The disposition of ^{14}C-CGA-163935 and the calculated absorbed doses are presented in Table 1.

TABLE 1: The Average Disposition of Doses of ^{14}C-CGA-163935 Following a Single Dermal Exposure (Expressed as

Duration of Exposure (hours)	Time Euthanized (hours)	Protective Appliance Washes (%)	Skin Washes (%)	Associated With Skin at Site (%)	Blood (%)	Urine (%)	Feces (%)	Cage Wash (%)	Carcass (%)	Average Recovery of Applied ^{14}C-CGA-163935 (%)	Average ^{14}C-CGA-Absorbed (Direct
						0.01 mg/cm^2					
2	2	0.6	41.71	22.11	0.54	25.61	0.14	8.79	3.87	103.37	38
4	4	1.23	40.92	22.64	0.31	35.37	0.00	5.06	2.33	107.86	43
10	10	1.65	21.85	21.03	0.85	48.52	0.04	6.92	0.97	101.03	56
24	24	2.01	15.43	25.73	0.03	61.66	0.26	2.24	0.71	108.07	64
						0.10 mg/cm^2					
2	2	0.13	89.34	11.07	0.39	10.91	<0.00	2.80	1.92	116.56	16
4	4	0.91	82.56	8.40	0.27	18.51	0.00	1.52	1.57	113.74	21
10	10	1.18	50.59	10.43	0.24	35.21	0.12	3.49	1.60	102.86	40
24	24	2.00	21.12	30.14	0.07	57.97	0.65	4.02	1.15	97.12	63
						1.0 mg/cm^2					
2	2	9.92	49.02	39.53	0.17	4.00	0.06	0.90	1.33	104.93	6
4	4	6.82	68.25	31.93	0.11	6.28	0.01	0.66	0.65	106.71	7
10	10	9.70	41.64	31.68	0.11	13.95	0.03	1.39	0.94	99.44	16
24	24	14.36	31.11	20.69	0.07	27.58	0.32	2.43	0.45	97.01	30

Source: CBI Tables 2, 3, and 4, pp. 36-38

Direct Procedure

A single dermal dose of 0.01, 0.1, and 1.0 mg/cm^2 ^{14}C-CGA-163935 administered to rats was rapidly absorbed, distributed and eliminated. The amount absorbed increased with duration of exposure. Using the direct procedure to calculate skin absorption, the average ^{14}C-CGA-163935 absorbed within 2 hours was 38.95, 16.02, and 6.46% of the applied dose for the low-, mid-, and high-dose groups, respectively. The average amount absorbed increased with time, such that by 24 hours, the amount absorbed was 64.9, 63.86, and 30.85% of the applied dose for the low-, mid-, and high-dose groups, respectively.

The absorption data indicated that ^{14}C-CGA-163935 penetrated the skin within the first 2 hours of exposure as indicated by the presence of 0.54, 0.39, and 0.17% of the applied dose in the blood (plasma and cellular fractions) for the low-, mid-, and high-dose groups, respectively. At all dose levels, the maximum concentrations, although quite low, were present after 2 hours of exposure, with levels gradually decreasing over the next 24 hours for the low- and mid-dose groups. At the high-dose level, the concentrations remained constant through the 10-hour exposure period and then decreased over the next 14 hours. In general, the concentrations in plasma ranged from 4- to 13-fold higher than concentrations in the cellular fractions. For example, in the low-dose group, the average amount in the cellular fractions was 0.07% of the dose while the average amount in plasma was 0.47% of the dose.

The ^{14}C was rapidly excreted in the urine within 2 hours of exposure as indicated by the recovery of 25.61, 10.91, and 4% of the applied dose in the urine for the low-, mid-, and high-dose groups, respectively. Recovery of the ^{14}C in the urine increased significantly over the 24 hour period. After 24 hours, 61.66, 57.97, and 27.58% of the applied dose was recovered in the urine for the low-, mid-, and high-dose groups, respectively. Very little ^{14}C (<0.7% of the applied dose) was excreted in the feces for all dose groups for all durations of exposure. ^{14}C was rapidly distributed to the carcass and was detected in the carcass within the first 2 hours at 3.87, 1.92, and 1.33% of the applied dose for the low-, mid- and high-dose groups, respectively. By 24 hours, however, the percentage recovered in the carcass decreased to 0.71, 1.15, and 0.45% of the applied dose for the low-, mid-, and high-dose groups, respectively.

