7-18-97

DATA EVALUATION RECORD

SODIUM SALT OF SAN 835H

Study Type: 83-3a; SAN 835H: Oral (Gavage) Prenatal Developmental Study in Rats

Work Assignment No. 3-01O (MRID 44170146)

Prepared for

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

Prepared by

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

Primary Reviewer:
William Spangler, Ph.D.

Signature: William J Spangler
Date: 07/18/97

Secondary Reviewer:
Sandy Daussin

Signature: S. Daussin
Date: 7/15/97

Project Manager:
Mary Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 7/18/97

Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 7/18/97

Disclaimer

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

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EPA Reviewer: Deborah Smegal, M.P.H.
Toxicology Branch II (7509C)

EPA Secondary Reviewer: Steven Dapson, Ph.D.
Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat

OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

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

TEST MATERIAL (PURITY): SAN 835H (98.1% a.i.)

SYNONYMS: Not provided

CITATION: Sharper, V.A. (1995) Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of SAN 835H Administered Orally via Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania. Laboratory Study No. 1819-001, February 28, 1995. MRID 44170146. Unpublished.

SPONSOR: Sandoz Agro, Inc., 1300 East Touhy Avenue, Des Plaines, IL

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 44170146), the sodium salt of SAH 835 H (98.1% a.i.) in 0.5% aqueous methylcellulose was administered by gavage to 25 female Crl:CD®BR VAF/Plus® (Sprague Dawley) rats/dose at dose levels of 0, 100, 300, or 1,000 mg/kg/day from days 6 through 15 of gestation.

Marginal maternal toxicity was noted at the limit dose of 1,000 mg/kg/day by a significant reduction in food consumption (6-14%, days 6-9, 9-12, and 6-12; p 0.01 or 0.05) and decrease in body weight gain during gestation days 6-9 [-43%] and gestation days

0-20 [-8%]. Similar effects were seen in the range-finding study. No treatment-related changes in body weight gain or food consumption were noted at 100 and 300 mg/kg/day compared to controls. No deaths occurred, there were no treatment-related gross pathologic findings, and no changes in clinical signs of toxicity were noted at any treatment level.

The maternal NOAEL is 300 mg/kg/day and the maternal LOAEL is 1000 mg/kg/day based on decreases in food consumption and weight gain.

Developmental effects, characterized as significantly lower fetal body weights in males(5%) and skeletal variations exhibited as incompletely ossified and unossified sternal centra and reduced fetal ossification sites for caudal vertebrae, were observed at 1,000 mg/kg/day.

The developmental LOAEL is 1,000 mg/kg/day, based on decreased fetal body weights and skeletal variations. The developmental NOAEL is 300 mg/kg/day.

This developmental toxicity study in the rat is classified Acceptable and satisfies the guideline requirement for a developmental toxicity study (83-3a) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SAN 835H
   Description: Beige powder
   Lot #: 5904-4
   Purity: 98.1% a.i.
   CAS #: Not provided
   Structure: Not provided

2. Vehicle: Aqueous 0.5% methylcellulose
   Description: Anhydrous as received
   Lot #: 922148
   Purity: Not reported

3. Test animals: Species: Rat
   Strain: Crl:CD®BR VAF/Plus® (Sprague-Dawley)
   Age at mating: 10 wks, females; 13 months, males
   Weight at mating: 211 - 257 g, females; 551 - 1169 g, males
   Source: Charles River Laboratories, Inc., Portage, Michigan
   Housing: Individually housed in wire bottomed cages except during mating.
   Diet: Certified Purina Rodent Chow® #5002M, ad libitum
   Water: Municipal tap water processed by reverse osmosis, ad libitum
   Environmental conditions:
   Temperature: 70-78 F±2%
   Humidity: 40-70±3%
   Air changes: 10/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): 5 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 09/06/94 End: 10/01/94

2. Mating: Females were paired on a 1:1 basis with stock males of the same strain. Day 0 of gestation was designated as the day a copulatory plug was found or sperm were found in a vaginal smear.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random by weight using

a computer-generated procedure.

Table 1. Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	25
Low (LDT)	100	25
Mid (MDT)	300	25
High (HDT)	1,000	25

4. Dose selection rationale: In a range-finding study included in the current submission, SAN 835H (97.1% a.i.) in 0.5% methylcellulose was administered orally by gavage to 8 presumed pregnant rats/dose at 0, 125, 250, 500, 750, or 1,000 mg/kg/day on days 6 through 15 of gestation. The rats were observed daily for viability, clinical signs of toxicity, body weights and food consumption. Dams were sacrificed and necropsied on day 20 of gestation. All corpora lutea were counted and the uterus of each pregnant dam was weighed and examined for number and placement of implantations, early and late resorptions, and live and dead fetuses. Each fetus was weighed, evaluated for gender, and examined for gross external alterations.

No mortality was observed at any dose. Salivation was increased at 500 mg/kg/day and above. Maternal body weight gains were decreased during days 0-6 of gestation for 750 and 1,000 mg/kg/day dams and for the overall dosing period (days 6-16) for 1,000 mg/kg/day dams. Gravid uterine weights and maternal body weights corrected for gravid uterine weights were decreased for 1,000 mg/kg/day dams compared to controls although the differences were not statistically significant. Food consumption was significantly reduced (p 0.01) for 1,000 mg/kg/day dams during dosing (34-42%, days 6-9, 9-12, and 6-12) with a significant (p 0.01) rebound (18%, days 18-20) during the post-dosing period.

At 750 and 1,000 mg/kg/day, the mean number of resorptions per litter were increased (p 0.01) and mean fetal body weights were decreased (statistically significant in 1000 mg/kg/day group; p<0.01). No other difference in embryo-fetal development or external fetal morphology was observed at any dose level.

Based on the results of this range-finding study, the subsequent full developmental toxicity study in rats was conducted at dosages of 0, 100, 300, or 1,000 mg/kg/day.

5. Dosage preparation and analysis: Test substance formulations were prepared daily by mixing appropriate amounts of test substance in 0.5% aqueous methylcellulose. Duplicate samples of each dosing suspension were analyzed on days 6 and 20 of the study. Three aliquots of test concentrations at 3 and 100 mg/mL were taken from the top, middle, and bottom of sample mixtures and analyzed for homogeneity by HPLC.

