Table. Clinical findings in post weaning F1 males

TABLE 64 (DETAILED PHYSICAL EXAMINATIONS) - F1 GENERATION
PROJECT NO.:MIL-370011A — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE 1
SPONSOR:SCHERING-PLOUGH — SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS
SPONSOR NO.:96386

----- M A L E -----

TABLE RANGE:	11-03-99 TO 01-10-00			
GROUP:	1	2	3	4
NORMAL				
-NO SIGNIFICANT CLINICAL OBSERVATIONS	238/25	229/25	241/25	216/25
DISPOSITION				
-FOUND DEAD	0/ 0	1/ 1	0/ 0	0/ 0
-SCHEDULED EUTHANASIA	25/25	24/24	25/25	25/25
BODY/INTEGUMENT				
-HAIR LOSS VENTRAL NECK	2/ 1	1/ 1	0/ 0	0/ 0
-DRIED RED MATERIAL RIGHT EYE	1/ 1	0/ 0	0/ 0	0/ 0
-HAIR LOSS RIGHT FORELIMB	20/ 2	25/ 3	12/ 2	19/ 3
-HAIR LOSS LEFT FORELIMB	11/ 2	20/ 4	15/ 4	12/ 3
-SCABBING FACIAL AREA	0/ 0	0/ 0	0/ 0	1/ 1
-SCABBING LEFT FORELIMB	0/ 0	1/ 1	1/ 1	0/ 0
-SCABBING RIGHT FORELIMB	0/ 0	0/ 0	4/ 1	0/ 0
-APPARENT UMBILICAL HERNIA	0/ 0	2/ 1	0/ 0	0/ 0
-DRIED YELLOW MATTING UROGENITAL AREA	0/ 0	0/ 0	0/ 0	1/ 1
EYES/EARS/NOSE				
-DRIED RED MATERIAL AROUND RIGHT EYE	3/ 1	1/ 1	0/ 0	2/ 2
-DRIED RED MATERIAL AROUND NOSE	3/ 3	6/ 4	1/ 1	5/ 4
-DRIED RED MATERIAL AROUND LEFT EYE	3/ 2	0/ 0	2/ 2	7/ 2

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Table. Clinical findings in post weaning in F1 females

TABLE 64 (DETAILED PHYSICAL EXAMINATIONS) - F1 GENERATION
PROJECT NO.:MIL-370011A — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE 3
SPONSOR:SCHERING-PLOUGH — SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS
SPONSOR NO.:96386

----- F E M A L E -----

TABLE RANGE:	11-03-99 TO 01-10-00			
GROUP:	1	2	3	4
NORMAL				
-NO SIGNIFICANT CLINICAL OBSERVATIONS	342/25	285/25	266/25	294/25
DISPOSITION				
-SENT TO LAB FOR SCHEDULED NECROPSY; LACTATION DAY 4	24/24	22/22	23/23	25/25
-TERMINAL NECROPSY; POST-MATING DAY 29	0/ 0	0/ 0	2/ 2	0/ 0
-TERMINAL NECROPSY; POST-MATING DAY 28	0/ 0	1/ 1	0/ 0	0/ 0
-SENT TO LAB FOR SCHEDULED NECROPSY; POST-COHABITATION DAY 25	1/ 1	2/ 2	0/ 0	0/ 0
BODY/INTEGUMENT				
-HAIR LOSS LEFT LATERAL THORACIC AREA	0/ 0	0/ 0	0/ 0	1/ 1
-HAIR LOSS VENTRAL NECK	0/ 0	0/ 0	1/ 1	1/ 1
-HAIR LOSS RIGHT FORELIMB	14/ 4	41/ 7	55/13	53/ 8
-HAIR LOSS LEFT FORELIMB	17/ 4	46/ 8	63/11	56/ 8
-SCABBING FACIAL AREA	0/ 0	2/ 2	0/ 0	0/ 0
-HAIR LOSS RIGHT HINDLIMB	0/ 0	13/ 3	1/ 1	4/ 1
-FIRM MOVEABLE MASS (5MM X 5MM X 5MM); RIGHT AXILLARY AREA	0/ 0	1/ 1	0/ 0	0/ 0
-SCABBING LEFT FORELIMB	2/ 1	4/ 2	0/ 0	1/ 1
-SCABBING RIGHT FORELIMB	3/ 2	4/ 2	0/ 0	1/ 1

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Estrous cycles: The mean length of estrus (4.1, 4.9, 5.1, 4.1 days) was not significantly different between groups.

Reproductive performance: No effects on mating indices, fertility indices and the number of days between pairing and coitus were observed. At low/mid doses there was a slight increase in animals that failed to deliver (1, 3, 2, 0 at 0, 100, 300, 1000 mg/kg/day failed to deliver) and these were euthanized on post mating day 25. All of these were internally normal and were nongravid, see Tables A-C.

