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8EHQ-0478-0134

(A)

Declassified

VELSICOL CHEMICAL CORPORATION

341 EAST OHIO STREET • CHICAGO, ILLINOIS 60611 • 312-670-4500

April 17, 1978

RECEIVED 20 APR 1978 JHW

Director of the Office of
Toxic Substances (WH-557)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Re: Chlorendic anhydride

Dear Sir:

The attached document is submitted for your files and in compliance with TSCA section 8(e) if the latter is necessary.

In summary, the document reports the results of laboratory toxicology studies.

For the record, the document is identified as follows:

"Acute Toxicity Studies in Rabbits and Rats", IRDC #163-512, March 21, 1978.

We make no judgment that this document contains information concerning a substantial risk. We reserve our right to contest the propriety of TSCA section 8(e).

Sincerely,

VELSICOL CHEMICAL CORPORATION

Thomas R. Loy

Thomas R. Loy
Manager,
Regulatory Activities
Environmental Sciences and
Regulatory Activities

TRL:eh
Enclosure

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88-7800134 see page 33 for alopecia 0134

8EHQ-0478-0134

International Research and Development Corporation

SPONSOR: Velsicol Chemical Corporation

TEST MATERIAL: Tech Ref. Std. Chlorendic Anhydride 98.81% (E1). Lot No. 3-12-206.

SUBJECT: Acute Toxicity Studies in Rabbits and Rats.

Wilson P. Dean, B.A.
Director of Acute Toxicology
and Special Studies
(Study Director)

Approved by: D. Clifford Jessup, Ph.D.
Associate Director of Research

Date: March 21, 1978

This document was declassified 5-22-78

[redacted]

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I. SYNOPSIS

Primary Eye Irritation Study in the Albino Rabbit

Based upon the data obtained and the regulations of the Federal Hazardous Substances Act, the test material would be considered a primary eye irritant.

Primary Skin Irritation Study in the Albino Rabbit

Primary Irritation Score = 0.8.
Not a primary skin irritant or a corrosive material.

Acute Dermal Toxicity Study in the Albino Rabbit

The minimum lethal dose by the dermal route of administration was found to be less than 20000 mg/kg but greater than 10000 mg/kg.

Acute Oral Toxicity (LD_{50}) Study in Rats

Male Rats: 2562 (2218-2961) mg/kg.
Female Rats: 2130 (1698-2671) mg/kg.
Combined Male and Female Rats: 2336 (2065-2643) mg/kg.

[redacted]

II. TEST MATERIAL

The test material was received from the Velsicol Chemical Corporation, Chicago, Illinois, on September 2 and December 5, 1977. The test material was received as a fine white powder containing some chunks and was identified as follows:

Tech Ref. Std.
Chlorendic Anhydride 6.19% unaccounted for!
93.81% (E1)
Lot No. 3-12-206

[redacted]

III. PRIMARY EYE IRRITATION STUDY IN THE ALBINO RABBIT

A. METHOD:

Three male and 3 female New Zealand White rabbits (obtained from Sweetwater Farms, Hillsboro, Ohio) were used for this study. The rabbits weighed from 2049 to 2523 grams at the beginning of the study. The rabbits were individually housed in hanging wire-mesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Rabbit Chow were available *ad libitum*.

Prior to test material administration, the eyes of each rabbit were examined with ultraviolet light following instillation of one drop of a 2.0 percent sodium fluorescein solution. Rabbits exhibiting corneal lesions were discarded.

The rabbits received 100 milligrams of the test material. The test material was placed into the cupped conjunctival sac of the right eye of each rabbit following which the eyelids were gently held together for one second. The left eye served as the untreated control for each rabbit. None of the rabbits received a washout.

[redacted]

The treated eyes were examined in accordance with the following schedule:

24 hours following instillation
48 hours
72 hours*
7 days*
14 days*

*Sodium fluorescein examinations were conducted at these intervals. After grading the eye reaction, one drop of sodium fluorescein was instilled directly onto the corneal surface of each rabbit. After a few seconds, excess stain was flushed from the corneal surface with distilled water and the eyes examined with ultraviolet light.

The grading system employed in this test procedure is presented on page 5.