Results -
Stability Analysis: Not reported

Homogeneity Analysis: Samples from the top, middle, and bottom of the low and high test concentrations ranged from 98-108% of nominal and 102-108% of nominal, respectively.

Concentration Analysis: The average concentrations of four samples per dose level were 98-106% of nominal.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 15, in a volume of 10 mL/kg of body weight/day. Control animals received aqueous 0.5% methylcellulose at 10 mL/kg/day. Dosing was adjusted daily, based on the most recent body weight determinations and was performed at approximately the same time each day.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality, moribundity, and clinical signs of toxicity, twice daily on day 0 and during treatment days 6-15 and once daily during the post-treatment period (days 15-20). Body weights were recorded twice during acclimation. Body weights and food consumption data were recorded daily on gestation days 0, during days 6-15 of dosing, and days 16-20 of the post-dosing period. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of a gross examination of the thoracic, abdominal and pelvic cavities. Abnormal tissues were preserved in 10% neutral buffered formalin. The reproductive tracts were removed and the following were recorded:
   - number of corpora lutea in each ovary
   - numbers and sites of implantations
   - numbers of live and dead fetuses
   - numbers of early and late resorptions

   An early resorption was defined as one in which organogenesis was not grossly evident whereas a late resorption was defined as one in which organogenesis was evident. A live fetus was defined as a term fetus that responded to mechanical stimuli.

2. Fetal Evaluations - Each fetus was weighed, sexed, and examined for external abnormalities. For each litter approximately half of the fetuses were examined for soft tissue alterations using a variation of the microdissection technique of Staples. The remaining fetuses were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: Pre-implantation and post-implantation loss indices (%) were calculated, by the reviewer, from cesarean section records of animals in the study.

   Pre-implantation loss indices were calculated as:
   (# corpora lutea - # implantations)/# corpora lutea x 100

Post-implantation loss indices were calculated as:
(# implantations - # live fetuses/# implantations x 100

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: No treatment-related deaths occurred at any dose level during the study. Clinical signs of toxicity, including missing or broken incisors, localized alopecia, and red substance, occurred in 1/25 or 2/25 of the 1,000 mg/kg/day test animals and were not considered to be treatment-related.

2. Body Weight - Body weight gain data are summarized in Table 2. During the first 3 days of treatment (gestational days 6-9) there was a decrease (43%) in body weight gain at the high-dose, compared to controls, that rebounded during the remainder of the treatment period. The overall difference (gestational days 0-20) was 8% and, corrected for gravid uterine weight, 11%. None of the differences in body weight gain noted in the high-dose group reached statistical significance. Mean body weights were comparable to the controls throughout treatment at all dose levels. No significant differences in maternal body weight gain were

observed at 100 or 300 mg/kg/day SAN 835H.

Table 2. Group mean maternal body weight gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (22)	100 (22)	300 (19)	1,000 (20)
Pre-treatment: Days 0-6	32.4±5.9	30.2±9.3	26.4±7.5	30.6±8.3
Treatment: Days 6-9	14.1±3.5	14.0±5.6	12.5±8.9	8.0±8.1
Treatment: Days 9-12	15.5±5.3	16.8±5.4	16.6±11.6	18.4±9.0
Treatment: Days 12-16	32.0±6.4	29.8±8.2	30.5±8.2	32.2±7.0
Post-treatment: Days 16-20	71.6±10.0	63.2±14.0	60.7±17.2	63.4±12.5
Overall treatment: Days 6-16	61.7±8.1	60.6±9.8	59.6±13.8	58.6±7.8
Overall pre-treatment through post-treatment: Days 0-20[b]	165.7±14.8 (71.6)	154.0±27.2 (65.5)	146.6±28.3 (65.6)	152.6±21.1 (63.4)

a Data extracted from study report Table 4, page 41.
b Data in parentheses, corrected for gravid uterine weights (net wt. change from day zero), were calculated by the reviewer from study data. Values for dams with single conceptus litters were excluded.

3. Food Consumption - Food consumption data, summarized in Table 3, are equivocal. A slight increase in food consumption preceded treatment (1%, not significant) for the high-dose group followed by significant (p 0.01 or p 0.05) decreases in food consumption during days 6-9 (14%) and 9-12 (6%) with recovery beginning at 12-16 days of treatment (2%). Overall (days 0-20), food consumption for the 1,000 mg/kg/day treatment group was reduced only slightly (2%, not significant) compared to controls. Food consumption values in the 100 and 300 mg/kg/day treatment groups were comparable to the controls throughout the study.

Table 3. Maternal food consumption (g/rat/day)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (22)	100 (22)	300 (19)	1,000 (20)
Pre-treatment: Days 0-6	21.5±1.5	21.5±1.5	21.0±1.8	21.8±2.3
Treatment: Days 6-9	23.0±1.8	23.1±2.1	22.0±1.7	19.8±2.4**
Treatment: Days 6-12	23.5±1.6	23.8±1.9	22.7±1.8	21.2±2.2**
Treatment: Days 9-12	24.1±1.6	24.4±1.8	23.4±2.2	22.6±2.4*
Treatment Days 12-16	25.9±1.9	26.3±2.7	25.7±2.0	26.4±1.9
Post-treatment: Days 16-20	28.6±2.8	27.9±2.5	27.4±2.4	28.4±2.6
Overall treatment: Days 6-16	24.5±1.5	24.8±2.1	23.9±1.8	23.3±1.8*
Overall treatment through post-treatment: Days 6-20	25.6±1.8	25.7±2.0	24.9±1.7	24.7±1.7
Overall pretreatment through post-treatment: Days 0-20	24.4±1.5	24.4±1.8	23.7±1.7	23.8±1.6

a Data extracted from study report Table 5, page 42.
* p 0.05, ** p 0.01.

4. Gross Pathology - There was no treatment-related gross pathologic finding upon necropsy for any dose level.

5. Cesarean Section Data - Cesarean section data are summarized in Table 4. At dose levels of 1,000 mg/kg/day, fetal body weights were reduced in both sexes (4-5%), but were significantly reduced (p 0.05) only in males (5%). Dams with resorptions were significantly higher at 100 and 300 mg/kg/day, but this finding was not dose-dependent and values were within the historical control range (0-83.3%). The litter averages for corpora lutea, implantations, litter sizes, live fetuses, late resorptions, percent male fetuses and percent resorbed conceptuses were comparable among the four dosage groups. The number of early resorptions was

significantly increased at 100 mg/kg/day (low-dose). However, because the number of early resorptions was comparable to the controls at the mid- and high-doses, the increase at the low-dose is not considered to be treatment-related.

