5-7-97

DATA EVALUATION RECORD

SODIUM SALT OF SAN 835H

Study Type: 82-2; 21-Day Repeated Dose Dermal Toxicity Study in the Rabbit

Work Assignment Study No. 2-65N (MRID 44170114)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Kathleen Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 5/7/97

Secondary Reviewer
Joan Harlin, M.S.

Signature: Joan L Harlin
Date: 5/2/97

Project Manager
Mary Menetrez, Ph.D.

Signature: Mary Menetrez
Date:

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date:

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

231

(1)

EPA Reviewer: William Greear, M.P.H., D.A.B.T. William Greear 5/28/97
Review Section IV, Toxicology Branch I (7509C)

EPA Secondary Reviewer: Marion Copley, D.V.M., D.A.B.T. Marion Copley 7/2/97
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Repeated dose dermal toxicity - 21-day rabbit
OPPTS Number: 870.3200 OPP Guideline Number: §82-2

DP BARCODE: D232811 SUBMISSION CODE: S516012
P.C. CODE: 005107 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): SAN 1269 H 70 WG 403 DP Formulation (20% sodium salt of SAN 835H and 51.0% dicamba by weight)

SYNONYMS: None

CITATION: Allan, S.A., P.L. Connolly, and C. Gopinath. (1996) SAN 1269 H 70 WG 403 DP Formulation. Twenty-one day dermal toxicity study in the rabbit. Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Project Number SNC 203/960162. May 21, 1996. MRID 44170114. Unpublished.

SPONSOR: Sandoz Agro Inc., Des Plaines, Illinois.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 44170114), SAN 1269 H 70 WG 403 DP Formulation (20% sodium salt of SAN 835H and 51.0% dicamba by weight; Lot# 6500-08) was applied to the shaved skin of five juvenile New Zealand White rabbits/sex/dose at dose levels of 0, 10, 30, or 100 mg/kg/day for 6 hours/day, 7 days each week, for 3 weeks.

Dermal irritation was observed in rabbits in all treatment groups. One male and four females in the 10 mg/kg/day treatment groups exhibited slight erythema without edema during the 21-day study. All rabbits in the 30 mg/kg/day treatment groups exhibited slight to well-defined erythema; 1/5 males and 4/5 females had accompanying slight to well-defined edema; and 4/5 males and 5/5 females exhibited trace to minimal diffuse epidermal acanthosis. All rabbits in the 100 mg/kg/day treatment groups exhibited moderate erythema and well-defined to moderate edema. In addition, rabbits in the 100 mg/kg/day treatment groups exhibited cracking (5/5 males, 5/5 females) and sloughing (3/5 males, 4/5 females) of the treated skin, minimal to moderate diffuse epidermal acanthosis (5/5 males, 5/5 females), diffuse

hyperkeratosis (1/5 males, 4/5 females), and diffuse inflammation of the superficial dermis (2/5 males, 4/5 females). In general, dermal responses were first noticed during the second week of treatment. For all treatment groups, there were no biologically significant, treatment-related differences in food consumption, hematological or clinical blood chemistry parameters, organ weights, or macroscopic or microscopic organ (other than treated skin) morphology between rabbits in the treated and the control groups. No significant systemic toxicity was observed as a result of treatment at any dose level. **The LOEL for dermal toxicity was 10 mg/kg/day, based on the occurrence of erythema in the treated skin of several rabbits in this treatment group. A NOEL was not determined. The NOEL for systemic toxicity is 100 mg/kg/day. A LOEL was not determined.**

This repeated dose dermal toxicity study is classified **acceptable (§82-2)** and satisfies the guideline requirements for a repeated dose dermal toxicity study in rabbits. Although a NOEL was not determined, the study is scientifically valid and effects observed at the lowest dose rate, 10 mg/kg/day, are indicative of mild dermal irritation, not toxicity.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

~~257~~
3

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SAN 1269 H 70 WG 403 DP Formulation
   Description: Beige powder
   Lot/Batch #: 6500-08
   Purity: 20% sodium salt of SAN 835H and 51.0% dicamba by weight
   Stability of compound: Expiration data reported to be March 31, 1997
   CAS #: Not provided
   Structure: Not provided

