7-18-97

DATA EVALUATION RECORD

SAN 835 H TECHNICAL

Study Type: 83-4; Two-Generation Reproduction Study - Rats

Work Assignment No. 3-01Q (MRID 44170148)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat
OPPTS Number: 870.3800 OPP Guideline Number: §83-4

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

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

SYNONYMS: Not provided

CITATION: Eschbach, B. (1996). SAN 835 H Technical Two Generation Reproduction Study in Rats. Sandoz Agro LTD., Muttenz, Switzerland. Laboratory study number 550R (BS8896), November 6, 1996. MRID 44170148. Unpublished.

SPONSOR: Sandoz Agro LTD., Basel, Switzerland

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID 44170148) SAN 835 H, 98.1% a.i. was administered continuously in the diet to 26 Wistar rats/sex/dose at dose levels of 0, 500, 2,000 or 8,000 ppm in the diet (0, 27.3-42.2, 113.1-175.9, or 466.2-742.0 mg/kg/day). Exposure to P generation animals (26/sex) began at 8 weeks of age and lasted for 10 weeks prior to mating to produce F_1 pups. At weaning, F_1 pups were selected to become the parents of the F_2 generation and were given the same concentration test diets as their dam. F_1 animals were given test diets for 12 weeks prior to mating.

Parental toxicity was demonstrated at 2,000 ppm in P generation males as treatment-related decreases in body weight gains (8-16%, $p<0.05$), and in F_1 generation females as treatment-related increases in food consumption (8-9%, $p<0.01$) during the pre-mating interval. In the P males food consumption was increased 5-9% during the latter half of the study ($p<0.05$ or 0.01). In

the mid-dose F_1 males food consumption was increased 9% for one week during premating ($p<0.05$). Increased food consumption was also recorded for P (10%, $p<0.05$) and F_1 generation females (21%, $p<0.01$) during pregnancy. F_1 generation males had increased absolute (12%, $p<0.05$) and relative (12-16%, $p<0.01$ or 0.05) seminal vesicle weights.

At 8,000 ppm, treatment-related decreases were noted in mean body weights (4-13%, $p<0.01$ or 0.05) and body weight gains (14-21%, $p<0.01$ or 0.05) in P and F_1 generation males. Food consumption was increased (5-16%, $p<0.01$ or 0.05) in males of both generations. Body weights for high-dose females were decreased (5-12%, $p<0.01$ or 0.05) and body weight gains were decreased (10-18%, $p<0.01$ or 0.05). In addition, decreases were noted in mean body weights (5-12%, $p<0.01$ or 0.05) and body weight gains (13-16%, $p<0.01$ or 0.05) during gestation for both generations. Food consumption was increased in F_1 generation females (11-22%, $p<0.01$) during the pre-mating period and (11-28%, $p<0.01$) during pregnancy in both generations. Increases were noted in relative seminal vesicle weights (15-24%, $p<0.01$ or 0.05) in P generation males, and absolute (13%, $p<0.05$) and relative (19-31%, $p<0.01$) seminal vesicle weights in F_1 generation males.

No systemic toxicity was observed at 500 ppm.

The systemic LOAEL is 2,000 ppm (113.1-175.9 mg/kg/day) based on reduced body weight gain, increased food consumption, and increased seminal vesicle weights. The systemic NOAEL is 500 ppm (27.3-42.2 mg/kg/day).

Reproductive toxicity was characterized at 8,000 ppm as lower live birth ($p<0.05$) and viability indices, significantly increased ($p<0.01$) total pre-perinatal loss (28 vs 6 for the controls), decreased body weights (12-14%, $p<0.01$) on day 21 of lactation, decreased body weight gains (17-18%, $p<0.01$) on days 4-21 of lactation, a higher proportion of runts, and a higher percentage of offspring with no milk in the stomach.

No significant reproductive toxicity was observed at 2,000 or 500 ppm.

The reproductive LOAEL is 8,000 ppm (466.2-742.0 mg/kg/day) based on lower live birth and viability indices, total pre-perinatal loss, reduced body weights and body weight gain during lactation,

a higher proportion of runts, and a higher percentage of offspring with no milk in the stomach. The reproductive NOAEL is 2,000 ppm (113.1-175.9 mg/kg/day).