Table A. reproductive performance in F1 female rats

TABLE 67 - F1 GENERATION
PROJECT NO.:WIL-370011A — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS — PAGE
SPONSOR:SCHERING-PLOUGH — SUMMARY OF REPRODUCTIVE PERFORMANCE
SPONSOR NO.:96386

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	25		25		25		25	
FEMALES THAT DIED DURING STUDY	0-A		0		0		0	
FEMALES ALLOWED TO DELIVER	25		25		25		25	
NONGRAVID	1	4.0	3	12.0	2	8.0	0	0.0
GRAVID	24	96.0	22	88.0	23	92.0	25	100.0
DELIVERED	24	100.0	22	100.0	23	100.0	25	100.0
FEMALES WITH TOTAL LITTER LOSS	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES WITH VIABLE PUPS	24	100.0	22	100.0	23	100.0	25	100.0
FEMALES WITH EVIDENCE OF MATING	23	92.0	21	84.0	23	92.0	25	100.0
NUMBER THAT DELIVERED	23	100.0	20	95.2	21	91.3	25	100.0
NUMBER THAT DID NOT DELIVER	0	0.0	1	4.8	2	8.7	0	0.0
FEMALES WITH NO EVIDENCE OF MATING	2	8.0	4	16.0	2	8.0	0	0.0
NUMBER THAT DELIVERED	1	50.0	2	50.0	2	100.0	0	0.0
NUMBER THAT DID NOT DELIVER	1	50.0	2	50.0	0	0.0	0	0.0
TOTAL FEMALES GRAVID	24	96.0	22	88.0	23	92.0	25	100.0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY
NOTE: POSITIVE EVIDENCE OF MATING DURING THE BREEDING PERIOD WAS CONFIRMED BY THE PRESENCE OF SPERM IN A VAGINAL SMEAR OR A COPULATORY PLUG.
A = ANIMAL NO. 25843-14 IN THE CONTROL GROUP WAS FOUND DEAD DURING STUDY WEEK 6 AND WAS REPLACED WITH A FEMALE FROM ANOTHER LITTER.

Table B. reproductive performance in F1 male rats

TABLE 67 - F1 GENERATION
PROJECT NO.:WIL-370011A — PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SPONSOR:SCHERING-PLOUGH — SUMMARY OF REPRODUCTIVE PERFORMANCE
SPONSOR NO.:96386

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
MALES ON STUDY	25		25		25		25	
MALES THAT DIED DURING STUDY	0		0		0		0	
MALES WITH EVIDENCE OF MATING	23	92.0	21	84.0	22	88.0	24	96.0
NO. THAT SIRED A LITTER	23	100.0	20	95.2	20	90.9	24	100.0
NO. THAT DID NOT SIRE A LITTER	0	0.0	1	4.8	2	9.1	0	0.0
MALES WITH NO EVIDENCE OF MATING	2	8.0	4	16.0	3	12.0	1	4.0
NO. THAT SIRED A LITTER	1	50.0	2	50.0	2	66.7	0	0.0
NO. THAT DID NOT SIRE A LITTER	1	50.0	2	50.0	1	33.3	1	100.0
MALES THAT SIRED MORE THAN ONE LITTER	0	0.0	0	0.0	1	4.8	1	4.8

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY
NOTE: MALES WERE CONSIDERED TO HAVE SIRED A LITTER IF THE PAIRED FEMALE WAS GRAVID, REGARDLESS OF DELIVERY STATUS
NOTE: POSITIVE EVIDENCE OF MATING DURING THE BREEDING PERIOD WAS CONFIRMED BY THE PRESENCE OF SPERM IN A VAGINAL SMEAR OR A COPULATORY PLUG.

Table C. Male and female fertility and mating indices in F1 generation rats

TABLE 67 - F1 GENERATION

PROJECT NO.:WIL-370011A
SPONSOR:SCHERING-PLOUGH
SPONSOR NO.:96386

PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SUMMARY OF REPRODUCTIVE PERFORMANCE

PAGE

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
MALE MATING INDEX	24/25	96.0	23/25	92.0	24/25	96.0	24/25	96.0
FEMALE MATING INDEX	24/25	96.0	23/25	92.0	25/25	100.0	25/25	100.0
MALE FERTILITY INDEX	24/24	100.0	22/23	95.7	22/24	91.7	24/24	100.0
FEMALE FERTILITY INDEX	24/24	100.0	22/23	95.7	23/25	92.0	25/25	100.0
MEAN PRE-COITAL INTERVALS (DAYS)	2.9	NA	3.4	NA	3.8	NA	3.7	NA
S.D.	1.18	NA	3.19	NA	3.41	NA	2.69	NA
N	23		21		23		25	

$$\text{MALE (FEMALE) MATING INDEX (\%)} = \frac{\text{NO. OF MALES (FEMALES) WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}}{\text{TOTAL NO. OF MALES (FEMALES) USED FOR MATING}} \times 100$$

$$\text{MALE FERTILITY INDEX (\%)} = \frac{\text{NO. OF MALES SIRING A LITTER}}{\text{TOTAL NO. OF MALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}} \times 100$$

$$\text{FEMALE FERTILITY INDEX (\%)} = \frac{\text{NO. OF FEMALES WITH CONFIRMED PREGNANCY}}{\text{TOTAL NO. OF FEMALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}} \times 100$$

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY
NOTE: MALES WERE CONSIDERED TO HAVE SIRED A LITTER IF THE PAIRED FEMALE WAS GRAVID, REGARDLESS OF DELIVERY STATUS
PRE-COITAL INTERVALS NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING DUNNETT'S TEST
MATING AND FERTILITY INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST
NA = NOT APPLICABLE

Body weights in F1 animals: Mean body weights or weigh gains were not effected up to day 70 in males or females. Sponsor mentions that BW gains at 300-1000 mg were transiently increased (19 g in both groups) during week 20-21 when compared to control (12 g), but this reviewer was unable to locate that data in any of the Tables mentioned (vol 1.37, pages 44-45), however this was not considered significant by the sponsor.