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	100	300	1,000
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant Pregnancy Rate (%)	22 (88.0)	22 (88.0)	19 (76.0)	20 (80.0)
# Nonpregnant	3	3	6	5
Maternal Wastage # Died # Aborted # Premature Delivery	 0 0 0	 0 0 0	 0 0 0	 0 0 0
Total # Corpora Lutea Corpora Lutea/Dam	367 16.7±2.3	378 17.2±3.4	314 16.5±4.5	340 17.0±2.9
Total # Implantations Implantations/Dam	334 15.2±2.5	317 14.4±4.1	274 14.4±5.2	306 15.3±2.7
Total # Litters	22	22	19	20
Total # Live Fetuses Live Fetuses/Dam	328 14.9±2.8	300 13.6±3.8	260 13.7±5.0	299 15.0±2.7
Total # Dead Fetuses Dead Fetuses/Dam	0 0	0 0	0 0	0 0
Total # Resorptions Early Late Resorptions/Dam Early Late Litters with Total Resorptions Dams with resorptions (%)	7 7 0 0.3±0.7 0.3±0.7 0.0±0.0 0 4 (18.2)	18 18 0 0.8±0.8* 0.8±0.8* 0.0±0.0 0 13 (59.1)**	13 13 0 0.7±0.7 0.7±0.7 0.0±0.0 0 10 (52.6)**	7 6 1 0.4±0.6 0.3±0.6 0.0±0.2 0 6 (30.0)
Mean Fetal Weight(g/litter) Males Females	3.45±0.16 3.54±0.17 3.36±0.20	3.43±0.25 3.49±0.20 3.36±0.28	3.49±0.26 3.56±0.26 3.38±0.30	3.30±0.26 3.38±0.24* 3.23±0.28
Sex Ratio (% Males/litter)	48.6±15.8	42.8±17.5	46.1±20.2	47.8±8.8
Pre-implantation Loss (%)[b]	9.0	16.1	12.7	10.0
Post-implantation Loss (%)[b]	1.8	5.4	5.1	2.3

a Data extracted from study report Table 7, pages 44-46.
b Calculated by reviewer.
* p 0.05, ** p 0.01.

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B. DEVELOPMENTAL TOXICITY

1. External Examination - No treatment-related alterations were found by external examination (Table 5a) at any dose level of SAN 835H tested.

Table 5a. Alterations found by external examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1,000
#Fetuses (#litters) examined	328 (22)	300 (22)	260 (19)	299 (20)
Jaw: Micrognathia[b]	0 (0)	0 (0)	0 (0)	0.3 (5.0)
Body:[b]				
Anasarca	0 (0)	0 (0)	0.4 (5.3)	0 (0)
Umbilical hernia	0 (0)	0 (0)	0.4 (5.3)	0 (0)
Tail:[b]				
Absent	0 (0)	0 (0)	0 (0)	0.3 (5.0)
Short	0 (0)	0 (0)	0.4 (5.3)	0 (0)

a Data extracted from study report Table 10, page 47
b %Fetuses (%litters)

2. Visceral Examination - No treatment-related soft tissue abnormalities or malformations were found by visceral examination (Table 5b) at any dose level.

Table 5b. Fetal soft tissue examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1,000
#Fetuses (#litters) examined	157 (22)	144 (21)	127 (17)	144 (20)
Brain:[b]				
Lateral ventricles, marked dilation	0 (0)	0 (0)	0.8 (5.9)	0 (0)
Third ventricle, moderate dilation	0 (0)	0 (0)	0.8 (5.9)	0 (0)
Eyes: Microphthalmia[b]	0 (0)	0 (0)	0.8 (5.9)	0 (0)

Bladder:[b] Displaced umbilical artery	0 (0)	0.7 (4.8)	0 (0)	0.7 (5.0)
Ureter:[b] White substance	0 (0)	0 (0)	0 (0)	0.7 (5.0)

a Data extracted from the study report Tables 11, pages 48.
b % Fetuses (% litters)

3. Skeletal Examination - The results of fetal skeletal examinations and fetal ossification sites are summarized in Tables 5c and 5d. Significant increases (p 0.01) in the fetal and litter incidence of incompletely ossified and/or unossified sternal centra were observed at the limit dose (fetal: 11.6% vs. 1.8% in controls; litter: 40% vs. 9.1% in controls). In addition, the average number of ossified caudal vertebra per fetus was reduced in the high-dose group (4.33 vs. 4.88 in controls, p 0.01). These reversible delays in sternal, caudal vertebral, and metacarpal ossification sites were considered to be treatment-related and correlated with the reduced body weights observed in fetuses from the 1,000 mg/kg/day treatment group. Significant reductions in average numbers of ossified sites of sternal centers and metacarpals noted in the 100 and/or 1,000 mg/kg/day groups were not dose-related and, therefore, not treatment related.

Table 5c. Fetal skeletal examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1,000
#Fetuses (#litters) examined	171 (22)	156 (22)	133 (19)	155 (20)
Skull:				
Fused mandible	0 (0)	0 (0)	0 (0)	0.6 (5.0)
Fused premaxillae	0 (0)	0 (0)	0 (0)	0.6 (5.0)
Vertebrae:				
Thoracic bifid centrum	1.2 (9.1)	0 (0)	0.8 (5.3)	1.3 (10.0)
Cervical; at 7th cervical rib	0.6 (4.5)	0 (0)	0 (0)	0.6 (5.0)

Observations	Dose (mg/kg/day)			
	0	100	300	1,000
Sternal centra: incompletely ossified and/or unossified	1.8 (9.1)	5.1 (22.7)	0 (0)	11.6** (40.0)**
No.litters with >1 fetus affected	2/22	5/22	0/19	8/20
Pelvis:				
inc. ossified pubes	5.8 (27.3)	9.0 (31.8)	0 (0)**	3.9 (30.0)
inc. ossified ischia	2.9 (18.2)	3.2 (18.2)	0 (0)	0.6 (5.0)

a Data extracted from study report Table 12, pages 49-50
b % fetuses (% litters)
* p 0.05, ** p 0.01