The reproductive study in the rat is classified acceptable and satisfies the guideline requirement for a 2-generation reproductive study (§83-4) in rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SAN 835 H
   Description: Technical, white powder
   Batch #: 5904-4
   Purity: 98.1% a.i.
   CAS #: Not provided
   Structure: Not provided

2. Vehicle: None

3. Test animals: Species: Rat
   Strain: Wistar
   Age at start of dosing: (P) 8 wks, (F_1) 8 wks
   Weight at start of dosing:
   (P) Males: 168-276 g, Females: 127-186 g
   (F_1) Males: 132-297 g, Females: 103-204 g
   Source: BRL Breeding Laboratories, Fullinsdorf, Switzerland
   Housing: Macrolon solid bottom plastic cages with sifted granular wood chips as bedding material; 1/cage, individually with litters during lactation
   Diet: KLIBA powdered diet no. 32-343-4, ad libitum
   Water: Tap water in plastic bottles, ad libitum
   Environmental conditions:
   Temperature: 21-25°C
   Humidity: 45-75%
   Air changes: Approximately 10-15/hour
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): 14-21 days,

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group. Females were examined daily and, if found to contain sperm or a copulation plug, they were removed from the male. If sperm or a copulatory plug were not found, the mating procedure was repeated for a maximum of 21 days. The P generation was allowed to produce two litters F_1a and F_1b. Sibling matings within the F_1 generation were avoided.

2. Study schedule: Starting at approximately 8 weeks of age, P generation animals were given test diets for 10 weeks before

they were mated. Upon weaning at 3 weeks of age, F_1a pups were selected to become parents of the F_2 generation and were given the same concentration test diets as their dam. F_1a animals were given test diets for 12 weeks prior to mating. P generation animals were mated 10 days after weaning the F_1a pups to produce the F_1b pups. Exposure of the test material to all animals was continuous in the diet throughout the study.

3. Animal assignment: P animals were allocated to treatment groups by a computer-generated cage distribution plan. One week before the start of treatment all animals were weighed and exchanged between groups when necessary to approximately equalize average group body weights. F_1 animals were selected randomly, avoiding brother/sister pairing. Group assignments are presented in Table 1.

Table 1. Animal assignment

Test Group	Dose in Diet [a] ppm	Animals/Group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	26	26	26	26
Low	500	26	26	26	26
Mid	2,000	26	26	26	26
High	8,000	26	26	26	26

a Diets were administered from the beginning of the study until sacrifice.

4. Dose selection rationale: Doses were selected from data obtained in a previously conducted pilot study (Sandoz study No. 483R). Information from the pilot study was not included with the current submission.

5. Dosage preparation and analysis

A 5% premix was prepared weekly by dilution of the powdered test substance with powdered diet. Dose formulations were prepared weekly by dilution of the premix with powdered

diet.

Stability and homogeneity of the test substance in the diet were evaluated prior to the current study. The analysis report was included with the current submission. In addition, homogeneity of the dose formulations from Week 1 of the study were analyzed and samples of diets were analyzed at 3-monthly intervals during the study for accuracy of mixing.

Results - The report of analyses performed prior to the current study on dose levels of 1,000, 5,000, 10,000, and 20,000 ppm indicated that the dose formulations were stable for up to two weeks at room temperature (86-102% of day 0 concentrations) and that homogeneity results were 83.5-117.0% of nominal concentrations. Analyses during the current study confirmed the homogeneity of the dose formulations (97.2-120.8% of nominal) and that actual concentrations of the test article during the study ranged from 85.2-120.8.%, 94.5-116.4%, and 97.5-112.4% of nominal concentrations at the 500, 2,000 and 8,000 ppm levels, respectively.

C. OBSERVATIONS

1. Parental animals: Animals were observed 1-2 times daily for mortality and clinical signs of toxicity. Detailed clinical examinations were performed weekly. Body weights of males were recorded weekly throughout the study. Females were weighed weekly prior to mating and on days 0, 7, 14 and 20 post coitum. Dams with litters were weighed on days 0, 7, 14, and 21 post partum. Food consumption values were recorded weekly at the same intervals used for recording body weights, except during cohabitation. Relative food consumption ratios and intake of test substance were calculated on a weekly basis. Vaginal smears were taken daily during the last 20 days of the premating period and for at least 10 days before the second mating period. Vaginal smears were also taken from all sperm negative females during the entire second mating period.

2. Litter observations: The litter observations are summarized in Table 2 below. In addition, the dams and pups were observed daily for behavioral abnormalities in nesting and

nursing. The age of vaginal opening and preputial separation were determined for F_1b pups (one animal/sex/litter).