2. Vehicle and/or positive control: None

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and weight at receipt: Approximately 10-12 weeks of age; body weight, 2.1-2.6 kg
   Source: Harlan UK Ltd., Bicester, Oxon, England
   Housing: Individually housed in metal cages with perforated floors
   Diet: SQC Rabbit diet, ad libitum
   Water: Municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 18-21 C
   Humidity: 44-72%
   Air Changes: 19/hour
   Photoperiod: 12-Hour light/dark cycle
   Acclimation period: 13 Days

B. STUDY DESIGN

1. In life dates - Start: 9/27/95 End: 10/20/95

2. Animal assignment

Rabbits (20/sex) were selected for use on the basis of their pretest bodyweight and observations during acclimatization. The selected rabbits were assigned to the test groups in Table 1 using a computerized random sort program to insure that body weight means for each group were comparable.

Table 1: Study design.[a]

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1 Control	0	5	5
2 Low	10	5	5
3 Mid	30	5	5
4 High	100	5	5

[a] Dose levels were selected based on the results of a preliminary dermal toxicity study in which rabbits (1-2/sex/dose) were treated with the test substance at 100, 300, or 1000 mg/kg/day for 8 days (SNC/202). Rabbits in the 300 and 1000 mg/kg/day treatment groups exhibited well-defined to moderate dermal irritation, accompanied by cracking and in some cases sloughing and/or brown staining. Rabbits treated at 100 mg/kg/day exhibited well-defined erythema, slight edema, brown staining, and in the female sloughing and cracking.

3. Preparation and treatment of animal skin

Approximately 24 hours before the initial exposure and as necessary during the experimental period, the fur on each rabbit was clipped from a 12-cm x 8-cm section of the dorsal surface, so that approximately 10% of the body surface was exposed. "The skin sites were not abraded."[page 13] The appropriate weight of the powdered test substance (based on the most recent body weight of the treated animal) was spread over the clipped skin and moistened with distilled water (2 mL/kg). The treated area was covered with an elastic adhesive dressing (Elastoplast) and backed with impervious plaster (Sleek). The rabbits were exposed to the test compound for approximately 6 hours/day, 7 days each week, for 3 weeks. After each exposure, the dressings were removed and the treated skin was washed with warm water, then blotted dry.

Rabbits in the control group were exposed to distilled water (2 mL/kg body weight) only, but otherwise handled as described for the treated animals.

4. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. If significant heterogeneity was found, the data were transformed logarithmically in an attempt to achieve less variance. If the variances of the original or transformed data were found to be equal, the data were analyzed using standard one-way ANOVA followed by

Williams' test for a dose related response. If variances proved to be unequal, the data were analyzed using the Kruskal-Wallis analysis of ranks followed by Shirley's test. Analysis of variance was followed by Student's "t" test and Williams's test or by their nonparametric equivalents. An analysis of covariance was conducted on organ weights and final body weights. Tests were conducted at the 5 and 1% levels.

C. METHODS

1. Observations

Animals were observed twice daily for mortality and moribundity, and three times daily for ill health, toxicosis, and changes in behavior. Dermal irritation was assessed prior to the first daily application of the test substance and daily thereafter using the Draize scoring system.

2. Body weight

Animals were weighed prior to dosing on day 1 and weekly thereafter.

3. Food consumption

Food consumption for each animal was determined weekly.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

5. Blood

Blood was collected prior to sacrifice from the median artery of the ear of all animals following overnight fasting. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Cell morphology
X	Blood clotting measurements* (Thrombotest)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
		X	A/G ratio
X	Alkaline phosphatase (AP)		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
	Gamma glutamyl transferase (GGT)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was not collected during the study.

7. Sacrifice and Pathology

All animals were sacrificed at the termination of the study and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. All tissues from the control and 100 mg/kg/day groups were examined; the treated and untreated skin of rabbits in the 10 and 30 mg/kg/day groups was also examined. The (XX) organs, in addition, were weighed.