Table 5d. Fetal ossification sites[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1,000
#Fetuses (#litters) examined	164 (21)	156 (22)	133 (19)	155 (20)
Caudal vertebrae[b]	4.88±0.39[c]	4.75±0.39	4.77±0.86	4.33±0.55**
Sternal centers[b]	3.71±0.25	3.46±0.31*	3.70±0.28	3.53±0.34
Metacarpals[b]	3.57±0.34	3.34±0.25*	3.52±0.34	3.35±0.36*

a Data extracted from study report Tables 13, page 51
b Sites per fetus per litter
c Mean±S.D.
* p 0.05, ** p 0.01

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study author concluded that oral administration of SAN 835H (97.1% a.i.) at 1,000 mg/kg/day to pregnant rats during organogenesis was associated with treatment-related reduced maternal body weight gain and food consumption values. The maternal NOAEL is 300 mg/kg/day. Developmental toxicity was observed as reduced fetal body weights and reversible delays in sternal and caudal vertebral ossification at 1,000 mg/kg/day of SAN 835H. The developmental NOAEL is 300 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: The reviewer agrees with the study author's determination of the maternal LOEL at 1000 mg/kg/day based on maternal body weight gain and food consumption data. The study author considered the decrease in body weight gain at the high-dose (8%; corrected for gravid weight 11%) to be treatment related. However the decreases observed were primarily due to a transient reduction during the initial treatment period (6-9 days). None of the differences were statistically significant and body weights were comparable to the controls throughout the study. Therefore, the reductions in weight gain at the high-dose are shown to be related to treatment. Likewise, food consumption data at the high-dose are treatment-related. There was a significant reduction in food consumption during days 6-9 and 9-12 with recovery beginning at 12-16 days. Although the reduction in food consumption at 1,000 mg/kg/day was transient, the reduction in food consumption at the high-dose is considered to be treatment-related. No difference in body weight gain or food consumption was observed at 100 or 300 mg/kg/day as compared to controls. No deaths, treatment-related clinical signs of toxicity, or gross pathologic findings were observed at any treatment level.

Maternal NOAEL = 300 mg/kg/day
Maternal LOAEL = 1000 mg/kg/day

2. DEVELOPMENTAL TOXICITY: Developmental effects characterized as lower fetal body weights and skeletal variations were noted for the 1,000 mg/kg/day treatment group.

a. Deaths/Resorptions: The numbers of resorption/dam and viable fetuses/dam for the treatment groups were not significantly different from the concurrent controls.

b. Altered Growth: Fetal body weights were lower (4-5%), but minimal in the 1,000 mg/kg/day treatment group compared to controls. Fetal body weight differences were significant (p 0.05) only for males (5% vs 4% in females).

c. Developmental Variations: Skeletal variations that were significantly increased (p 0.01) were incompletely ossified and unossified sternal centra at 1,000 mg/kg/day

(fetal: 11.6% vs. 1.8% in controls; litter: 40% vs. 9.8% in controls). Also at 1,000 mg/kg/day, fetal ossification sites/fetus were significantly reduced (p 0.01) for caudal vertebrae (4.33 vs. 4.89 in controls).

d. Malformations: There were no treatment-related developmental malformations noted at any dose level.

Developmental NOAEL = 300 mg/kg/day
Developmental LOAEL = 1,000 mg/kg/day

C. STUDY DEFICIENCIES Stability analyses for test doses were not performed. Because doses were mixed daily and other analytical data were obtained for dose concentration and mixing procedure, this deficiency is not likely to have affected the study outcome.

7-18-97

DATA EVALUATION RECORD

SODIUM SALT OF SAN 835H

Study Type: 83-3b; SAN 835H: Oral (Gavage) Prenatal Developmental Study in Rabbits

Work Assignment No. 3-01P (MRID 44170147)

Prepared for

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

Prepared by

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

Primary Reviewer: William Spangler, Ph.D.	Signature: [signature] Date: 07/05/97
Secondary Reviewer: Sandy Daussin	Signature: [signature] Date: 7/7/97
Project Manager: Mary Menetrez, Ph.D.	Signature: [signature] Date: 7/18/97
Quality Assurance: Steven Brecher, Ph.D.	Signature: [signature] Date: 7/18/97

Disclaimer

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

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Chemical Name: Developmental Toxicity Study in Rabbits
BASF. 1995. MRID No. 44170147
HED Doc. No. None

EPA Reviewer: Deborah Smegal, M.P.H.
Toxicology Branch II (7509C)

EPA Secondary Reviewer: Steven Dapson, Ph.D.
Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit
OPPTS Number: 870.3700 OPP Guideline Number: §83-3b

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

TEST MATERIAL (PURITY): SAN 835H (98.1% a.i.)

SYNONYMS: Not provided

CITATION: Sharper, V.A. (1995). Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of SAN 835H Administered Orally via Gavage to New Zealand White Rabbits. Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, Pennsylvania. Laboratory Study No. 1819-008, February 23, 1995. MRID 44170147. Unpublished.

SPONSOR: Sandoz Agro, Inc., 1300 East Touhy Avenue, Des Plaines, IL

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 44170147), the SAN 835 H (98.1% a.i.) in 0.5% aqueous methylcellulose was administered by gavage to 20 female New Zealand White [Hra:(NZW)SPF] rabbits/dose at dose levels of 0, 30, 100, or 300 mg/kg/day from days 6 through 19 of gestation.

Three treatment-related deaths occurred at 300 mg/kg/day of SAN 835H. Treatment-related clinical signs of toxicity associated with the deaths were abnormal feces, persistent weight loss, and reduced food consumption (24%) during the treatment period (gestational days 6-19). Neither decreased weight gain or

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reduction in feed consumption was statistically significant at the high-dose. At 300 mg/kg/day, there was a significant (p 0.01), treatment-related increase in abortions (5/17 vs. 0/19 for controls). At 100 mg/kg/day, no treatment-related deaths, abortions or gross pathologic findings were observed, although there was a minimal reduction in body weight gain, with no reduction in food consumption. In addition, there were differences in the incidence ratios for abnormal feces (soft or liquid feces, dried, or no feces) in the 100 mg/kg/day group compared to controls. No statistically significant changes in body weight gain, food consumption, mortality, abortions, clinical signs of toxicity (fecal abnormalities), or gross pathologic findings were observed at 30 mg/kg/day.

The maternal LOAEL is 100 mg/kg/day, based on minimal reductions in body weight gain with no reduction in food consumption and clinical signs of toxicity (abnormal feces). The maternal NOAEL is 30 mg/kg/day.

