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Registration Action Branch I (7509C)

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Registration Action 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 836 H 86 SP 401 DP Formulation (sodium salt of SAN 835 H containing 86.5% SAN 835 H by weight)

SYNONYMS: None

CITATION: Allan, S.A., P.L. Connolly, and C. Gopinath. (1996) SAN 836 H 86 SP 401 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 201/960143. May 24, 1996. MRID 44307447. Unpublished.

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

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 44307447), SAN 836 H 86 SP 401 DP Formulation (sodium salt of SAN 835 H containing 86.5% SAN 835 H by weight, Lot# 6150-17) was applied in distilled water to the shaved skin of five juvenile New Zealand White rabbits/sex/dose at dose levels of 0, 100, 300, or 1,000 mg/kg/day for 6 hours/day, 7 days each week, for 3 weeks.

Dermal irritation was observed in rabbits in all treatment groups after 5 days. Rabbits in the control group exhibited no erythema, edema, or other treatment-related dermal reactions during the study. During the 21-day study duration following signs of skin reactions were observed. Slight to well-defined erythema (4/5 and 1/5) and slight edema (1/5) were observed in male rabbits treated 100 mg/kg/day. Female rabbits treated at 100 mg/kg/day exhibited slight to moderate erythema (5/5) and slight well defined edema (3/5). All male rabbits treated 300 and 1000 mg/kg/day exhibited well-defined to moderate erythema and slight to well-defined edema (5/5) at 300 mg/kg/day and slight to moderate edema at 1000 mg/kg/day. All female rabbits treated at 300 and 1000 mg/kg exhibited slight to moderate erythema and

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edema. In general, the incidence and severity of the response increased with increasing dose rate. In addition, 2 out of 5 male rabbits in the 300 and 1000 mg/kg/day treatment groups exhibited cracking of the treated skin. Female rabbits in the 100, 300 and 1000 mg/kg/day also exhibited cracking of the treated skin (1/5, 4/5 and 2/5, respectively). Sloughing of the treated skin was observed in males at 300 and 1000 mg/kg/day dose levels (2/5 and 1/5) and in females at dose levels 100, 300 and 1000 mg/kg/day (1/5, 4/5 and 4/5, respectively). All rabbits treated with the test material exhibited a yellow staining in the area of treatment.

No other significant differences that are considered toxicologically significant were observed in body weight gain, food consumption, signs of toxicity, hematological and clinical parameters and macroscopic and microscopic findings in treated rabbits compared to controls.

The No Observed Effect Level (SYSTEMIC NOEL) = or > 1000 mg/kg/day (HDT; males and females)

The Lowest Observed Effect Level (SYSTEMIC LOEL) >1000 mg/kg/day (HDT; males and females)

This repeated dose dermal toxicity study is classified **acceptable/guideline (§82-2)** and satisfies the guideline requirements for a repeated dose dermal toxicity study in rabbits.

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

I. **MATERIALS AND METHODS**

A. MATERIALS

1. Test Material: SAN 836 H 86 SP 401 DP Formulation
   Description: A grey powder
   Lot/Batch #: 6150-17
   Purity: sodium salt of SAN 835 H containing 86.5% SAN H by weight
   Stability of compound: Expiration data reported to be November 9, 1997
   CAS #: Not provided
   Structure: Not provided

2. Vehicle and/or positive control: 2 mL/kg distilled water used as vehicle to moisten the powder

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age and weight at receipt: Approximately 10-12 weeks of age; body weight, 2.2-2.7 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 - September 27, 1995-February 20, 1996.

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	100	5	5
3 Mid	300	5	5
4 High	1,000	5	5

[a] Dose levels were selected based on the results of a preliminary dermal toxicity study performed at the laboratory (study number SNC/200).

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

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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
	Blood clotting measurements*		
X	(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.

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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 1,000 mg/kg/day groups were examined; the treated and untreated skin of rabbits in the 100 and 300 mg/kg/day groups was also examined. The (XX) organs, in addition, were weighed.