[redacted]

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Scale for Scoring Ocular Lesions*

(1) Cornea

(A) Opacity-degree of density (area most dense taken for reading)

No opacity	0
Dulling of corneal surface	0.5
Scattered or diffuse area, details of iris clearly visible	1
Slightly translucent areas, not well defined	1.5
Easily discernible translucent areas, details of iris slightly obscurred	2
Well defined translucent areas, iris moderately obscured	2.5
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opalescent areas, pupil/iris appear as a shadow, details not discernible	3.5
Opaque, iris invisible	4

(B) Area of cornea involved

Very small area, less than 5%	0.5
One quarter (or less) but greater than 5%	1
25 - 35%	1.5
Greater than one quarter, but less than half (35 - 50%)	2
50 - 60%	2.5
Greater than half, but less than three quarters (60 - 75%)	3
75 - 90%	3.5
Greater than three quarters, up to whole area (90 - 100%)	4

Score equals A x B x 5 [redacted] Total maximum = 80

(2) Iris

(A) Values

Normal	0
Doubtful or barely perceptible (same conditions as 1)	0.5
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

Score equals A x 5 Total maximum = 10

(3) Conjunctivae

(A) Redness (refers to palepebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Doubtful or barely perceptible injection	0.5
Vessels definitely injected above normal	1
Severe injection or slight diffuse redness, well defined redness	1.5
More diffuse, deeper crimson red, individual vessles not easily discernible	2
Same as 2, but areas of diffuse beefy red	2.5
Diffuse beefy red	3

*Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Assoc. Food and Drug Officials of the U.S., Austin, Texas, 1959, page 51, Modified according to revision of 1964. Edited by A. J. Lehman.
Further modified (inclusion of 0.5 scores) at International Research and Development Corporation.

(B) Chemosis

No swelling	0
Doubtful or barely perceptible	0.5
Any swelling above normal (includes nictitating membrane)	1
Definite swelling, very slight eversion of lids	1.5
Obvious swelling with partial eversion of lids	2
Definite eversion of lids, one quarter to one half closed	2.5
Swelling with lids about half closed	3
More than half, but less than three quarters	3.5
Swelling with lids three quarters to completed closed	4

(C) Discharge

No discharge	0
Very slight, barely perceptible	0.5
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Slight moistening of lids and adjacent area	1.5
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Moistening of moderate area around eye	2.5
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score equals (A + B + C) x 2 Total maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctivae. Total maximum score possible = 110

[redacted]

B. RESULTS:

Table 1 presents the grade of effect with time. Table 2 shows the group average scores. Table 3 presents the individual rabbit scores.

Based upon the data, the test material would be considered a primary eye irritant.

The test material would be further classified, based upon the scores obtained, as being severely irritating to the eye when evaluated using the method described on page 7.

[redacted]

Classification of Test Materials
Based on Eye Irritation Properties

Rating	Range	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	Greater than 0.5 - 2.5	To maintain this rating, all scores at the 24-hour reading must be zero; otherwise, increase rating one level.
Minimally Irritating	Greater than 2.5 - 15.0	To maintain this rating, all scores at the 72-hour reading must be zero; otherwise, increase rating one level.
Mildly Irritating	Greater than 15.0 - 25.0	To maintain this rating, all scores at the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	Greater than 25.0 - 50.0	To maintain this rating, scores at 7 days must be less than or equal to 10 for 60% or more of the animals. Also, mean 7-day score must be less than or equal to 20. If 7-day mean score is less than or equal to 20 but less than 60% of animals show scores less than 10, then no animal among those showing scores greater than 10 can exceed a score of 30 if rating is to be maintained; otherwise, increase rating one level.
Severely Irritating	Greater than 50.0 - 80.0	To maintain this rating, scores at 7 days must be less than or equal to 30 for 60% or more of the animals. Also, mean 7-day score must be less than or equal to 40. If 7-day mean score is less than or equal to 40 but less than 60% of the animals show scores less than or equal to 30, then no animal among those showing scores greater than 30 can exceed a score of 60 if rating is to be maintained, otherwise, increase rating one level.
Extremely Irritating	Greater than 80.0 - 110.0	[redacted]

Chlorendic Anhydride

Primary Eye Irritation Study in the Albino Rabbit.