Developmental effects, characterized as significant increases (p 0.01) in the incidence of supernumerary thoracic rib pair ossification sites (12.74 vs. 12.54 for controls) occurred at 300 mg/kg/day of SAN 835H. Consequently, thoracic vertebrae ossification sites were significantly (p 0.05) increased (12.79 vs. 12.47 for controls) and lumbar vertebrae ossification sites were significantly (p 0.05) decreased (6.20 vs. 6.45 for controls) at the high-dose. No treatment-related developmental effects were noted at the low- or mid-doses of SAN 835H.

The developmental LOAEL is 300 mg/kg/day based on increased skeletal variations (supernumerary rib ossification sites). The developmental NOAEL is 100 mg/kg/day.

This developmental toxicity study in the rabbit is classified Acceptable and satisfies the guideline requirement for a developmental toxicity study (83-3b) in rabbits.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SAN 835H
   Description: Beige powder
   Lot #: 5904-4
   Purity: 98.1% a.i.
   CAS #: Not provided
   Structure: Not provided

2. Vehicle: Aqueous 0.5% methylcellulose
   Description: Anhydrous as received
   Lot #: 922148
   Purity: Not provided

3. Test animals: Species: Rabbit
   Strain: New Zealand White [Hra:(NZW)SPF]
   Age at mating: 11 months
   Weight on day 0 of gestation: 2.8-5.0 kg
   Source: HRP, Inc., Denver, Pennsylvania
   Housing: Individually housed. All cages were in compliance with the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23.
   Diet: Certified Rabbit Diet® #5322 (Purina Mills, Inc.),150 g from days 1-6 and 180 g from days 6-19.
   Water: Municipal tap water processed by reverse osmosis. Chlorine was added to the processed water as a bacteriostat, ad libitum
   Environmental conditions:
   Temperature: 61-72 F±2%
   Humidity: 40-60±3%
   Air changes: 10/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): approximately 2 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 09/02/94 End: 09/30/94

2. Mating: Females were purchased from the supplier premated and presumed pregnant.

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3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random by weight using a computer-generated procedure.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	20
Low (LDT)	30	20
Mid (MDT)	100	20
High (HDT)	300	20

4. Dose selection rationale: Dosages were selected on the basis of two range-finding studies included in the current submission.

In the first study, SAN 835H (94.7% a.i.) in 0.5% methylcellulose was administered orally by gavage to 5 artificially inseminated New Zealand White rabbits/dose at 0, 250, 500, 750, or 1,000 mg/kg/day on days 6 through 18 of presumed pregnancy. The rabbits were observed daily for viability, clinical signs of toxicity, body weights, food consumption, abortions and premature deliveries. Dams were sacrificed on gestational day 29 and a gross necropsy of the thoracic and abdominal viscera was performed. All corpora lutea were counted and the uterus of each pregnant dam was examined for number and placement of implantations, early and late resorptions, and live and dead fetuses. Each fetus was weighed, sacrificed, evaluated for gender, and examined for gross external alterations. No gross external fetal alterations were found and all fetuses were discarded.

At the 750 and 1,000 mg/kg/day treatment levels 4/5 dams died and one 1,000 mg/kg/day dam was sacrificed moribund. Clinical signs of toxicity at 750 mg/kg/day were dried

feces, decreased motor activity, red substance in cage pans, impaired righting reflex and/or emaciation with body weight reduction and reduced food consumption generally beginning on gestational day 7 and continuing through death or moribund sacrifice. Three does each in the 750 and 1,000 mg/kg/day treatment groups had litters that consisted only of early resorptions. The remaining dams that died had litters consisting of early resorptions and conceptuses that appeared normal. There were no surviving, pregnant does in the 750 and 1,000 mg/kg/day treatment groups after 19 and 15 days of gestation, respectively.

At 500 mg/kg/day, SAN 835H caused maternal body weight loss and reduced food consumption during the treatment period. Post-dosing body weight losses recovered somewhat and food consumption was comparable to controls, however, the overall body weight gain and food consumption values were reduced for days 6 to 29 and 0 to 29 of gestation. No mortality occurred at 500 mg/kg/day and clinical signs of toxicity were dried or no feces, red substance in cage pans, and orange urine. One 500 mg/kg/day doe aborted on gestational day 23. Increased resorption of fetuses and reduced fetal body weights were observed at 500 mg/kg/day.

At 250 mg/kg/day, no effect on body weight, body weight gain, food consumption, or cesarean section parameters was observed compared to controls. No mortality occurred and no clinical signs of toxicity were noted.

In the second range-finding study, SAN 835H in 0.5% methylcellulose was administered orally by gavage to 2 female New Zealand White rabbits/dose at 300, 350, 400, 450, and 500 mg/kg/day for up to 12 consecutive days. The rabbits were observed daily for viability, clinical signs of toxicity, body weight, and food consumption. The animals were, presumably, not pregnant and teratogenicity was not evaluated.

One death occurred in each of the 400, 450, and 500 mg/kg/day dosage groups. Prior to death on days 9-11, these animals exhibited severe weight loss, severe reduction of

food consumption and no feces. Common signs of toxicity for all treatment groups were dried, absent, and/or soft or liquid feces. Weight loss occurred in each dosage group on each study day except days 1-2 for the 350 and 400 mg/kg/day treatment groups. Food consumption was reduced in all treatment groups throughout the treatment period.

At necropsy, a trichobezoar was found in the stomach of one or two rabbits/treatment group. Other necropsy observations included discolored lungs in both 450 mg/kg/day rabbits and one 500 mg/kg/day rabbit, the cardiac and fundic regions of the stomach were reddened in one rabbit each of the 300 and 350 mg/kg/day dosage groups, there was an accessory spleen in one 350 mg/kg/day rabbit, and there were three pale spots on the left lateral lobe of the liver of the dead 400 mg/kg/day rabbit. There were no changes in mean absolute or relative organ weights among the treatment groups.

Based on the results of these range-finding studies, the subsequent full developmental toxicity study in rabbits was conducted at dosages of 0, 30, 100, or 300 mg/kg/day.

5. Dosage preparation and analysis: Test substance formulations were prepared daily by mixing appropriate amounts of test substance in 0.5% aqueous methylcellulose. Two samples each of test dose concentrates at 3.0, 10.0 and 30.0 mg/mL were analyzed in duplicate to compare nominal and actual concentrations of test substance. Three aliquots of test concentrations at 3 and 100 mg/mL were taken from the top, middle, and bottom of sample mixtures and analyzed for homogeneity by HPLC.