~~254~~ 7

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow		Spinal cord
X	Stomach		(sternum	X	Pituitary
X	Duodenum	X	Lymph nodes	X	Eyes
X	Jejunum	XX	Spleen		
X	Ileum	X	Thymus		
X	Cecum				GLANDULAR
X	Colon				
	Rectum		UROGENITAL	XX	Adrenal gland
XX	Liver*+				Lacrimal gland
X	Gall bladder	XX	Kidneys*+	X	Mammary gland
X	Pancreas	X	Urinary bladder	X	Thyroids with
		XX	Testes*+ with		parathyroids
			epididymides		
	RESPIRATORY	X	Prostate		
			Seminal vesicle		OTHER
X	Trachea	XX	Ovaries		
X	Lungs*	X	Uterus	X	Bone* (sternum)
	Nose	X	Vagina	X	Skeletal muscle*
X	Pharynx			X	Skin* (treated
X	Larynx				and untreated)
				X	All gross lesions
					and masses*

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.
+ Organ weight required in repeated dose dermal toxicity studies.

II. RESULTS

A. Observations

1. Mortality - No rabbits died during the study.

2. Clinical Signs - No obvious treatment-related abnormalities other than the dermal reactions noted below were observed in any treatment group during the study.

Rabbits in the control group exhibited no erythema, edema, or other treatment-related dermal reactions during the study (Table 2). One male and four females in the 10 mg/kg/day treatment groups exhibited slight erythema (score of 1) without edema during the 21-day study. All animals in the 30 and 100 mg/kg/day treatment groups exhibited slight to moderate erythema (scores of 1-3) during the 21-day study; one male and four females in the 30 mg/kg/day treatment group and all animals in the 100 mg/kg/day groups had accompanying edema. The incidence and severity of the response increased with increasing dose rate. In addition, animals in the 100 mg/kg/day treatment groups exhibited cracking (5/5 males, 5/5 females) and sloughing (2/5 males, 4/5 females) of the treated skin. All rabbits treated with the test material exhibited a brown staining in the area

of treatment. In general, dermal responses were first noticed during the second week of treatment.

Table 2. Dermal reactions in the treated skin of rabbits (total 5 rabbits/sex/dose).[a]

Observation/severity	Dose (mg/kg body weight/day)			
	0	10	30	100
Males				
Erythema, slight	0/5	1/5	5/5	0/5
well-defined	0/5	0/5	0/5	0/5
moderate	0/5	0/5	0/5	5/5
Edema, slight	0/5	0/5	1/5	0/5
well-defined	0/5	0/5	0/5	1/5
moderate	0/5	0/5	0/5	4/5
Brown staining	0/5	5/5	5/5	5/5
Cracking	0/5	0/5	0/5	5/5
Sloughing	0/5	0/5	0/5	3/5
Female				
Erythema, slight	0/5	4/5	2/5	0/5
well-defined	0/5	0/5	3/5	0/5
moderate	0/5	0/5	0/5	5/5
Edema, slight	0/5	0/5	3/5	0/5
well-defined	0/5	0/5	1/5	2/5
moderate	0/5	0/5	0/5	3/5
Brown staining	0/5	5/5	5/5	5/5
Cracking	0/5	0/5	0/5	5/5
Sloughing	0/5	0/5	0/5	4/5

[a] Data obtained from Appendix, pages 45-52, in the study report. Scoring classifications in this table are based on the most severe response seen in individual animals.

B. Body weight and weight gain

There were no significant differences between the body weights and body weight gains of rabbits in the control group and treatment groups during the study. Mean terminal body weights of male rabbits were 2.70-2.85 kg, and of females were 2.88-3.00 kg. Body weight gains during the 3 weeks of treatment ranged from 186-233 g for males and from 148 to 250 g for females.

C. Food consumption

No biologically significant treatment-related differences were observed in food consumption by the treated and control groups.

D. Ophthalmoscopic examination

Ophthalmoscopic examinations were not performed.

E. Blood work

1. Hematology - No significant treatment-related differences were observed between hematology parameters of rabbits in the treated and control groups.

2. Clinical Chemistry - Males in the 30 and 100 mg/kg/day treatment group had glucose levels 13 and 16% higher, respectively, than the controls; the difference was significant ($p<0.05$) for the high dose group. Glucose levels of females at these dose levels were nearly identical to the controls. No other differences were observed between the clinical blood chemistry of rabbits in the treated and control groups.

F. Urinalysis

Urine was not collected during the study.

G. Sacrifice and Pathology

1. Organ weight - No treatment-related differences in the absolute or relative organ weights were observed between rabbits in the treated and the control groups.