TABLE 1. Group Observations

	Observations		Examination Interval (No. Positive/No. Dosed) Hours 24	Hours 48	Hours 72	Days 7	Days 14
I. DRAIZE SCORING VALUES							
Cornea:	Cornea Normal		4/6	4/6	1/6	1/6	1/6
	Dulling Normal Corneal Luster		1/6		2/6	3/6	
	Corneal Opacity:	very slight	1/6	2/6	3/6	2/6	5/6
		slight					
		moderate					
		marked					
Iris:	Iris Normal		1/6	1/6	1/6	2/6	2/6
	Iridal Irritation		5/6	5/6	5/6	4/6	4/6
Conjunctivae:	Redness:	normal				1/6	1/6
		very slight			1/6	1/6	
		slight		2/6	3/6	4/6	5/6
		moderate	6/6	4/6	2/6		
		marked					
	Chemosis:	normal					1/6
		very slight		4/6	4/6	6/6	5/6
		slight	6/6	2/6	2/6		
		moderate					
		marked					
	Discharge:	normal			1/6	2/6	1/6
		very slight				1/6	1/6
		slight		3/6	2/6	3/6	3/6
		moderate	6/6	3/6	3/6		1/6
		marked					
II. SODIUM FLUORESCEIN/ULTRA-VIOLET LIGHT EXAMINATION							
	Negative (normal):		---	---	1/6	1/6	1/6
	Positive (corneal injury):		---	---	5/6	5/6	5/6
III. OTHER FINDINGS							
	Clear Discharge		[redacted] 6/6	2/6	0/6	0/6	0/6
	Purulent Discharge			4/6	5/6	3/6	4/6
	Dulling of the Corneal Surface		1/6		2/6	3/6	0/6
	Corneal Epithelial Damage, Peeling				5/6	5/6	5/6
	Vascularization of the Corneal Surface						2/6
	Pannus						2/6

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 2. Group Average Scores.

Area		Average Scores (Range) Period: Period 24	Period 48	Period 72	Days 7	Days 14
Cornea:	A	0.2 (0-1.0)	0.3 (0-1.0)	0.5 (0-1.0)	0.3 (0-1.0)	1.0 (0-1.5)
	B	0.3 (0-1.5)	1.3 (0-4.0	1.5 (0-4.0)	1.0 (0-4.0)	1.5 (0-3.0)
	Score	0.3	2.0	3.8	1.5	7.5
Iris:	A	0.8 (0-1.0)	0.8 (0-1.0)	0.8 (0-1.0)	0.7 (0-1.0)	0.7 (0-1.0)
	Score	4.0	4.0	4.0	3.5	3.5
Conjunctivae:	A	2.1 (2.0-2.5)	1.8 (1.5-2.0)	1.3 (0.5-2.0)	0.9 (0-1.5)	0.8 (0-1.0)
	B	2.3 (2.0-2.5)	1.5 (1.0-2.0)	1.4 (0.5-2.0)	0.9 (0.5-1.5)	0.9 (0-1.5)
	C	2.1 (2.0-2.5)	1.7 (1.0-2.5)	1.3 (0-2.0)	0.7 (0-1.5)	1.0 (0-2.0)
	Score	13.0	10.0	8.0	5.0	5.4
Total Scores		17.3	16.0	15.8	10.0	16.4

[redacted]

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. Individual Rabbit Scores.

Area			Rabbit Number: 30402, Female — Observations — Hours 24	Hours 48	Hours 72	Days 7	Days 14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	0	0	0	0	
	Area	(B)	0	0	0	0	
	Subtotal	(A x B x 5)	0	0	0	0	0
Iris:	Irritation	(A)	1.0	1.0	1.0	0	0
	Subtotal	(A x 5)	5.0	5.0	5.0	0	0
Conjunctivae:	Redness	(A)	2.0	2.0	1.5	0	0
	Chemosis	(B)	2.0	2.0	1.5		0
	Discharge	(C)	2.5	2.5	2.0	0	0
	Subtotal	(A + B + C) x 2	13.0	13.0	10.0	1.0	0
Total Scores:			18.0	18.0	15.0	1.0	0
II. SODIUM FLUORESCEIN EXAM			---	---	Negative	Negative	Negative
III. OTHER FINDINGS							
Purulent Discharge				X	X		
Clear Discharge			X				

[redacted]

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. (Cont.) Individual Rabbit Scores.