Table 2. F_1/F_2 litter observations.[a]

Observation	Time of observation (lactation day)				
	Day 0	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Pup weight	X	X	X	X	X
Gross anomalies	X	X	X	X	X
Number of dead pups	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X

a Data extracted from the study report pages 459-556.

Litters were not standardized. Dead pups were examined externally and internally for gross abnormalities and F_1a pups not selected for mating were sacrificed and preserved.

3. Postmortem observations:

1) Parental animals: All parental animals were sacrificed when they were no longer necessary for the assessment of reproductive effects. Terminally sacrificed animals and those that died during the study were examined macroscopically for any structural abnormalities or pathological changes. Sperm motility was videorecorded at necropsy, the left testis was stored at -70 C for sperm counts, and slides were prepared for sperm morphology evaluation. However, sperm parameters were not investigated because fertility parameters were unaffected by treatment. Implantation sites were counted and assigned to the corresponding mating, if possible.

Gross necropsy consisted of complete macroscopic examinations, with special attention to the reproductive organs.

The following tissues (X) were collected from all parental animals; the (XX) organs were weighed:

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X	Pituitary Gland	X	Mammary gland
XX	Ovaries	XX	Epididymides
XX	Uterus (w/cervix)	XX	Prostate
X	Vagina	XX	Testes
XX	Seminal vesicles	(with coagulating gland)	
X	Lesions	XX	Brain
XX	Liver	XX	Kidney
XX	Adrenal gland	XX	Spleen
XX	Thymus		

In addition, the vagina, uterus with cervix, ovaries with oviducts, testis, intact epidydmis, seminal vesicles, prostate, coagulating gland, mammary gland, and the livers, pituitary and any grossly abnormal tissue of both sexes of both generations in the control and high dose groups were examined microscopically.

2) Offspring: On Day 21 postpartum 2 pups/litter (1 male, 1 female) were sacrificed and necropsied. Ovaries, testes, brain, liver, kidneys, adrenal glands, spleen and thymus were weighed. The weighed organs, the oviduct, and grossly abnormal tissue were preserved for histopathology. For the F_1a generation the whole body was fixed. The remaining pups were sacrificed, necropsied, and checked for abnormalities.

D. DATA ANALYSIS

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

2. Indices:

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

mating index = (# of females for which mating was confirmed/# of females paired) x 100
fertility (pregnancy) index = (# of females pregnant/# of females mated) x 100
gestation index = (# of females that delivered litters containing viable pups/# of pregnant females) x 100

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Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

pup livebirth index = (# of pups born alive/# of pups delivered) x 100
pup viability index = (# of pups alive on day 4/# of pups born live) x 100
pup lactation index = (# of pups alive on day 21/# of pups alive on day 4) x 100

3. Historical control data: No historical control data were provided for this study.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: No treatment-related clinical signs or increases in mortality were noted in the P or F_1 adults at any dose level.

2. Body weight, body weight gain and food consumption: Selected body weight, weight gain and food consumption data from the study are summarized in Tables 3a and 3b. Food consumption data are presented as g/kg/day; absolute food consumption values as g/animal/day were not given in the submission. During the pre-mating interval, treatment-related decreases were noted in mean body weights and body weight gains in the high-dose P and F_1 males and females. This trend continued in these females during pregnancy. Treatment-related reduced body weight gains were also noted in the mid-dose P males. Increased food consumption was noted in mid- and high-dose P males and in high-dose F_1 males. Mid- and high-dose P females had increased food consumption during pregnancy while mid- and high-dose F_1 females had increased food consumption during the pre-mating interval and during pregnancy.

For the high-dose P and F_1 males, decreases were noted in mean body weights (4-8%, $p<0.05$ or $p<0.01$ and 12-13%, $p<0.01$, respectively) during weeks 1-10. For the high-dose females, mean body weights were decreased in the P

generation (5-6%, weeks 7-10, $p<0.05$) and in the F_1 generation (11-12%, weeks 0-12, $p<0.01$). Body weight gains were decreased in high-dose P males (17-21%, weeks 0-5 and 5-10, $p<0.01$), F_1 males (14%, weeks 0-5, $p<0.05$), P females (18%, weeks 0-5 and 0-10, $p<0.01$), and F_1 females (10 and 11%, weeks 0-5 and 0-12, $p<0.05$). Body weight gains were also decreased in mid-dose P males (8%, weeks 0-32 and 16%, weeks 5-10, $p<0.05$), although mean body weights were comparable to the controls at this dose level.