Gestation/lactation in F1 females: Mean Bw or weight gains were not different throughout gestation or during lactation in F1 females. Gestation lengths were not effectd (21.7, 21.8, 21.8, 21.6 days respectively).

F2 litter data: No effects on mean F2 litter survival, number of pups born/litter, percentages of male/females per litter at birth, or on pup survival were observed. The pup % survival on PND 0-4 was 96, 97.4, 96.1, 97.3% respectively. One pup at 300 mg/kg/day was euthanized on PND-1 with lacerations on the head. Clinical findings showed higher subcutaneous hemorrhage at mid–high doses (total number of findings/number of pups with findings 0/0, 0/0, 3/3, 2/2 respectively). Also, tail was missing in 3 pups at mid doses vs none in other groups, animals were small in size at low mid doses (1/1, 4/3, 2/2, 0/0 respectively) see Table. Body weights of pups or pups found dead (13, 3, 10, 8 pups respectively or in 9, 2, 6, 6 litters respectively) were not different from controls. Overall the number of pups with a milk spot present was not significantly different in pups/litters (0/0, 0/0, 1/1, 1/1 respectively)

Table. Clinical observations in F2 generation rats.

TABLE 59 - F1 GENERATION

PROJECT NO.:WIL-370011A
SPONSOR:SCHERING-PLOUGH
SPONSOR NO.:96386

PRENATAL/POSTNATAL DEVELOPMENT STUDY OF SCH 58235 IN RATS
SUMMARY OF PUP CLINICAL OBSERVATIONS

ALL OBSERVATION PERIODS
TOTAL NUMBER OF FINDINGS/NUMBER OF PUPS WITH FINDING

FINDING	GROUP : 1	2	3	4
NORMAL				
NO REMARKABLE OBSERVATIONS	685/345	596/301	624/316	706/358
DISPOSITION				
FOUND DEAD	13/ 13	3/ 3	9/ 9	8/ 8
EUTHANIZED IN EXTREMIS	0/ 0	0/ 0	1/ 1	0/ 0
MISSING	2/ 2	6/ 6	4/ 4	2/ 2
SCHEDULED EUTHANASIA (PND 4)	341/341	299/299	312/312	356/356
BEHAVIOR/CNS				
LETHARGIC	1/ 1	0/ 0	0/ 0	0/ 0
BODY/INTEGUMENT				
SUBCUTANEOUS HEMORRHAGE(S)	0/ 0	0/ 0	3/ 3	2/ 2
SMALL IN SIZE	1/ 1	4/ 3	2/ 2	0/ 0
TAIL MISSING	0/ 0	0/ 0	6/ 3	0/ 0
LACERATION(S)	0/ 0	0/ 0	1/ 1	0/ 0
PALE IN COLOR	0/ 0	0/ 0	1/ 1	4/ 4
MALROTATION	0/ 0	1/ 1	0/ 0	0/ 0
CARDIO-PULMONARY				
LABORED RESPIRATION	0/ 0	0/ 0	1/ 1	0/ 0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

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Key study findings: In the rat Segment III study (100, 300, 1000 mg/kg/day, n=25/group), the NOAEL for maternal toxicity was 1000 mg/kg/day (or 1000 fold the human dose, based on body surface area), although some clinical signs (dried red material around nose in 0/25, 1/25, 5/25, 1/25 rats respectively, hair loss in 0/25, 0/25, 0/25, 2/25 respectively) and necropsy findings (hair loss in at mid-high doses in 0/23, 0/24, 1/23, 2/24) were observed at 300-1000 mg/kg/day. The NOAEL for peri/post natal toxicity was <100 mg/kg/day (or <100 fold the human dose, based on body surface area), as all doses produced toxicity, including clinical signs in F1 pups. Clinical signs were observed at all doses in F1 pups. These included subcutaneous hemorrhage (number of findings/number of pups with findings 0/0, 1/1, 1/1, 4/3), missing tail (in 1 pup at a mid dose), uneven hair growth (in 0/0, 0/0, 1/1, 2/2 respectively), animals small in size (0, 2, 1, 4 respectively). Post weaning clinical signs were also increased in F1 animals (dry material around eyes/ears/nose in 1/1, 5/3, 0/0, 3/3 respectively, increased hair loss in left and/or right forelimbs in male rats 11/2, 28/4, 15/4, 12/3, and females rats 17/4, 46/8, 63/11, 56/8 respectively). Post natal learning and memory developmental tests in F1 rats showed decreased mean time intervals in these tests on days 5-7 in males (suggesting faster learning in males), but increased time intervals in these tests on days 3-7 in females (suggesting some delay in learning in females) at all drug doses. **One F1 male died at the lowest dose of 100 mg/kg/day on day 155, this death was attributed to the drug administration in mothers.** Scheduled necropsy of F1 rats again showed hair loss in 0/24, 2/22, 0/23, 1/25. In F2 pups, tail was missing in 3 pups at mid doses vs none in other groups, and subcutaneous hemorrhage was higher at mid high doses (0/0, 0/0, 3/3, 2/2 respectively). In summary the NOAEL for peri/post natal toxicity was <100 mg/kg/day, since one F1 male rat died (on day 155) at 100 mg/kg/day, and 300 and/or 1000 mg/kg/day produced increased subcutaneous hemorrhages and missing tails in F1 and F2 pups.