	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). No dermal irritation was observed during the first five days. During the 21-day study duration following signs of skin reactions were observed. Slight to well-defined erythema (4/5 and 1/5) and slight edema (1/5) were observed in male rabbits treated 100 mg/kg/day. Female rabbits treated at 100 mg/kg/day exhibited slight to moderate erythema (5/5) and slight well defined edema (3/5). All male rabbits treated 300 and 1000 mg/kg/day exhibited well-defined to moderate erythema and slight to well-defined edema (5/5)at 300 mg/kg/day and slight to moderate edema at 1000 mg/kg/day. All female rabbits treated at 300 and 1000 mg/kg exhibited slight to moderate erythema and edema. In general, the incidence and severity of the response increased with increasing dose rate. In addition, 2 out of 5 male rabbits in the 300 and 1000 mg/kg/day treatment groups exhibited cracking of the treated skin. Female rabbits in the 100, 300 and 1000 mg/kg/day also exhibited cracking of the treated skin (1/5, 4/5 and 2/5, respectively). Sloughing of the treated skin was observed in males at 300 and 1000 mg/kg/day dose levels (2/5 and 1/5) and in females at dose levels 100, 300 and 1000 mg/kg/day (1/5, 4/5 and 4/5, respectively). All rabbits treated with the test material exhibited a yellow staining in the area 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	100	300	1000
Males				
Erythema, slight	0/5	4/5	0/5	0/5
well-defined	0/5	1/5	4/5	2/5
moderate	0/5	0/5	1/5	3/5
Edema, slight	0/5	1/5	1/5	1/5
well-defined	0/5	0/5	4/5	1/5
moderate	0/5	0/5	0/5	3/5
Yellow staining	0/5	5/5	5/5	5/5
Cracking	0/5	0/5	2/5	2/5
Sloughing	0/5	0/5	2/5	1/5
Female				
Erythema, slight	0/5	1/5	1/5	0/5
well-defined	0/5	3/5	0/5	4/5
moderate	0/5	1/5	4/5	1/5
Edema, slight	0/5	2/5	1/5	3/5
well-defined	0/5	1/5	3/5	1/5
moderate	0/5	0/5	1/5	1/5
Yellow staining	0/5	5/5	5/5	5/5
Cracking	0/5	1/5	4/5	2/5
Sloughing	0/5	1/5	4/5	4/5

[a] Data obtained from Appendix 1, pages 43-50, 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 2.58-2.77 kg, and of females were 2.73-2.94 kg. Body weight gains during the 3 weeks of treatment ranged from 108-213 g for males and from 131 to 264 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. Statistically significantly higher than control monocyte counts and lymphocyte counts were recorded for the high dose group males and females, respectively. Since the total white blood cells count were not affected and a smaller magnitude of change or large individual variations, these effects were not considered treatment-related.

2. Clinical Chemistry - No significant treatment-related differences were observed between clinical chemistry parameters 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 - The macroscopic examination performed at termination revealed yellow staining of the skin in some rabbits at all dose levels. No other treatment-related gross postmortem differences were observed between rabbits in the treated and the control groups.

3. Microscopic pathology

a) Non-neoplastic - Males and females in the 300 and 1000 mg/kg/day treatment groups had a higher incidence of trace to minimal diffuse epidermal acanthosis than rabbits in the 100 mg/kg/day treatment groups or the controls (Table 3). Diffuse inflammation of the superficial dermis was slightly increased in some male rabbits at all dose levels and in some females at 300 and 1000 mg/kg/day dose levels compared to controls.

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	100	300	1000
Males				
Diffuse epidermal acanthosis:				
Total	0/5	3/5	5/5**	4/5*
Trace	0/5	2/5	3/5	1/5
Minimal	0/5	1/5	2/5	3/5
Diffuse inflammation, superficial dermis				
Total	0/5	2/5	3/5	3/5
Trace	0/5	1/5	1/5	1/5
Minimal	0/5	1/5	2/5	2/5
Female				
Diffuse epidermal acanthosis:				
Total	0/5	3/5	4/5*	5/5**
Trace	0/5	2/5	1/5	2/5
Minimal	0/5	1/5	3/5	3/5
Diffuse inflammation, superficial dermis				
Total	0/5	0/5	1/5	2/5
Trace	0/5	0/5	0/5	1/5
Minimal	0/5	0/5	1/5	1/5

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

* $p<0.05$ ** $p<0.01$ with Fisher's Exact Test

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 with inflammation of the superficial dermis 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 1000 mg/kg/day, and that a NOEL had not been determined.

B. Reviewer's Discussion

RAB 1 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 836 H 86 SP 401 DP. However, in this study **the limit dose of 1000 mg/kg/day** was included and the guideline does not require to conduct studies at higher doses. All treated rabbits exhibited erythema ranging from slight to moderate in severity. Slight to moderate edema was observed in male rabbits at 100, 300 and 1000 mg/kg/day dose levels (1/5, 5/5 and 5/5, respectively) and also in female rabbits (3/5, 5/5 and 5/5, respectively). Cracking and sloughing of the treated skin was observed at 300 and 1000 mg/kg/day in male rabbits (cracking 2/5 at both doses and sloughing 2/5 and 1/5). Cracking and sloughing of the treated skin was observed in female rabbits at all dose levels. Minimal to trace diffuse acanthosis was seen in the majority of male and female rabbits from all treated groups and this effect was statistically significant at 300 and 1000 mg/kg/day. A diffuse inflammation of superficial dermis (trace to minimal) was observed in some treated male rabbits at all dose levels and in some female rabbits at 300 and 1000 mg/kg/day dose levels.

No other significant differences that are considered toxicologically significant were observed in body weight gain, food consumption, signs of toxicity, hematological and clinical parameters and macroscopic and microscopic findings in treated rabbits compared to controls.

In conclusion,

The No Observed Effect Level (SYSTEMIC NOEL) = or > 1000 mg/kg/day (HDT; males and females)

The Lowest Observed Effect Level (SYSTEMIC LOEL) >1000 mg/kg/day (HDT; males and females)

IV. **STUDY DEFICIENCIES**

No scientific or guideline deficiencies were noted with this study.

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