Results -
Stability Analysis: Not reported

Homogeneity Analysis: Samples from the top, middle, and bottom of the low and high test concentrations ranged from 98-108% of nominal and 102-108% of nominal, respectively.

Concentration Analysis: The average concentrations for duplicate analyses of two samples per dose concentrate (3.0, 10.0, and 30.0 mg/mL) were 2.80, 10.3, and 31.4 mg/mL (93, 103, and 105% of nominal), respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (±10% of target value).

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 19, in a volume of 10 mL/kg of body weight/day. Control animals received aqueous 0.5% methylcellulose at 10 mL/kg/day. Dosing was adjusted daily, based on the most recent body weight determinations and was performed at approximately the same time each day.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked twice daily for abortions, premature delivery, and clinical signs of toxicity during the dosing period and once daily during the post-treatment interval. All animals were observed for mortality twice daily throughout the study. Body weights were recorded once during the pre-dosing period, daily through the dosing period, and on days 20, 24, and 29 of the post-dosing period. Food consumption was recorded daily throughout the study. Rabbits that aborted, delivered and were sacrificed, and rabbits that were found dead were necropsied on the same day the event occurred. The remaining dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of a gross examination of the thoracic, abdominal and pelvic cavities. Abnormal tissues were preserved in 10% neutral buffered formalin. The reproductive tracts were removed and the following were recorded:
   - number of corpora lutea in each ovary
   - numbers and sites of implantations
   - numbers of live and dead fetuses
   - numbers of early and late resorptions

   Uteri from does that appeared nonpregnant were stained with 10% ammonium sulfide to confirm the absence of implantation sites.

2. Fetal Evaluations - Each fetus was weighed, sexed, and examined for external abnormalities. For all fetuses,

cavitated organs, including the brain, were evaluated by dissection and the brain was free-hand cross-sectioned and examined for hydrocephaly. Fetal gross lesions were preserved in neutral buffered 10% formalin. All fetuses were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations. Late resorptions were examined to the extent possible.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: Pre-implantation and post-implantation loss indices (%) were calculated, by the reviewer, from cesarean section records of animals in the study.

   Pre-implantation loss indices were calculated as:
   (# corpora lutea - # implantations)/# corpora lutea x 100

   Post-implantation loss indices were calculated as:
   (# implantations - # live fetuses/# implantations x 100

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: Deaths occurred at all dose levels during the study (1, 2, 1, and 4 at 0, 30, 100, and 300 mg/kg/day, respectively), however only 3 deaths at 300 mg/kg/day were treatment-related. The deaths of the control, one low-dose, the mid-dose, and one high-dose animal were due to intubation accidents. One low-dose animal died of unknown causes. Clinical signs of toxicity, exhibited by the high-dose animals that died were abnormal feces, persistent weight loss, and reduced food consumption from the beginning of the dosing period. There was a significant increase (p 0.01) in the number of abortions at 300 mg/kg/day (5/17) compared to controls (0/19). Increased

incidences of abnormal feces (dried and/or no feces) were also observed in the high-dose animals that aborted. No statistically significant treatment-related clinical signs of toxicity were observed in the 30 or 100 mg/kg/day treatment groups.

2. Body Weight - Body weight gain data are summarized in Table 2. These values exclude dams that died, aborted, or prematurely delivered. At the high-dose, there was a marginal (not statistically significant) decrease in weight gain during the treatment period (days 6-20), recovery during the post-treatment period (days 20-29), and a slight overall (days 0-29) decrease in body weight which never achieved statistical significance. Effects on body weight gain at the mid-dose are variable, with marginal increases and decreases throughout the treatment and post-treatment interval compared to controls. Body weights were comparable to the controls throughout the study at the mid-dose. No significant difference in maternal body weight gain was observed at the low-dose (30 mg/kg/day SAN 835H), except for an increase on treatment days 15-20.

Table 2. Group mean maternal body weight gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)[b]			
	0 (17-18)	30 (15)	100 (17-18)	300 (9-16)
Pre-treatment: Days 0-6	30±90	40±60	10±90	30±120
Treatment: Days 6-9	-10±50	-20±50	-20±60	-30±80
Treatment: Days 9-12	-20±60	0±70	10±60	-40±90
Treatment: Days 12-15	20±70[c]	-10±90[c]	10±80[c]	-40±80[c]
Treatment: Days 15-20	-50±120[c]	40±90[c,*]	-80±140[c]	-10±130[c]
Post-Treatment: Days 20-24	20±140[c]	80±80[c]	30±120[c]	-70±110[c]
Post-treatment: Days 24-29	-20±120[c]	20±110[c]	10±130[c]	110±230[c,d]
Overall treatment: Days 6-20	-40±220[c]	20±220[c]	-50±220[c]	-180±310[c]
Post-treatment: Days 20-29	20±190[c]	100±180[c]	50±240[c]	80±280[c,d]
Overall treatment through post-treatment: Days 6-29	-20±30[c]	120±350[c]	20±390[c]	60±430[c,d]
Overall pre-treatment through post-treatment: Days 0-29	250±550[c]	410±540[c]	230±600[c]	190±580[c,d]

a Data extracted from study report Table 5, page 60.
b Number of does included in analysis.
c Weights for does that died, aborted, or prematurely delivered are excluded from means.
d Only 9 does included in these means.
* p 0.05.

3. Food Consumption - Food consumption data are summarized in Table 3. Food consumption in the high-dose group was decreased throughout dosing with the severity increasing over time (17-35%, days 6-9, 9-12, 12-15, 15-20, and 6-20) and rebounded after treatment (12%, days 20-29). None of these differences were statistically significant. Food consumption was significantly reduced (p 0.05) in the high-dose group on gestational days 7 to 8. At the 30 and 100 mg/kg/day treatment levels, no treatment-related differences in food consumption were observed at any test interval.