Food consumption was increased for high-dose P males (5-16%, weeks 0-32, 5-7%, weeks 3-4, and 5-10, and 6-16%, weeks 13-32, $p<0.05$ or 0.01) and F_1 males (7-16%, weeks 0-7, and 8-12, $p<0.01$). Food consumption was increased in the high-dose F_1 females (11-22%, weeks 0-3, 5-7, and 9-12, $p<0.01$) and in the mid-dose F_1 females (8-9%, weeks 5-7, $p<0.05$). In the mid-dose P males food consumption was increased 5-9% during the latter half of the study (weeks 13-15, 16-17, 19-21, and 24-30, $p<0.05$ or 0.01). In the mid-dose F_1 males food consumption was increased for only one week during premating (9%, week 11-12, $p<0.05$). There were no treatment-related changes in food consumption noted for the P generation females at any dose level during the pre-mating interval.

For the P and F_1 low-dose males and females, body weights, body weight gains, and food consumption were comparable to the controls.

Table 3a. Body weight, body weight gain and food consumption values - P generation pre-mating/mating (males) and pre-mating (females).[a]

Observations/study week	Dose Group (ppm)			
	0	500	2,000	8,000
P Generation Males - Pre-mating/mating				
Mean body weight (g)/Weeks 1-10@	423.4	416.4	404.0	389.6**
Mean body weight (g)/Weeks 0-32@	442.5	438.6	424.6	407.0
Mean weight gain (g)/Weeks 0-5	124.4	121.1	117.4	103.5**
Mean weight gain (g)/Weeks 5-10	58.9	56.6	49.7*	46.8**
Mean weight gain (g)/Weeks 0-32	296.5	288.7	272.1*	242.9**
Mean food consumption (g/kg/day)/Weeks 0-32	54.6	54.7	56.5*	58.3*
P Generation Females - Pre-mating				
Mean body weight (g)/Weeks 0-10@	206.9	203.5	202.3	197.9
Mean body weight (g)/Weeks 7-10@	230.7	228.0	224.8	218.0*
Mean weight gain (g)/Weeks 0-5	54.7	53.8	51.0	45.0**
Mean weight gain (g)/Weeks 0-10	79.5	80.7	74.4	65.5**
Mean food consumption (g/kg/day)/Weeks 0-10	83.2	84.5	84.6	83.7

a Data extracted from study report pages 22-24, 50-53, 56-57, 77-79, and 82-84.
* Statistically different from the controls, $p<0.05$
** Statistically different from the controls, $p<0.01$
@ Calculated by reviewer

Table 3b. Body weight, body weight gain and food consumption values - F_1 generation pre-mating.[a]

Observations/study week	Dose Group (ppm)			
	0	500	2,000	8,000
F_1 Generation Males - Pre-mating				
Mean body weight (g)/Weeks 1-10@	357.3	357.7	343.9	313.5**
Mean body weight (g)/Week 0-20	396.1	397.7	380.6	347.4**
Mean weight gain (g)/Week 0-5	116.9	114.0	110.4	100.7*
Mean weight gain (g)/Week 0-20	230.5	233.4	217.1	199.8**
Mean food consumption (g/kg/day)/Week 0-12@	66.3	67.6	68.8	74.6**
Mean food consumption (g/kg/day)/Week 0-20	61.2	62.1	63.2	69.0**
F_1 Generation Females - Pre-mating				
Mean body weight (g)/Weeks 0-12@	216.3	212.5	207.5	190.6**
Mean body weight gain (g)/Weeks 0-5	53.0	54.2	51.6	47.1*
Mean weight gain (g)/Weeks 0-12	82.6	83.5	78.7	74.2*
Mean food consumption (g/kg/day)/Weeks 5-7@	77.9	78.0	84.5*	88.0**
Mean food consumption (g/kg/day)/Weeks 0-12	82.4	84.0	87.9*	92.7**

a Data extracted from the study report pages 22-24, 54-55, 58-59, 61, and 80.
* Statistically different from the controls, $p<0.05$.
** Statistically different from the controls, $p<0.01$.
@ Calculated by reviewer.