Indirect Procedure

Using the indirect procedure to calculate skin absorption, the average ^{14}C-CGA-163935 absorbed within 2 hours was 35.58, -0.54, and 1.53% of the applied dose for the low-, mid-, and high-dose groups, respectively. (The study author indicated that the direct procedure for calculation of absorption was more realistic, as the indirect calculations produced some negative numbers. Therefore, emphasis should be placed on the direct absorption values.) Skin absorption increased with exposure time. By 24 hours of exposure, the absorption values were comparable to those calculated by the direct procedure. For the low-, mid-, and high-dose groups, the average ^{14}C-CGA-163935 absorbed was 56.83, 66.74, and 33.84% of the applied dose, respectively.

A majority of the unabsorbed ^{14}C-CGA-163935 was associated with the skin washes. For the low-, mid-, and high-dose groups, 41.71, 89.34, and 49.02%, respectively, of the applied dose was absorbed within 2 hours of exposure. The amount of ^{14}C associated with skin washes decreased after 24 hours of exposure to 15.43, 21.12, and 31.11%, for the low-, mid-, and high-dose groups, respectively. The amount of ^{14}C associated with the skin site at 2 hours of exposure was 22.11 and 11.07% of the applied dose for the low- and mid-dose groups, respectively. These levels stayed somewhat level or increased slightly over 24 hours of exposure to 25.73 and 10.14% for the low- and mid-dose groups, respectively. The amount associated with the skin site in the high-dose group decreased significantly over 24 hours, with 39.52% of the applied dose recovered after 2 hours of exposure, decreasing to 20.69% of the applied dose recovered after 24 hours. The amount of ^{14}C associated with the protective appliance washes was generally low but increased with time. In the low-dose and mid-dose groups, the amount recovered was <1% of the applied dose at 2 hours and increased to 2% of the applied dose by 24 hours of exposure. In the high-dose group, the amount recovered from the protective appliance washes was higher than that recovered from the other dose groups. After 2 hours of exposure, 9.92% of the applied dose was recovered and 14.36% of the applied dose was recovered at 24 hours of exposure.

Total average recoveries ranged from 101 to 108% in the low-dose group, 97-117% in the mid-dose group, and 97-107% in the high-dose group. The study author indicated that, for the high-dose group, a significant amount of ^{14}C was associated with the protective appliance (average of all rats in the high-dose group, 10.2%). This was attributed to the larger dose volume (150 µL) applied to these animals (110 µL was applied to the low- and mid-dose groups). However, since total recoveries for the high-dose group were excellent, the study author felt that this did not influence the integrity or validity of the study.

A Good Laboratory Practice Compliance Statement and Quality Assurance Statement were included.

C. REVIEWERS' COMMENTS

The reviewers note that the study would have been more complete if an additional group of four rats were included which had the skin washed at the end of the 24-hour exposure and then absorption of that remaining on the skin followed for an additional 72 hours. However, this is not required since 30-60% was absorbed.

This study was classified as Acceptable. Although this study does not fulfill all the requirements suggested in Dr. R. Zendzian's draft "Procedure for Studying Dermal Absorption," the deficiencies are not major and do not affect interpretation of the data. The following deficiencies were noted: (1) the skin wash was taken after the animals were killed. The general procedures state that the skin wash should be collected before the animals are killed, as up to three fold differences have been observed in the ability of skin on the live animal and skin on the dead animal to bind test compounds; (2) only 16 animals were used per dose group. The paper discussing the general procedure for dermal absorption studies recommends using 24 animals per dose group;

(3) although only three dose groups were used for this study, the lower dose range was not used as recommended; (4) The general draft procedures recommend anesthetizing animals at 0.5-, 1-, 2-, 4-, 10-, and 24-hour intervals; however, in this study the animals were not anesthetized at the 0.5- and 1-hour intervals.