Area			Rabbit Number: 30405, Male — Observations: Hours 24	Hours 48	Hours 72	Days 7	Days 14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	0	0	0	0	1.0
	Area	(B)	0	0	0	0	2.0
	Subtotal	(A x B x 5)	0	0	0	0	10.0
Iris:	Irritation	(A)	1.0	1.0	1.0	1.0	1.0
	Subtotal	(A x 5)	5.0	5.0	5.0	5.0	5.0
Conjunctivae:	Redness	(A)	2.5	2.0	1.0	1.0	1.0
	Chemosis	(B)	2.5	1.0	1.0	1.0	1.0
	Discharge	(C)	2.0	1.0	1.0	1.0	1.0
	Subtotal	(A + B + C) x 2	14.0	8.0	6.0	6.0	6.0
Total Scores:			19.0	13.0	11.0	11.0	21.0
II. SODIUM FLUORESCEIN EXAM			---	---	Positive	Positive	Positive
(% of Corneal Surface Positive)					20	20	15
III. OTHER FINDINGS							
Clear Discharge				X			
Purulent Discharge					X	X	X
Dulling of the Corneal Surface					X	X	
Vascularization of the Corneal Surface							X
Corneal Epithelial Damage, Peeling					X	X	X

[redacted]

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. (Cont.) Individual Rabbit Scores.

Rabbit Number: 30406, Female

			Observations				
			Hours			Days	
Area			24	48	72	7	14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	1.0	1.0	1.0	1.0	1.5
	Area	(B)	1.5	4.0	4.0	4.0	3.0
	Subtotal	(A x B x 5)	7.5	20.0	20.0	20.0	22.5
Iris:	Irritation	(A)	1.0	1.0	1.0	1.0	1.0
	Subtotal	(A x 5)	5.0	5.0	5.0	5.0	5.0
Conjunctivae:	Redness	(A)	2.0	2.0	2.0	1.0	1.0
	Chemosis	(B)	2.0	1.5	2.0	1.0	1.0
	Discharge	(C)	2.0	2.0	2.0	0.5	1.5
	Subtotal	(A + B + C) x 2	12.0	11.0	12.0	5.0	7.0
Total Scores:			24.5	36.0	37.0	30.0	34.5
II. SODIUM FLUORESCEIN EXAM			---	---	Positive	Positive	Positive
(% of Corneal Surface Positive):					50	40	30
III. OTHER FINDINGS							
Purulent Discharge				X	X	X	X
Clear Discharge			X				X
Vascularization of the Corneal Surface							X
Corneal Epithelial Damage, Peeling					X	X	X

[redacted]

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. (Cont.) Individual Rabbit Scores.

Rabbit Number: 30407, Male

Area			Observations 24	48	72	Days 7	14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	0	0	0	0	1.0
	Area	(B)	0	0	0	0	1.0
	Subtotal	(A x B x 5)	0	0	0	0	5.0
Iris:	Irritation	(A)	0	0	0	0	0
	Subtotal	(A x 5)	0	0	0	0	0
Conjunctivae:	Redness	(A)	2.0	1.5	0.5	1.5	1.0
	Chemosis	(B)	2.0	1.0	0.5	1.0	1.0
	Discharge	(C)	2.0	1.0	0	1.0	0.5
	Subtotal	(A + B + C) x 2	12.0	7.0	2.0	7.0	5.0
Total Scores:			12.0	7.0	2.0	7.0	10.0
II. SODIUM FLUORESCEIN EXAM			---	---	Positive	Positive	Positive
(% of Corneal Surface Positive):					30	30	20
III. OTHER FINDINGS							
Dulling of the Corneal Surface					X	X	
Pannus							X
Corneal Epithelial Damage, Peeling					X	X	X
Clear Discharge			X	X		X	X
Vocalization Following Dosing			X				

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. (Cont.) Individual Rabbit Scores.