The AUC exposures are not available at lower doses of 100-300 mg/kg/day in segment III study in rats, and TK studies have shown that these do not increase proportionally with the dose. However, the AUC values of the total drug (parent + metabolite) at 1000

mg/kg/day in mothers in segment III in rats are available and these on gestation day 20 (12.2 µg.h/ml) was 18-fold, the human AUC at 10 mg/day (0.68 µg.h/ml). The AUC values of the total drug at 1000 mg/kg/day in rat fetal plasma on gestation day 20 (18.7 µg.h/ml) was 28-fold, the human AUC at 10 mg/day (0.68 µg.h/ml). The AUC values of the total drug at 1000 mg/kg/day in lactating rats on lactation day 12 (23.1 µg.h/ml) was 34-fold, the human AUC at 10 mg/day (0.68 µg.h/ml). The AUC values of the total drug at 1000 mg/kg/day in rat pups on lactation day 12 (11.3 µg.h/ml) was 17-fold, the human AUC at 10 mg/day 0.68 µg.h/ml).

Thus based on 1000 mg/kg/day doses in segment III study in rats, the AUC exposures in rat maternal plasma was 18 fold, in fetal plasma was 28-fold, and on lactation day 12 in mothers it was 34 fold and in lactating fetuses on day 12 was 17 fold, all of these are based on human doses of 10 mg/day (0.68 µg.h/ml).

In summary maternal NOAEL was 1000 mg/kg/day (or ≈ 1000-fold the human dose of 10 mg/day based on body surface area) and pre-post natal NOAEL was <100 mg/kg/day (or < 100 fold the human dose of 10 mg/day based on body surface area).

Reproductive and Developmental Toxicology Summary

Following reproductive toxicity studies are summarized here: segment I fertility study in rats, segment II teratology studies in rats and rabbits, segment III peri-postnatal study in rats. Also a TK study was conducted at one high dose of 1000 mg/kg/day in rats (during gestation day 6 through lactation day 12) and in rabbits (during gestation day 7 through lactation day 22), where a drug exposure was examined in mother and fetal plasma as well as during lactation in mothers and pups . Note that repro tox studies in animals were carried out using a gavage route, while most standard tox studies in rats/dogs (3-12 months) were conducted using a dietary route. The gavage route has lower expsoures in animals vs the dietary route (see 2-week TK studies in rats and mice)

In a segment I fertility study in rats (n=25/group), animals were given oral (by gavage) SCH 58235 at doses of 0, 250, 500, 1000 mg/kg/day. Females were given the drug for 2-weeks prior to mating, throughout mating, and from days 0 to day 7 of gestation, and sacrificed on GD-14. Males were given the drug for 3 weeks prior to mating, during mating, until necropsy. Clinical signs (chromorhinorrhea, reduced fecal pellet, urogenital statining, broken teeth) were increased during gestation in females (observed in total 8 females at mid/high doses) and also during treatment, prior to gestation (observed in total 4 females at mid/high doses). Doses of 250-500 mg/kg/day produced some broken or loose teeth in both sexes, but did not have any affect on the fertility or on the general maternal or paternal reproductive performance, or on the progression of pregnancy in rats. The percentage of resorptions were higher at a high dose (4, 5.4, 6.4, 7.5% respectively), but were not significant and within historical control range. However, less animals were pregnant at the highest dose (male/female fertility index was decreased from 96% to 84% respectively). Since decreased pregnancy index, as well as increased clinical signs were observed at a high dose in females, the NOAEL dose in this fertility study was 500 mg/kg/day (or 500 times the human dose of 10 mg/day, based on body surface area). **Note that the recommended human dose is 10 mg/day.**

In a segment II teratology study in rats, pregnant animals (n=25/group) were given oral SCH 58235 by gavage at doses of 0, 250, 500, 1000 mg/k/day from day 6 to day 15 of gestation. Females were sacrificed on day 21 PC and necropsied. Maternal exposures of the total drug on GD 15 were 3.1, 4.2, 4.9 µg.h/ml at 250, 500, 1000 mg/kg/day respectively. Maternal NOAEL was 1000 mg/kg/day (≈ 1000 times the human dose, based on body surface area) in rats, as no toxicity was observed even at the highest dose of 1000 mg/kg/day. Malformations with low incidences were observed in fetuses and litters at 250-500 mg/kg/day. These included a small size heart (in one fetus and litter at 250 mg/kg/day), short filamentous tail (in one fetus and litter at 500 mg/kg/day), vestigial right kidney (in one fetus and litter at 500 mg/kg/day). Soft tissue variations were observed in one fetus at low dose (anasarca), and in 3 fetuses (a dilated renal pelvis) two at a low dose and 1 at a high dose. However, increased skeletal observations were noted at a high dose. These included extra pair of thoracic ribs (fetal incidences 11 vs 7 in controls, or 5.5% vs 3.8% in controls), unossified cervical vertebral centra (fetal incidences 41% vs 20% in controls, litter incidences 92% vs 56% in controls), and shortened ribs (fetal/litter incidences 2-4% vs 1-2% in controls). The historical control means have not been provided by the sponsor. Since malformations (short filamentous tail) were observed at a mid dose and increased skeletal variations were observed at a high dose in fetuses, the embryo-fetal NOAEL was 250 mg/kg/day (or 4-fold the human dose of 10 mg/day based on exposures, and ≈ 250-fold based on body surface area), and maternal NOAEL was 1000 mg/kg/day (or 8-fold the human dose of 10 mg/day based on exposures and ≈ 1000 fold based on body surface area). Some developmental toxicity (increased malformations in head and kidney) or soft tissue variations (anasarca, and dilated renal pelvis) was noted in 1-2 fetuses at 250-1000 mg/kg/day, but there was not dose related trend.