At 8,000 ppm, treatment-related decreases were noted in mean body weights (5-12%, $p<0.05$ or <0.01) and body weight gains (13-16%, $p<0.05$ or $p<0.01$) during gestation for both generations. During the first two weeks of lactation, body weights were decreased (6-11%, $p<0.05$ or $p<0.01$), and throughout lactation body weight gains were increased ($p<0.01$) for both generations. Food consumption was increased during gestation (11-28%, $p<0.01$) for both generations. During lactation, food consumption was comparable to the controls for both generations.

At 2,000 ppm, mean body weights and body weight gains during gestation and lactation, and food consumption during lactation were comparable to controls. Food consumption was increased during the last week of gestation (10%, $p<0.05$) for the P generation and during the first week of gestation (21%, $p<0.01$) for the F_1 generation.

3. Test Substance Intake: Based on food consumption and the nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the entire study (males) and pre-mating period (females) for the P and F_1 generations are presented in Table 4.

Table 4. Test substance intake ranges (means mg/rat/day). [a]

Male			Female		
500 ppm	2,000 ppm	8,000 ppm	500 ppm	2,000 ppm	8,000 ppm
P Generation					
27.3	113.1	466.2	42.2	169.2	669.6
F_1 Generation					
31.1	126.4	552.2	42.0	175.9	742.0

a Data extracted from study report page 24.

4. Reproductive function:

a. Estrous cycle length and periodicity: Vaginal smear data were not included in this submission. However, there were no indications of treatment-related effects on these parameters during the study.

b. Sperm measures: Sperm motility, sperm count, and morphology data were not included with this submission. However, there were no indications of treatment-related male fertility abnormalities during the study.

c. Sexual maturation: Individual data pertaining to vaginal opening and preputial separation in the F_1b litters were included with this submission. There were no biologically relevant effects.

5. Reproductive performance: The pre-coital interval was significantly shorter at 8,000 ppm in the second P generation mating period. This finding is considered incidental, because pre-coital intervals for all other matings were comparable to the controls at the high dose. There were no treatment-related effects noted in mating, fertility, and gestation performance of the P or F_1 adults. Results for the parental animals are summarized from the report in Table 5.

Table 5. Reproductive performance.[a]

Observation (units)	Dose Group (ppm)			
	0	500	2,000	8,000
P Generation - Litter F_1				
Mating Index (%)	100	100	100	100
Fertility (Pregnancy) Index (%)[b]	100	96	100, 92	100, 96
Gestation Index (%)[b]	100	96	92, 100	96, 100
Pre-coital Interval (days)[b]	2.5, 3.0	2.7, 2.4	2.9, 2.4	2.7, 1.8*
Gestation Length (days)[b]	21.8, 21.9	22.0, 21.9	21.9, 22.0	21.8, 21.7
Litters (numbers)[b]	26, 25	24, 22	24, 22	25, 23
F_1 Generation - Litter F_2				
Mating Index (%)	96	100	100	100
Fertility (Pregnancy) Index (%)	92	100	100	100
Gestation Index (%)	100	100	100	96
Pre-coital Interval (days)	2.7	2.7	2.6	2.2
Gestation Length (days)	22.0	21.8	22.0	22.0
Litters (numbers)	23	26	26	25

a Data extracted from the study report pages 25-26, 117, 120, and 123.
b Values for first and second matings presented when they differ.
* Statistically different from the controls, $p<0.05$

5. Parental postmortem results

a) Organ weights: Organ weight data are presented in Table 6. At 8,000 ppm, increases in relative (to body and brain) seminal vesicle weights were observed in the P generation (15-24%, $p<0.05$, $p<0.01$). At 2,000 and 8,000 ppm, increases in absolute and relative seminal vesicle weights were observed in F_1 generation males (12-31%, $p<0.05$, $p<0.01$). There were no correlated histopathologic changes in these organs. Since this finding was observed in both generations and the severity increased in the second generation, the increase in seminal vesicle weight is considered treatment-related.

Table 6. Organ weight data.[a]

Observation (units)	Dose Group (ppm)			
	0	500	2,000	8,000
P Generation - Litter F_1				
Absolute seminal vesicle weight (g)	1.56	1.47	1.63	1.74
Relative (to body) seminal vesicle weight (g)	0.29	0.28	0.32	0.36**
Relative (to brain) seminal vesicle weight (g)	73.4	70.5	77.5	84.3*
F_1 Generation - Litter F_2				
Absolute seminal vesicle weight (g)	1.51	1.53	1.69*	1.70*
Relative (to body) seminal vesicle weight (g)	0.32	0.32	0.37**	0.42**
Relative (to brain) seminal vesicle weight (g)	73.9	74.0	82.8*	87.7**

a Data extracted from the study report page 28.
* Statistically different from the controls, $p<0.05$
** Statistically different from the controls, $p<0.01$

b) Pathology

1) Macroscopic examination: There were no treatment-related macroscopic findings for either the P or F_1 parental generations at any dose level.