2. Gross pathology - No macroscopic treatment-related gross postmortem differences were observed between rabbits in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Males and females in the 30 and 100 mg/kg/day treatment groups had a higher incidence of trace to moderate diffuse epidermal acanthosis than rabbits in the 10 mg/kg/day treatment groups or the controls (Table 3). Diffuse hyperkeratosis was observed only in the 100 mg/kg/day treatment groups. Diffuse inflammation of the superficial dermis was slightly increased in the 100 mg/kg/day treatment groups; focal inflammation was most pronounced at the 30 mg/kg/day treatment level.

No other microscopic treatment-related gross postmortem differences were observed between rabbits in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

Table 3. Microscopic observations of the treated skin of rabbits (total 5 rabbits/sex/dose).[a]

Observation/severity	Dose (mg/kg body weight/day)			
	0	10	30	100
Males				
Diffuse epidermal acanthosis:				
Trace	0/5	0/5	2/5	0/5
Minimal	1/5	0/5	2/5	5/5
Moderate	0/5	0/5	0/5	0/5
Diffuse hyperkeratosis				
Minimal	0/5	0/5	0/5	1/5
Diffuse inflammation, superficial dermis				
Trace	0/5	0/5	0/5	1/5
Minimal	1/5	0/5	0/5	1/5
Focal inflammation, superficial dermis				
Trace	0/5	0/5	2/5	0/5
Minimal	0/5	0/5	0/5	1/5
Female				
Diffuse epidermal acanthosis:				
Trace	0/5	2/5	3/5	0/5
Minimal	1/5	0/5	2/5	4/5
Moderate	0/5	0/5	0/5	1/5
Diffuse hyperkeratosis				
Minimal	0/5	0/5	0/5	4/5
Diffuse inflammation, superficial dermis				
Trace	1/5	0/5	0/5	1/5
Minimal	0/5	0/5	0/5	3/5
Focal inflammation, superficial dermis				
Trace	0/5	2/5	3/5	0/5
Minimal	1/5	0/5	2/5	0/5

[a] Data obtained from page 24 in the study report.

b) Neoplastic - No neoplastic tissue was observed in any rabbits during the study.

III. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that dermal irritation was observed in all treatment groups in a dosage-related degree. The diffuse acanthosis and hyperkeratosis observed in the intermediate and high dose animals were attributed to an adaptive response of the skin to an irritant test substance and were not considered to be adverse in nature. The study authors determined that the NOAEL was 100 mg/kg/day, and

that a NOEL had not been determined.

B. Reviewer's Discussion

We agree with the study authors that a NOEL was not established in this study, since mild to moderate dermal irritation was observed in all rabbits treated with the formulated product SAN 1269 H 70 WG 403 DP. Rabbits (1/5 males, 4/5 females) in the 10 mg/kg/day treatment groups exhibited slight erythema. All rabbits in the 30 mg/kg/day treatment groups exhibited slight to minimal erythema; 1/5 males and 4/5 females had accompanying edema; and 4/5 males and 5/5 females exhibited diffuse epidermal acanthosis. Rabbits in the 100 mg/kg/day treatment groups exhibited erythema and edema (5/5 males, 5/5 females), cracking (5/5 males, 5/5 females) and sloughing (3/5 males, 4/5 females) of the treated skin, diffuse epidermal acanthosis (5/5 males, 5/5 females), diffuse hyperkeratosis (1/5 males, 4/5 females), and diffuse inflammation of the superficial dermis (2/5 males, 4/5 females).

Although males in the 30 and 100 mg/kg/day treatment groups had increased glucose levels (13 and 16% higher, respectively), it was uncertain if this was a response to treatment. There were no accompanying histopathological changes, and glucose levels of females at these dose levels were nearly identical to the controls. No historical data were provided for comparison. No significant systemic toxicity was observed as a result of treatment at any dose level.

In conclusion, the LOEL for dermal toxicity in this study is 10 mg/kg/day based on dermal irritation at the treatment site. A NOEL was not determined. The NOEL for systemic toxicity is 100 mg/kg/day. The LOEL was not determined.

IV. STUDY DEFICIENCIES

No scientific or guideline deficiencies were noted with this study.