Table 3. Maternal food consumption (g/rabbit/day)[a]

Interval	Dose in mg/kg/day (# of pregnant Dams)[b]			
	0 (17-18)	30 (15)	100 (17-18)	399 (9-16)
Treatment: Days 6-9	144.9±33.0	156.1±29.2	141.8±36.4	120.8±53.1
Treatment: Days 9-12	127.7±34.4	137.0±33.4	126.7±33.6	86.8±76.6
Treatment: Days 12-15	94.6±57.6[b]	103.8±55.1[b]	103.1±49.6[b]	65.9±71.3[b]
Treatment: Days 15-20	77.9±70.8[b]	109.4±54.7[b]	78.1±65.1[b]	50.3±70.8[b]
Treatment: Days 20-24	88.5±73.7[b]	122.8±54.8[b]	89.0±62.2[b]	58.4±77.1[b]
Treatment: Days 24-29	70.3±44.8[b]	85.9±49.8[b]	95.3±57.2[b]	101.2±63.0[b,c]
Overall treatment: Days 6-20	106.1±46.2[b]	124.1±39.3[b]	107.3±44.4[b]	83.6±61.6[b]
Post-treatment: Days 20-29	80.8±51.3[b]	102.3±50.0[b]	94.8±55.3[b]	90.5±61.7[b,c]
Treatment and post-treatment: Days 6-29	98.0±42.8[b]	115.6±39.5[b]	104.5±42.5[b]	105.6±53.7[b,c]

a Data extracted from study report Table 6, page 62.
b Mean values exclude does that died, aborted, or prematurely delivered.
c Only 9 does included in these means.

4. Gross Pathology - A significant increase in gastric trichobezoars was observed in the high-dose group (6/19 vs. 2/20 for controls, p 0.01). All of these animals died and/or aborted. This finding was attributed to the thick

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consistency of the high-dose suspension and was correlated with reduced food consumption in the high-dose group. There were no other treatment-related gross pathologic findings upon necropsy for any dose level.

5. Cesarean Section Data - Cesarean section data are summarized in Table 4. At the 300 mg/kg/day treatment level, there was a significant (p 0.01), treatment-related increase in abortions (5/17 vs. 0/19 in controls). All other cesarean section parameters were comparable among the four dosage groups.

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	30	100	300
# Animals Mated	20	20	20	20
# Animals Pregnant Pregnancy Rate (%)	19 (95)	16 (80)	19 (95)	17 (85)
# Nonpregnant	1	4	1	3
Maternal Wastage # Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	 1 1 0 0 1	 2 1 1 0 0	 1 1 0 1 0	 4 4 0 5** 0
Total # Corpora Lutea Corpora Lutea/Dam	160 9.4±1.3	158 10.5±2.4	153 9.0±1.9	82 9.1±1.0
Total # Implantations Implantations/Dam	134 7.9±1.8	129 8.6±2.0	119 7.0±2.6	68 7.6±1.9
Total # Litters	17	15	17	9
Total # Live Fetuses Live Fetuses/Dam	130 7.6±1.7	111 7.4±3.3	105 6.2±2.2	63 7.0±2.5
Total # Dead Fetuses Dead Fetuses/Dam	0 0.0±0.0	7 0.5±1.8	0 0.0±0.0	1 0.1±0.3
Resorptions/Dam Early Late	0.3±0.5 0.0±0.0 0.3±0.5	0.7±1.4 0.3±1.3 0.4±0.7	0.9±1.3 0.0±0.2 0.8±1.3	0.4±0.7 0.0±0.0 0.4±0.7
Litters with any Resorptions	5	5	8	3
Litters with Total Resorptions	0	1	0	0
Does with Viable Fetuses	17	14	17	9
Mean Fetal Weight (g) Males Females	40.84±8.69 40.82±8.45 39.54±9.41	45.8±5.83 46.53±7.03 45.08±5.51	43.82±8.62 44.60±8.29 42.53±10.2	40.13±9.17 43.92±8.08 38.88±8.71
Sex Ratio (% Male)/litter	45.1±26.5	48.0±19.4	47.5±19.5	42.9±22.2
Pre-implant. Loss (%)[b]	16	18	22	17
Post-implant. Loss (%)[b]	3	14	12	7

a Data extracted from study report Tables 8 and 9, pages 64-65.
b Calculated by reviewer.
** includes the rabbits that aborted and the rabbit that aborted and was found

dead

B. DEVELOPMENTAL TOXICITY

1. External Examination - No treatment-related alterations or malformations was found by external examination (Table 5a) at any dose level of SAN 835H tested.

Table 5a. Malformations found by external examinations[a]

Observations	Dose (mg/kg/day)			
	0	30	100	300
#Fetuses(#litters) examined	130 (17)	111 (14)	105 (17)	63 (9)
%Fetuses(%litters) affected	0.8 (5.9)	0.9 (7.1)	0 (0.0)	0 (0.0)
Hind paws:[b] Digits curled in	0 (0.0)	0.9 (7.1)	0 (0.0)	0 (0.0)
Tail:[b] Short	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)

a Data extracted from study report Table 11, page 67.
b %Fetuses (%litters)

2. Visceral Examination - No treatment-related soft tissue abnormalities or malformations were found by visceral examination (Table 5b) at any dose level. A statistically significant decrease (p 0.01) in agenesis of the intermediate lung lobe was noted in the fetal incidence values for all treatment groups. However, a decrease in a fetal finding is not considered biologically significant.

Table 5b. Visceral examinations for alterations[a]

Observations	Dose (mg/kg/day)			
	0	30	100	300
#Fetuses (# litters) examined	130 (17)	111 (14)	105 (17)	63 (9)
Fetuses examined: Live (dead)	130 (0)	111 (7)[c]	105 (0)	63 (1)[c]
Vessels:[b] Persistent truncus arteriosus	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Lungs:[b] Small lobes	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Intermediate lobe absent	6.9 (29.4)	0.9** (7.1)	1.9** (11.8)	1.6** (11.1)
Diaphragm:[b] Herniated	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Kidneys:[b] Pelvis, slight dilation	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Pelvis, marked dilation	0.8 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)

a Data extracted from the study report Tables 12, page 68.
b % Fetuses (%litters)
c Dead fetuses excluded from group means and statistical analyses
** p 0.01

3. Skeletal Examination - The results of fetal skeletal examinations are summarized in Tables 5c and 5d. The 300 mg/kg/day treatment group exhibited significant increases (p 0.01) in the incidence of supernumerary thoracic rib pairs (12.74 vs. 12.47 for controls). In addition, related significant changes (p 0.05) in the average numbers of thoracic and lumbar vertebrae (increases: 12.79 vs. 12.54 for controls and decreases: 6.20 vs. 6.45 for controls, respectively), were observed. No other treatment-related differences in skeletal alterations were observed at any treatment level.