2) Microscopic examination: There were no treatment-related histopathologic effects noted in either generation.

B. OFFSPRING

1. Viability and clinical signs: No clinical signs were noted in either the F_1 or F_2 generation pups. Lower live birth ($p<0.05$) and viability indices were noted in the F_2 generation at 8,000 ppm (one dam delivered all pups stillborn). The total pre-perinatal (days 0-4 of lactation) loss was significantly increased in the F_2 generation at 8,000 ppm (28 vs 6 for the controls, $p<0.01$). There was no effect on lactation index. These differences are considered treatment-related. In the F_1b generation, a significantly decreased viability index was noted at 500 ppm. However, similar findings were not observed at higher dose levels. Mean litter size and sex ratios were similar to controls in both generations. Mean litter size and viability results from pups during lactation are summarized from the report in Tables 7a and 7b.

Table 7a. Mean litter size and viability.[a]

Observation (units)	Dose Group (ppm)			
	0	500	2,000	8,000
	F_1a, F_1b Generations			
Mean litter size (number)				
Day 0	11.3, 10.7	9.9, 8.6	11.5, 11.0	11.0, 9.8
Day 4	11.2, 10.4	9.9, 7.9	11.4, 11.2	10.7, 10.0
Day 7	11.0, 10.3	9.7, 7.8	11.4, 11.1	10.6, 10.0
Day 14	11.0, 10.3	9.3, 7.6*	11.3, 11.1	10.6, 10.0
Day 21	11.0, 10.3	9.3, 7.6*	11.3, 11.1	10.6, 10.0
Live pups (number)@				
Day 0	293, 268	238, 190	277, 241	274, 226
Day 4	290, 260	228, 174	274, 235	267, 220
Day 7	286, 258	223, 171	274, 234	264, 220
Day 14	285, 258	213, 168	272, 234	264, 220
Day 21	285, 257	213, 168	272, 233	264, 220

Deaths (number)				
Days 0-4	3, 8	10*,15*	3, 6	7, 6
Days 4-21	5, 3	15**, 6	2, 2	3, 0
Survival indices				
Live birth index (%)	96, 97	96, 95	98, 94	97, 99
Viability index (%)	99, 97	96, 92	99, 98	97, 97
Lactation index (%)	98, 99	93, 97	99, 100	99, 100

a Data extracted from the study report pages 117-122.
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
@ Calculated by reviewer

Table 7b. Mean litter size and viability.[a]

Observation (units)	Dose Group (ppm)			
	0	500	2,000	8,000
F_2 Generation				
Mean litter size (number)				
Day 0	11.0	11.6	10.9	9.3
Day 4	11.2	11.4	10.7	9.3
Day 7	11.2	11.1	10.6	9.3
Day 14	11.1	11.0	10.5	9.0
Day 21	11.1	11.0	10.5	9.0
Live pups (number)				
Day 0	253	302	282	242
Day 4	247	295	277	214
Day 7	246	289	275	214
Day 14	245	286	273	208
Day 21	245	285	272	208
Deaths (number)				
Days 0-4	6	7	5	28**
Days 4-21	2	10	5	6
Survival indices				
Live birth index (%)	98	99	98	93*
Viability index (%)	98	98	98	88
Lactation index (%)	99	97	98	97

a Data extracted from the study report pages 123-125.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

2. Body weight and weight gain: Treatment-related decreases were noted in pup body weights and body weight gains at 8,000 ppm in both sexes of the F_1a generation, and in body weight gains in males of the F_1b generation.

At 8,000 ppm, the F_1a generation pups had decreased body weights on lactation day 21: 14% ($p<0.01$) in males and 12% ($p<0.01$) in females. Body weight gains were decreased 18% and 17% from lactation days 4-21 for males and females, respectively. For the F_1b generation at 8,000 ppm, body weight gains in males were decreased 16% ($p<0.01$) on

lactation days 14-21. Body weights and body weight gains were comparable to the controls for the F_2 pups.