Area			Rabbit Number: 30408, Female — Observations: Hours 24	Hours 48	Hours 72	Days 7	Days 14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	0	0	1.0	0	1.0
	Area	(B)	0	0	1.0	0	1.0
	Subtotal	(A x B x 5)	0	0	5.0	0	5.0
Iris:	Irritation	(A)	1.0	1.0	1.0	1.0	1.0
	Subtotal	(A x 5)	5.0	5.0	5.0	5.0	5.0
Conjunctivae:	Redness	(A)	2.0	1.5	1.0	0.5	1.0
	Chemosis	(B)	2.5	1.5	1.5	0.5	1.0
	Discharge	(C)	2.0	1.0	1.0	0	1.0
	Subtotal	(A + B + C) x 2	13.0	8.0	7.0	2.0	6.0
Total Scores:			18.0	13.0	17.0	7.0	16.0
II. SODIUM FLUORESCEIN EXAM			---	---	Positive	Positive	Positive
(% of Corneal Surface Positive):					25	20	10
III. OTHER FINDINGS							
Purulent Discharge				X	X		X
Clear Discharge			[redacted]				
Pannus							X
Corneal Epithelial Damage, Peeling					X	X	X
Dulling of the Corneal Surface						X	

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Chlorendic Anhydride: Primary Eye Irritation Study in the Albino Rabbit.

TABLE 3. (Cont.) Individual Rabbit Scores.

Rabbit Number: 30409, Male

Area			Observations: Hours 24	Hours 48	Hours 72	Days 7	Days 14
I. DRAIZE SCORING VALUES							
Cornea:	Opacity	(A)	0	1.0	1.0	1.0	1.5
0	Area	(B)	0	4.0	4.0	2.0	2.0
	Subtotal	(A x B x 5)	0	20.0	20.0	10.0	15.0
Iris:	Irritation	(A)	1.0	1.0	1.0	1.0	1.0
	Subtotal	(A x 5)	5.0	5.0	5.0	5.0	5.0
Conjunctivae:	Redness	(A)	2.0	2.0	2.0	1.5	1.0
	Chemosis	(B)	2.5	2.0	2.0	1.5	1.5
	Discharge	(C)	2.0	2.5	2.0	1.5	2.0
	Subtotal	(A + B + C) x 2	13.0	13.0	12.0	9.0	9.0
Total Scores:			18.0	38.0	37.0	24.0	29.0
II. SODIUM FLUORESCEIN EXAM			---	---	Positive	Positive	Positive
(% of Corneal Surface Positive):					40	40	30
III. OTHER FINDINGS							
Purulent Discharge				X	X	X	X
Clear Discharge			X				
Vascularization of the Corneal Surface							X
Corneal Epithelial Damage, Peeling					X	X	X
Dulling of the Corneal Surface			X				

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IV. PRIMARY SKIN IRRITATION STUDY IN THE ALBINO RABBIT

A. METHOD:

Three male and 3 female New Zealand White rabbits (obtained from Sweetwater Farms, Hillsboro, Ohio) were used for this study. The rabbits weighed from 2174 to 2861 grams at the beginning of the study. They were individually housed in hanging wire-mesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Rabbit Chow were available ad libitum.

The hair was removed from the back of each rabbit (20-30% of the body surface) with an electric clipper. The skin of 3 of the rabbits was abraded with a scalpel blade. The abrasions penetrated the stratum corneum, but were not deep enough to cause bleeding.

Five hundred milligrams of the test material was applied to the back of each rabbit. The application sites were then covered with gauze bandaging and occluded with Saran Wrap. Four hours later the bandages were removed and the sites were washed with tepid tap water and examined for skin irritation (in accordance with the scale on page 18) and for corrosion. These examinations were repeated at 24, 48 and 72 hours.

B. RESULTS:

The table on the following page presents the grade of effect with time. Table 4 presents the individual rabbit scores and the computed primary irritation score of 0.8.

Based upon the results obtained, the test material would not be considered a primary skin irritant or a corrosive material.

The test material would be further classified, based upon the scores obtained, as being slightly irritating to the skin when evaluated using the method described on page 19.