In a segment II teratology study in rabbits, pregnant animals (n=20/group) were given oral SCH 58235 by gavage at doses of 0, 250, 500, 1000 mg/k/day from day 7 to day 19 of gestation. Females were sacrificed on day 30 PC. Maternal exposures of the total drug on GD 19 were 71.5, 95.7, 113 µg.h/ml at 250, 500, 1000 mg/kg/day respectively. Maternal NOAEL was 500 mg/kg/day as in females, since mean resorptions were increased at 1000 mg/kg/day (9.9% vs 4.1% in controls). Developmental NOAEL was <250 mg/kg/day, as all doses (250-1000 mg/kg/day) increased incidence of malformations in fetuses and litters. These included exencephaly (in one fetus and litter at 250 mg/kg/day), agenesis of tail (in one fetus and litter at 250 mg/kg/day), head malformed (in one fetus and litter at 500 mg/kg/day), omphalocele (i.e intestinal and viscera protruding in 1-3 fetuses and litters at all doses vs 1 fetus/litter in controls), and shortened tail in one fetus and litter at 1000 mg/kg/day. Skeletal observations were noted at all doses. These included reduced ossifications in parietals (fetal incidences 0, 3, 5, 0, litter incidences 0, 2, 2, 0) and frontals (fetal/litter incidences 0, 1, 1, 0). Increased focal thickening of ribs at mid/high doses (fetal/litter incidences 0, 0, 2, 1), increased unossified distal humeral epiphysis at a mid dose (fetal 1, 0, 4, 0, litter 1, 0, 2, 0). Extra pair of thoracic ribs were increased at all doses of the drug (fetal incidences 90-107 vs 67 in controls, litter incidences 16-19 vs 14 in controls). Scoliosis was observed in 1 fetus/litter at a high dose (fetal incidences 0.8, litter incidences 5.9 vs none in other groups). Thus maternal NOAEL was 500 mg/kg/day (or 140-fold the human dose of 10 mg/day based on exposures, and ≈ 1000-fold the human dose of 10 mg/day based on body surface area), as increased resorptions (9.9% vs 4.1% in controls) and altered sex distribution (ratios of male/female fetuses was increased at MD & HD 1.2 & 1.4 vs 0.96 in controls). The embryo-fetal NOAEL was 250 mg/kg/day (or 100-fold the human dose

of 10 mg/day based on exposures, and ≈ 500-fold the human dose of 10 mg/day based on body surface area), as increased focal thickening of ribs and scoliosis were observed at 500 and/or 1000 mg/kg/day.

Toxicokinetic parameters in segment II study with SCH 58235 in rats and rabbits at doses of 250, 500, 1000 mg/kg/day

	AUC (0-24 Hrs) µg.h/ml		
Rat seg II study Maternal GD 15	**Total SCH 58235 (parent+metabolite)**	**Parent (SCH 58235) Unconjugated**	**Metabolite (glucuronide) conjugated**
Mg/kg/day			
250	3.09	0.03	3.10
500	4.23	na	4.3
1000	4.93	0.05	5.3
Rabbit seg II study Maternal GD 19			
250	71.5	0.045	71.5
500	95.7	0.058	95.6
1000	113.1	0.072	112.9

Toxicokinetic parameters in segment III study in rats and rabbits with SCH 58235 at one dose of 1000 mg/kg/day

	AUC (0-24 Hrs) µg.h/ml		
Rat	**Total SCH 58235 (parent+metabolite)**	**Parent (SCH 58235) Unconjugated**	**Metabolite (glucuronide) conjugated**
Maternal GD 10	5.8	0.08	5.7
Maternal GD 20	12.2	4.2	8.1
Fetal GD 20	18.7	1.2	17.5
Maternal LD 12	23.1	6.4	16.7
Fetal LD 12	11.3	0.10	11.2
Rabbit			
Maternal GD 10	180.7	0.129	180.6
Maternal GD 22	157.9	0.122	157.8
Fetal GD 22	4.7	0.169	4.5