Mean pup body weight and weight gain data are presented in Table 8.

Table 8. Mean pup body weights and body weight gains (g).[a]

Day of lactation	Dose Group (ppm)			
	0	500	2,000	8,000
F_1a, F_1b Generations				
Males				
Body weights				
Day 0	5.9, 6.1	6.0, 6.3	6.0, 6.0	5.7, 5.9
Day 4	9.6, 10.0	10.1, 10.6	9.7, 10.0	9.1, 9.6
Day 7	14.0, 14.9	14.5, 16.1	14.0, 14.8	13.1, 14.0
Day 14	25.9, 27.5	27.0, 30.1	25.6, 27.8	23.4, 25.8
Day 21	41.4, 43.5	43.7, 46.0	40.1, 42.6	35.7**, 39.4
Weight gain, Days 0-21	35.5, 37.5	37.7, 39.7	34.1, 36.6	30.0**, 33.5
Females				
Body weights				
Day 0	5.6, 5.8	5.7, 6.0	5.7, 5.7	5.5, 5.6
Day 4	9.1, 9.5	9.7, 10.2	9.3, 9.6	8.7, 9.2
Day 7	13.4, 14.4	13.8, 15.7	13.6, 14.0	12.8, 13.4
Day 14	25.2, 27.1	25.8, 29.8	24.8, 26.8	23.0, 24.4
Day 21	40.3, 41.8	41.6, 45.4	39.2, 41.5	35.3**, 38.2
Weight gain, Days 0-21	34.7, 36.0	35.9, 39.4	33.4, 35.8	29.8**, 32.5
F_2 Generation				
Males				
Body weights				
Day 0	5.9	6.0	6.2	5.9
Day 4	9.6	9.7	10.3	10.0
Day 7	14.0	14.1	14.9	14.2
Day 14	25.6	26.3	27.4	26.4
Day 21	39.1	40.4	41.6	40.3
Weight gain, Days 0-21	33.3	34.4	35.4	34.3
Females				
Body weights				
Day 0	5.8	5.6	5.9	5.5
Day 4	9.4	9.1	10.0	9.5
Day 7	13.8	13.5	14.8	13.7
Day 14	25.4	25.6	27.4	25.8
Day 21	38.6	39.4	41.4	39.3

a Data extracted from the study report pages 127-141.
** Statistically different from the controls, $p<0.01$

3. Offspring postmortem results:

a) Organ weights: At 8,000 ppm, decreased absolute (19%, $p<0.01$) and relative (to body) (10%, $p<0.05$) thymus weights were noted in F_1b generation males. Decreased absolute (14%, $p<0.05$) and relative (to body) (6%, $p<0.05$) kidney weights were noted in F_1b generation females. Decreased relative (to body) (5%, $p<0.05$) kidney weights were also noted in F_2 generation females. These organ weight changes may be attributed to treatment with SAN 835 H.

b) Pathology

1) Macroscopic examination: At 8,000 ppm, a higher proportion of runts was observed in the F_1a (8% vs 0.4% for controls, $p<0.01$) and F_1b (14% vs 0% for controls, $p<0.01$) generations. In the F_2 generation a higher percentage of offspring were found to have no milk in the stomach (8.4% vs 1.2% for controls, $p<0.01$).

2) Microscopic examination: Histopathology was not done on either the F_1 or F_2 pups at any dose level.

III. DISCUSSION

A. INVESTIGATOR CONCLUSIONS: The study author concluded that a dose level of 8,000 ppm resulted in lower weight gains and higher food consumption in both sexes during the premating periods, and in pregnant dams. Higher absolute and relative weights of seminal vesicles in F_1 parental males were not correlated with microscopic findings. The F_1 generation pups had lower weight gains during the lactation period, a higher proportion of runts at 21 days post-partum, and lower absolute and relative kidney (females) and thymus (males) weights at

necropsy. The F_2 generation had higher pre-perinatal losses, lower live birth and viability indices, and a higher percentage of pups with no milk in the stomach at necropsy. At 2,000 ppm, lower total weight gains and higher food consumption was noted in P parental males. Higher food consumption was also noted in F_1 females during the pre-mating period, and in pregnant P and F_1 dams. Higher absolute and relative weights of seminal vesicles in F_1 parental males were not correlated with microscopic findings. There were no treatment-related effects noted on the offspring at 2000 ppm. There were no treatment-related effects noted at 500 ppm in the reproductive or systemic parameters. On the basis of the results obtained in this study, the NOAEL for SAN 835 H technical was 2,000 ppm (approximately 90-500 mg/kg body weight/day).