[redacted]

Number of Animals Exhibiting Signs with Scoring "Value"

Observations	Value	Intact Skin Hours 4	24	48	72	Abraded Skin Hours 4	24	48	72
Erythema,	0								
Eschar	1	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Formation	2								
	3								
	4								
Edema	0	2/3	2/3	2/3	3/3	3/3	2/3	2/3	2/3
Formation	1	1/3	1/3	1/3			1/3	1/3	1/3
	2								
	3								
	4								

[redacted]

Primary Skin Irritation Scoring Scale

	Value
Erythema and Eschar Formation:	
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
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 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

Mean Primary Irritation Score Evaluation

(Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

[redacted]

Primary Skin Irritation

Individual Rabbit Scores and Score Calculation

Chlorendic Anhydride:

TABLE 4. Intact Skin

	Erythema						Edema					
	Observation Period (Hours)						Observation Period (Hours)					
Animal No./Sex	4	24	48	72			4	24	48	72		
30367, Male	1.0	0.5	0.5	0.5			1.0	0.5	0.5	0		
30395, Male	0.5	0.5	0.5	0.5			0	0	0	0		
30398, Female	0.5	0.5	0.5	0.5			0	0	0	0		
Total	2.0	1.5	1.5	1.5			1.0	0.5	0.5	0		

Abraded Skin

	Erythema						Edema					
	Observation Period (Hours)						Observation Period (Hours)					
Animal No./Sex	4	24	48	72			4	24	48	72		
30382, Female	0.5	0.5	0.5	0.5			0	0	0	0		
30401, Male	0.5	1.5	1.0	1.0			0	1.0	1.0	0.5		
30414, Female	0.5	0.5	0.5	0.5			0	0	0	0		
Total	1.5	2.5	2.0	2.0			0	1.0	1.0	0.5		

Calculation for Primary Skin Irritation Score (Score = the average of all the erythema scores plus the average of all the edema scores): (14.5 ÷ 24) + (4.5 ÷ 24) = 0.8.

V. ACUTE DERMAL TOXICITY STUDY IN THE ALBINO RABBIT

A. METHOD:

Four male and 4 female New Zealand White rabbits were used for this study. The rabbits weighed from 2302 to 2557 grams at the beginning of this study. They were individually housed in hanging wire-mesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". Water and Purina Rabbit Chow were available ad libitum.

The rabbits were divided into 2 groups containing 2 male and 2 female rabbits each.

The hair was removed from the back of each rabbit (20-30% of the body surface) with an electric clipper. The skin of one male and one female in each group was abraded with a scalpel blade. The abrasions penetrated the stratum corneum, but were not sufficiently deep to cause bleeding.

The test material was applied once only to the backs of the rabbits at the following dosage levels: 10000 and 20000 mg/kg.

The area of application was wrapped with gauze bandaging and occluded with Saran Wrap. Twenty-four hours later the bandages were removed and the backs were washed with tepid tap water. They were observed at 24 hours and daily thereafter for a total of 14 days for mortality, pharmacotoxic signs and dermal irritation (in accordance with the scale on page 19).

Body weights were measured immediately preceding dosing (control weight) and at 7 and 14 days. All rabbits which died on study were subjected to gross necropsy examination.

[redacted]

B. RESULTS:

1. Mortality:

Both male rabbits at the 20000 mg/kg dosage level died during the 14-day observation period. None of the other rabbits died.

The minimum lethal dose by the dermal route of administration was found to be less than 20000 mg/kg but greater than 10000 mg/kg.

2. Pharmacotoxic Signs:

The following pharmacotoxic signs were observed during the 14-day observation period: (see the following pages).

[redacted]

Chlorendic Anhydride:

10000 mg/kg

Males

	Normal
Days 1-14	2/2

Females

	Normal	Diarrhea
Day 1	1/2	1/2
Days 2-14	2/2	

[redacted]

Chlorendic Anhydride:

20000 mg/kg

Males

	Lacrimation	Bradypnea	Hyp.activity	Decreased Limb Tone	Ataxia	Phonation	Death
Day 1	2/2					2/2	
Days 2-3	2/2						
Days 4	1/2						1/2
Day 5	1/1						
Day 6		1/1	1/1	1/1	1/1		
Day 7							1/1

Females	Normal	Lacrimation	Phonation	Purulent Nasal Discharge
Days 1-2		2/2		1/2
Days 3-5			2/2	
Days 6-14	2/2			

[redacted]

3. Dermal Irritation Signs:

The following signs of dermal irritation were observed during the 14-day observation period: (see the following pages).