In the rat Segment III study, SCH 58235 was given to pregnant female rats, orally by gavage on gestation days 6 to lactation day 21 (100, 300, 1000 mg/kg/day, n=25/group). The NOAEL for maternal toxicity was 1000 mg/kg/day (or <0.1 times the human dose, based on body surface area). Some clinical signs (dried red material around nose in 0/25, 1/25, 5/25, 1/25 rats respectively, hair loss in 0/25, 0/25, 0/25, 2/25 respectively) and necropsy findings (hair loss in at mid-high doses in 0/23, 0/24, 1/23, 2/24) were observed at 300-1000 mg/kg/day in mothers. Pre/post natal toxicity was observed at all doses. Clinical signs were observed at all doses in F1 pups. These included subcutaneous hemorrhage (number of findings/number of pups with findings 0/0, 1/1, 1/1, 4/3) and uneven hair growth (in 0/0, 0/0, 1/1, 2/2 respectively). Missing tail was observed in one F1 pup at a mid dose. Post weaning clinical signs were also increased in F1 animals (dry material around eyes/ears/nose in 1/1, 5/3, 0/0, 3/3 respectively,

increased hair loss in left and/or right forelimbs in male rats (11/2, 28/4, 15/4, 12/3), and females rats (17/4, 46/8, 63/11, 56/8 respectively). One F1 male died at the lowest dose of 100 mg/kg/day on day 155, this death was attributed to the drug administration in mothers. Scheduled necropsy of F1 rats again showed hair loss in 0/24, 2/22, 0/23, 1/25. In F2 pups, tail was missing in 3 pups at mid doses vs none in other groups, and subcutaneous hemorrhage was higher at mid/high doses. (0/0, 0/0, 3/3, 2/2 respectively). Thus maternal NOAEL was 1000 mg/kg/day (or 1000-fold the human dose of 10 mg/day, based on body surface area). The embryo-fetal NOAEL was <100 mg/kg/day (or <100 fold the human dose of 10 mg/day, based on body surface area). The latter is based on one fetal death (F1 male died on day 155) at 100 mg/kg/day, and subcutaneous hemorrhages & tail missing at 300-1000 mg/kg/day in both F1 and F2 pups.

NOAEL values in reproductive/developmental toxicity studies with SCH 58235:

Repr-tox study	**NOAEL's**	
Segment I study in rats	500 mg/kg/day (≈ 500 times the human dose of 10 mg/day, based on body surface area).	
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day (or ≈ 1000 times the human dose of 10 mg/day, based on body surface area, **and 8-fold based on AUC exposures).**	250 mg/kg/day (or ≈ 250 times the human dose of 10 mg/day, based on body surface area, **and 4-fold based on AUC exposures).**
Segment II study in rabbits	500 mg/kg/day (or ≈ 1000 times the human dose of 10 mg/day, based on body surface area, **and 140 fold based on AUC exposures)**	250 mg/kg/day (or ≈ 500 times the human doses of 20 mg/day, based body surface area, **and 100 times based on AUC exposures).**
Segment III study in rats	1000 mg/kg/day (or 1000 times the human dose of 10 mg/day, based on body surface area).	**Pre/post natal NOAEL in F1/F2 rats** was <100 mg/kg/day (or (≈ <100 times the human doses of 10 mg/day, based on body surface area).

Following Table summarizes the clinical signs, deaths and malformations in reproductive/developmental toxicity studies with SCH 58235 monotherapy. Note that the incidental deaths may be attributed to drug related hemorrhages, as clinical signs have shown dried red material around eyes/ears/nose, and subcutaneous hemorrhages in F1/F2 rats. In rabbits the deaths may have something to do with lungs

Repr-tox study	
Clinical signs	
Segment I study in rats	Chromorhinrrhea, reduced fecal pellet, urogenital statining, broken teeth at 1000 mg/kg/day (≈ 1000 times the human dose of 10 mg/day, based on body surface area)
Segment III study in rats	In F1 rats at 300-1000 mg/kg/day (dried red material around nose, subcutaneous hemorrhage, hair loss). In F2 rats, at all doses of 100-1000 mg/kg/day (pre weaning; subcutaneous hemorrhage, uneven hair growth, post weaning; dry material around eyes/ears/nose, increased hair loss in left and/or right forelimb).
Malformations	
Segment II study in **rats**	Short filamentous tail at 500 mg/kg/day
Segment II study in **rabbits**	Agenesis of tail at 250 mg/kg/day, & shortened tail at 1000 mg/kg/day
Segment III study in rats	Missing tail in F1& F2 pups at 300 mg/kg/day
Deaths	
Segment I study in rats	1/25 female rats died at 500 mg/kg/day (on day 7, it was considered accidental, no findings available
Segment II study in rabbits	1/20 rabbits died at 500 mg/kg/day (on GD 9, it was considered incidental, it had its thoracic cavity filled with blood and had dark red focal discoloration and foamy exudate in trachea)
Segment III TK study in rabbits	2/30 rabbits died at 1000 mg/kg/day, one on GD 16 (died shortly after dosing, so necropsy was performed) and the other on GD-17 (necropsy showed white material in the thoracic cavity adhered to all lobes of the right lung and the thoracic wall). Sponsor considers these incidental, since none were seen in the standard segment II in rabbits, although 1/20 rabbit died in that study
Segment III study in rats	1/25 F1 male rat died on day 155 at the lowest dose of 100 mg/kg/day, it had no clinical signs, the animal was internally normal, sponsor acknowledges that this death was attributed to the drug administration in F0 mot hers.