B. REVIEWER'S DISCUSSION: Over the course of the 2-generation reproduction study, SAN 835 H was administered continuously in the diet to Wistar rats at dose levels of 0, 500, 2,000 or 8,000 ppm (0, 27.3-42.2, 113.1-175.9 or 466.2-742.0 mg/kg/day). Exposure to P animals (26/sex) began at 8 weeks of age and lasted for 10 weeks prior to mating to produce F_1 pups. At weaning, F_1 pups were selected to become the parents of the F_2 generation and were given the same concentration test diets as their dam. F_1 animals were given test diets for 12 weeks prior to mating.

1. Parental Toxicity Parental toxicity was demonstrated at 2,000 ppm in P generation males as treatment-related decreases in body weight gains (8-16%, weeks 5-12, 0-32; $p<0.05$), and in F_1 generation females as treatment-related increases in food consumption (8-9%, weeks 5-7, $p<0.01$) during the pre-mating interval. In the P males food consumption was increased 5-9% during the latter half of the study (weeks 13-15, 16-17, 19-21, and 24-30, $p<0.01$ or 0.05). In the mid-dose F_1 males food consumption was increased for only one week during premating (9%, week 11-12, $p<0.05$). Increased food consumption was also recorded for the P (10%, $p<0.05$) and F_1 generations (21%, $p<0.01$) during pregnancy. Increased absolute (12%, $p<0.05$) and relative (12-16%, $p<0.01$ or 0.05) seminal vesicle weights were noted in F_1 generation males.

At 8,000 ppm, treatment-related decreases were noted in mean body weights (4-13%, $p<0.01$ or 0.05) and body weight gains (14-21%, $p<0.01$ or 0.05) in P and F_1 generation males. Food consumption was increased (5-16%, $p<0.01$ or 0.05) in males of both generations. Body weights for high-dose females of both generations were decreased (5-12%, $p<0.01$ or 0.05) as were body weight gains (10-18%, $p<0.01$ or 0.05). In addition, decreases were noted in mean body weights (5-12%, $p<0.01$ or 0.05) and body weight gains (13-16%, $p<0.01$ or 0.05) during gestation for both generations. Food consumption was increased in F_1 generation females (11-22%, $p<0.01$) during the pre-mating period and increased (11-28%, $p<0.01$) during pregnancy in both generations. Increased relative seminal vesicle weights (15-24%, $p<0.01$ or 0.05) in P generation males, and increased absolute (13%, $p<0.05$) and relative (19-31%, $p<0.01$) seminal vesicle weights in F_1 generation males were noted.

No systemic toxicity was observed at 500 ppm.

The systemic LOAEL is 2,000 ppm (113.1-175.9 mg/kg/day) based on reduced body weight gain, increased food consumption, and increased seminal vesicle weights. The systemic NOAEL is 500 ppm (27.3-42.2 mg/kg/day).

2. Reproductive Toxicity. The F_2 generation pups dosed at 8,000 ppm had lower live birth ($p<0.05$) and viability indices and the total pre-perinatal loss (28 vs 6 for the controls) was significantly increased ($p<0.01$). Body weights were decreased (12-14%, $p<0.01$) in F_1a generation males and females on day 21 of lactation, and body weight gains were decreased (17-18%, $p<0.01$) on days 4-21 of lactation in both F_1a sexes. The F_1a and F_1b generations had a higher proportion of runts and the F_2 generation had a higher percentage of offspring with no milk in the stomach.

No reproductive toxicity was observed at 2,000 or 500 ppm.

The reproductive LOAEL is 8,000 ppm (466.2-742.0 mg/kg/day) based on lower live birth and viability indices, total pre-perinatal loss, reduced body weights and body weight gain during lactation, a higher proportion of runts, and a higher

percentage of offspring with no milk in the stomach. The reproductive NOAEL is 2,000 ppm (113.1-175.9 mg/kg/day).

C. STUDY DEFICIENCIES: Deficiencies with the submitted 2-generation reproduction study in the rat included a lack of historical control data. However, as it was possible to determine a systemic and reproductive LOAEL from the submitted data, these deficiencies do not affect the acceptability of the study.

DER #5

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