Table 5c. Skeletal examinations for alterations[a]

Observations	Dose (mg/kg/day)			
	0	30	100	300
#Fetuses (#litters) examined	130 (17)	111 (14)	105 (17)	63 (9)
Skull:[b] Irregular ossification	16.9 (52.9)	15.3 (71.4)	20.0 (58.8)	17.5 (66.7)
Hyoid bone:[b] Ala, angulated	4.6 (35.3)	4.5 (35.7)	1.0 (5.9)	6.3 (33.3)
Caudal vertebrae:[b] Misaligned	2.3 (17.6)	0.9 (7.1)	0 (0.0)	3.2 (11.1)
Ribs:[b] Flat	2.3 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Sternal centra:[b] Fused	0.8 (5.9)	5.4 (28.6)	3.8 (11.8)	0 (0.0)
1st incompletely ossified	2.3 (11.8)	0 (0.0)	1.0 (5.9)	0 (0.0)

a Data extracted from study report Tables 13, pages 69-74.
b % Fetuses (% litters).

Table 5d. Fetal ossification sites[a]

Observations	Dose (mg/kg/day)			
	0	30	100	300
#Fetuses (#litters) examined	130 (17)	111 (14)	105 (17)	63 (9)
Hyoid bone:	0.99±0.05	1.00±0.00	0.99±0.05	1.00±0.00
Vertebrae:				
Cervical	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00
Thoracic	12.54±0.20	12.42±0.30	12.67±0.32	12.79±0.14*
Lumbar	6.45±0.21	6.56±0.31	6.32±0.32	6.20±0.14*
Sacral	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
Caudal	16.82±0.58	16.75±0.48	16.88±0.54	16.86±0.31
Ribs: Thoracic pairs	12.47±0.19	12.36±0.27	12.61±0.31	12.74±0.18**
Sternum:				
Manubrium	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Sternal centers	3.90±0.15	3.89±0.14	3.86±0.26	3.92±0.12
Xiphoid	0.95±0.08	0.96±0.08	0.90±0.18	0.93±0.13
Forelimb:[d]				
Carpals	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Metacarpals	4.95±0.16	5.00±0.00	4.95±0.19	4.86±0.26
Digits	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Phalanges	13.95±0.09	13.92±0.13	13.80±0.68	13.77±0.37

Observations	Dose (mg/kg/day)			
	0	30	100	300
Hindlimb:[d]				
Tarsals	2.00±0.00	2.00±0.00	1.99±0.05	2.00±0.00
Metatarsals	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Digits	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Phalanges	11.99±0.05	12.00±0.00	11.90±0.39	11.98±0.07

a Data extracted from study report Table 14, page 75.
b Sites per fetus per litter.
c Mean±S.D.
d Calculated as average per limb.
* p 0.05, ** p 0.01

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study author concluded that oral administration of SAN 835H (98.1% a.i.) at 100 and 300 mg/kg/day to pregnant rabbits during organogenesis was associated with treatment-related reduced maternal body weight gain and increased incidences of abnormal feces for the entire dosing period. The 300 mg/kg/day dosage also caused deaths and abortions, gastric trichobezoars, reduced food consumption values for the entire dosing period, and increased weight gain during the post-treatment period. The maternal NOAEL is 30 mg/kg/day and the maternal LOAEL is 100 mg/kg/day. The developmental LOAEL is 300 mg/kg/day based on increased incidences of supernumerary thoracic rib ossification sites in fetuses, a variation in fetal ossification that commonly occurs at maternally toxic dosages. There were no adverse effects on embryo-fetal viability, fetal body weight, sex, fetal malformations, and fetal external or soft tissue morphology at 300 mg/kg/day. The developmental NOAEL is 100 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following oral administration of the test substance, SAN 835H (98.1% a.i.) at 0, 30, 100, or 300 mg/kg/day to pregnant rabbits on days 6-19 of gestation, deaths occurred at all dose levels during the study (1, 2, 1, and 4 at the respective dose levels), however only 3 deaths at 300 mg/kg/day were treatment-related. Clinical signs of toxicity were abnormal feces (dried and/or no feces). Trichobezoars were observed as related gross pathological

changes. At the high-dose, there was also a significant (p 0.01), treatment-related increase in abortions (5/17 vs. 0/19 for controls). There were marginal decreases in body weight gain and food consumption with rebound during the post-treatment period. Neither of these effects was statistically significant at any observation period.

At 100 mg/kg/day, there was a minimal (but non significant) reduction in body weight gain, with no reduction in food consumption. There were differences in the incidence ratios for abnormal feces (soft or liquid feces, dried, or no feces) at 100 mg/kg/day compared to controls.

No treatment related effects were observed at 30 mg/kg/day.

Maternal NOAEL = 30 mg/kg/day
Maternal LOAEL = 100 mg/kg/day

2. DEVELOPMENTAL TOXICITY: Developmental effects characterized as significant increases (p 0.01) in the incidence of supernumerary thoracic rib pair ossification sites (2%) and related significant changes (p 0.05) in the average numbers of thoracic and lumbar vertebrae ossification sites(2% increase and 4% decrease, respectively) were noted for the 300 mg/kg/day treatment group.

   a. Deaths/Resorptions: The numbers of resorptions/dam and viable fetuses/dam for the treatment groups were not significantly different from the concurrent controls.

   b. Altered Growth: Fetal body weights were not significantly different from concurrent controls in either sex at any dose level.

   c. Developmental Variations: Skeletal variations were characterized as significantly increased (p 0.01) incidences of supernumerary thoracic rib pair ossification sites at 300 mg/kg/day (12.74 vs. 12.47 for controls). Consequently, thoracic vertebrae ossification sites were significantly (p 0.05) increased (12.79 vs. 12.54 for controls) and lumbar vertebrae ossification sites were significantly (p 0.05) decreased (6.20 vs. 6.45 for controls).

d. Malformations: There were no treatment-related developmental malformations noted at any dose level.

Developmental NOAEL = 100 mg/kg/day
Developmental LOAEL = 300 mg/kg/day

C. STUDY DEFICIENCIES Stability analyses for test doses were not performed. Because doses were mixed daily and other analytical data were obtained for dose concentration and mixing procedure, this deficiency is not likely to have affected the study outcome.