VII. B. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY WITH WITH COMBINATION (SCH 58235 + STATINS):

1. Oral Gavage Rat Embryo Fetal Developmental Toxicity and TK of SCH 58235 and Pravastatin (SCH 57096)

Key study findings:

- **Exposure to SCH 58235 increased when 500 mg/kg pravastatin was co-administered. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235**
- **Exposure to pravastatin free acid when co-administered with SCH 58235 increased > dose proportional as the pravastatin dose increased from 125 to 500 mg/kg resulting in pravastatin free acid exposure increasing 1.7X**
- **The sponsor attributes increased salivation to pravastatin at doses ≥250 mg/kg, decreased body weight gain was attributed to the 500 mg/kg pravastatin dose. Discolored stool was attributed to SCH 58235**
- **SCH 58235 co-administered with pravastatin 500 mg/kg resulted in a small increased in fetal visceral variation of dilated ureters which the sponsor attributes to pravastatin**
- **Skeletal variations consisting of reduced ossification of sternebrae, parietal bone, proximal phalanges (hind paws) generally increased in incidence (fetal/litter) with 1000 mg/kg SCH 58235 + 500 mg/kg pravastatin compared to pravastatin alone (500 mg/kg/day). These increases exceed the historical mean but are within the sponsor's historical range.**
- **Maternal NOAEL=1000 mg/kg SCH 52835 + 250 mg/kg pravastatin based on statistically significant body weight gain decrements in the 1000 SCH + 500 pravastatin mg/kg group**
- **Developmental NOAEL=1000 mg/kg SCH 52835 + 250 mg/kg pravastatin based on the increased incidence of reduced skeletal ossification observed with 1000 mg/kg SCH 52835 + 500 mg/kg pravastatin compared to pravastatin alone (500 mg/kg/day).**

Study no.:99495
Volume #, and page #: 1.144, pg.1
Conducting laboratory and location: Safety Evaluation Center; Schering-Plough Research Institute; Lafayette, NJ
Date of study initiation: 4/18/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH 58235 97-58235-X-02 purity was not provided
Pravastatin 75793-008
Formulation/vehicle: 0.4% aqueous methylcellulose; 10 ml/kg vehicle control, others 5 ml/kg

Methods:

Species/strain: Crl:CD(SD)IGS BR VAF/plus Sprague-Dawley

Doses employed: 1000 mg/kg SCH 58235 + Pravastatin sodium 131, 263, 526 equivalent to 125, 250, 500 mg/kg pravastatin free acid, controls received vehicle or 500 pravastatin
Route of administration: oral gavage

Study design: daily dosing GD6-17
Number/sex/group: 25F/group and 12F/group for TK
Parameters and endpoints evaluated:

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Pravastatin (SCH 57096) Administered Orally by Gavage in Rats (SN 99495): **Observations and Measurements**	
Investigation	**Performed**
Clinical Observations	Daily
Body Weight	Gestation Days 0, 6, 9, 12, 15 and 17 (and gestation Day 21 for toxicity portion animals)
Food Consumption	Recorded for the intervals of gestation Days 0-6, 6-12, 12-17 and 17-21 for toxicity portion rats.
Plasma Analysis for SCH 58235 and Pravastatin	Gestation Day 17 (1, 2, 4, 6, 8 and 24 hours postdose)
Necropsy[a]/C-Section	Gestation Day 21 (or after the final bleed for Toxicokinetic animals)
Reproductive Parameters	Yes
Fetal Body Weight	Yes
Determination of Fetal Sex	Yes
Fetal External/Visceral/ Skeletal Examinations	Yes
a: Toxicokinetic animals were only examined to confirm pregnancy status; no other necropsy examinations were performed.	

Results:

Mortality: One female from each group given 1000/250 and 1000/500 were sacrificed moribund Day 14 and found dead after dosing Day 17 respectively. Adverse signs included: thick whitish liquid around mouth, rales, urogenital staining, chromorhinorrea and a perforated esophagus consistent with a gavage induced trauma.

Clinical signs:	Dose SCH58235/Pravastatin			
	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Salivation	5/25		7/25	8/25
Pale stool		25/25	24/25	

Salivation was attributed to pravastatin and pale stool was previously reported with SCH 52835 according to the sponsor.

Body weight:	Dose SCH58235/Pravastatin				
	Vehicle	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Gain GD6-17 (g)	72±11.2	64±12.5	70±12.1	69±15.3	61*±13.9

The reduced gain observed with pravastatin 500 mg/kg/day was not associated with decreased food consumption or decreased gravid uterine weight.

Food consumption: unremarkable

Toxicokinetics:

Table 1 Mean Pharmacokinetic Parameters of Total, Conjugated and Unconjugated SCH 58235 on Day 17 of Gestation Following Multiple Oral Gavage Administration (Gestation Days 6 to 17) of 1000 mg SCH 58235/kg in Combination with 131, 263 or 526 mg Pravastatin/kg to Female Rats