[redacted]

Chlorendic Anhydride:

Dermal Irritation

10000 mg/kg

	Erythema					Edema			Atonia			Desquamation				Coriaceousness				
	None	Very Slight	Slight	Moderate	Marked	None	Slight	Moderate	None	Very Slight	Slight	None	Very Slight	Slight	Moderate	None	Very Slight	Slight	Blanching	Subcutaneous Hemorrhaging
Day 1			2/4		2/4	1/4		3/4	2/4		2/4	4/4				2/4	2/4		0/4	2/4
Day 2			2/4	1/4	1/4	2/4	1/4	1/4	2/4		2/4	4/4				2/4	2/4		1/4	2/4
Day 3		2/4		1/4	1/4	2/4	2/4		2/4		2/4	4/4				2/4	2/4		1/4	0/4
Day 4	1/4	1/4		2/4		2/4	2/4		2/4		2/4	2/4	1/4	1/4		2/4		2/4	1/4	0/4
Days 5-6	1/4	1/4		2/4		2/4	2/4		2/4		2/4	1/4		3/4		2/4		2/4	1/4	0/4
Days 7-10	2/4		1/4	1/4		3/4	1/4		3/4		1/4			2/4	2/4	2/4		2/4	0/4	0/4
Day 11	2/4	1/4	1/4			4/4			3/4		1/4			2/4	2/4	2/4		2/4	0/4	0/4
Day 12-13	3/4		1/4			4/4			3/4		1/4	1/4		3/4		4/4			0/4	0/4
Day 14	3/4		1/4			4/4			3/4	1/4		1/4		3/4		4/4			0/4	0/4

[redacted]

80-1000134

Chlorendic Anhydride:

Dermal Irritation

20000 mg/kg

	Erythema				Edema				Atonia				Desquamation				Coriaceousness			Fissuring		
	None	Very Slight	Slight	Moderate	None	Very Slight	Slight	Moderate	None	Very Slight	Slight	Moderate	None	Very Slight	Slight	Moderate	None	Very Slight	Slight	None	Very Slight	Slight
Day 1				4/4			2/4	2/4			3/4	1/4	4/4				4/4			4/4		
Day 2				4/4			3/4	1/4			4/4		4/4				4/4			4/4		
Day 3			4/4			3/4	1/4				3/4	1/4		3/4	1/4			3/4	1/4	4/4		
Day 4*			1/3	2/3		1/3	2/3				3/3			1/3	2/3			1/3	2/3		2/3	1/3
Day 5			1/3	2/3		1/3	2/3				3/3			1/3	2/3			1/3	2/3		2/3	1/3
Day 6			2/3	1/3			3/3				3/3			2/3	1/3			1/3	2/3			3/3
Day 7**			1/2	1/2			2/2				2/2			1/2	1/2				2/2			2/2
Days 8-9			2/2				2/2			2/2					2/2				2/2	1/2		1/2
Day 10			2/2			1/2	1/2			2/2					1/2	1/2		1/2	1/2	1/2		1/2
Day 11		1/2	1/2			1/2	1/2			1/2	1/2				1/2	1/2		1/2	1/2	1/2		1/2
Day 12		1/2	1/2		1/2		1/2		1/2		1/2				1/2	1/2	1/2		1/2	1/2		1/2
Day 13		2/2			1/2		1/2		1/2	1/2					1/2	1/2		1/2	1/2	1/2		1/2
Day 14	1/2	1/2			2/2				1/2	1/2					2/2		2/2			2/2		

* one of four rabbits dead

** two of four rabbits dead

[redacted]

163-512

33

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4. Body Weights:

The following body weights were obtained during the 14-day observation period:

Dosage Level (mg/kg)	Individual Rabbit No.	Sex	Control Weight (grams)	7-Day Weight (grams)	14-Day Weight (grams)
10000	32505	Male	2473	2795	2954
	32507	Male	2479	2700	2899
	32506	Female	2315	2032	2333
	32514	Female	2302	2198	2521
20000	30305	Male	2490	Died	Died
	30319	Male	2557	Died	Died
	30308	Female	2333	2330	2447
	30320	Female	2430	2417	2499

5. Necropsy Findings:

Gross necropsy findings in the rabbits which died on study were as follows:

20000 mg/kg

Lungs, congestion	1/2 males
Lungs, consolidation	1/2 males
Stomach, diffuse erythema and dark red foci	1/2 males
Cecum mucosa, congestion	1/2 males
Kidneys, pale, mottled coloration	1/2 males

[redacted]