Total SCH 58235 (ng/mL)				
		1000/131[a]	1000/263[a]	1000/526[a]
Cmax	ng/mL	2400	2030	3400
Tmax	hr	1	1	1
tf	hr	24	24	24
AUC(0-24 hr)	ng-hr/mL	9780	11200	30000
Conjugated SCH 58235 (ng/mL plasma)[b]				
Cmax	ng/mL	2390	2020	3390
Tmax	hr	1	1	1
tf	hr	24	24	24
AUC(0-24)	ng.hr/mL	9710	11100	29800
Unconjugated SCH 58235 (ng/mL plasma)				
Cmax	ng/mL	8.42	11.0	13.4
Tmax	hr	1	1	4
tf	hr	8	24	8
AUC(tf)	ng.hr/mL	43.5	68.2	84.8
AUC(0-24 hr)	ng.hr/mL	73.0[c]	68.2	189[d]
% Total[e]		0.746	0.609	0.631

a: Dose SCH 58235/ Dose Pravastatin (as mg/kg; pravastatin dose expressed as sodium salt)

b: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents

c: Percent AUC extrapolated is 67.9%

d: Percent AUC extrapolated is 123%

e: Calculated as [unconjugated SCH 58235 AUC(0-24 hr)/total SCH 58235 AUC(0-24 hr)] x 100

Table 2 Comparison of Dose Mean AUC (0-24 hr) Ratios of Total, Conjugated and Unconjugated SCH 58235 on Gestation Day 17 Following Multiple Oral Gavage Administration (Gestation Days 6-17) of 1000 mg SCH 58235/kg with 131, 263 or 526 mg Pravastatin/kg to Female Rats.

Group	Total Daily Dose (mg/kg)	SCH 58235 Dose Ratio[a]	Mean SCH 58235 AUC (0-24 hr) Ratio[a]		
			Total	Conjugated	Unconjugated
3 SCH 58235 Pravastatin[b]	1000 131	1	1	1	1
4 SCH 58235 Pravastatin	1000 263	1	1.14	1.15	0.934
5 SCH 58235 Pravastatin	1000 526	1	3.06	3.07	2.59

a: Normalized to Group 3

b: Expressed as sodium salt

Table 3 Mean Pharmacokinetic Parameters of Pravastatin on Day 17 of Gestation Following Multiple Oral Gavage Administration (Gestation Days 6 to17) of 526 mg Pravastatin Alone or 131, 263 or 526 mg Pravastatin/kg in Combination with 1000 mg SCH 58235/kg to Female Rats

Time	Unit	0/526[a]	1000/131[a]	1000/263[a]	1000/526[a]
		Mean	Mean	Mean	Mean
Cmax	ng/mL	1830	943	1570	3810
Tmax	hr	2	1	1	1
tf	hr	24	24	24	24
AUC(0-24 hr)	ng-hr/mL	10900	1970	4820	18200

a: Dose SCH 58235/ Dose Pravastatin (as mg/kg; pravastatin dose expressed as sodium salt)

Table 4 Comparison of Dose Mean AUC (0-24 hr) Ratios of Pravastatin on Gestation Day 17 Following Multiple Oral Gavage Administration (Gestation Days 6-17) of 1000 mg SCH 58235/kg in Combination with 131, 263 or 526 mg Pravastatin/kg to Female Rats.

Group	Total Daily dose (mg/kg)	Pravastatin Dose Ratio[a]	Mean Pravastatin AUC (0-24 hr) Ratio[a]
3 SCH 58235 Pravastatin[b]	1000 131	1	1
4 SCH 58235 Pravastatin	1000 263	2	2.45
5 SCH 58235 Pravastatin	1000 526	4	9.24

a: Normalized to Group 3
b: Expressed as sodium salt

For embryofetal development studies:

In-life observations:

Offspring:	Dose SCH58235/Pravastatin				
	Vehicle control	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Decreased fetal body weight	5.8±0.29	5.6*±0.26	5.8±0.26	5.7±0.33	5.7±0.35

The decreased fetal body weight was considered incidental by the sponsor since it was not observed in the 1000/500 mg/kg/day group. Fetal external observations were unremarkable.

Terminal and necroscopic evaluations:

Dams	Dose SCH58235/Pravastatin				
	Vehicle control	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Perforated esophagus drug in thoracic cavity				1/25	1/25

Reproductive data including: viable fetuses (sex ratio), corpora lutea, implantation sites, preimplantation loss, postimplantation loss, resorptions (early, late) are unremarkable.

Offspring:	Dose SCH58235/Pravastatin				
Visceral Malform./ variations	Vehicle control	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Litters Fetuses	24 151	25 160	25 152	23 145	24 147
Fetal Malformations (by litter)	4 (3)	4 (2)	4 (2)	4 (3)	3 (2)
Fetal Variations (by litter)	4 (3)	9 (5)	5 (3)	3 (2)	8 (5)
Visceral variation: Dilated ureter (by litter)	2 (1;4.2%)	9 (5;20%)	2 (2;8%)	3 (2;8.7%)	5 (3;12.5%)

No visceral malformations were observed. The increased incidence of dilated ureter was attributed to pravastatin by the sponsor.

Offspring:	Dose SCH58235/Pravastatin				
Skeletal Malform./ Variations	Vehicle control	0/500 mg/kg/day	1000/125 mg/kg/day	1000/250 mg/kg/day	1000/500 mg/kg/day
Litters Fetuses	24 149	25 161	25 154	23 146	24 148
Fetal Malformations (by litter)				1 (1;4.3%)	
Fetal	104; 69.8%	126; 78.3%	126; 81.8%	118; 80.8%	125;84.5%

Variations (by litter)	(23; 95.8%)	(25; 100%)	(25; 100%)	(23;100%)	(24; 100%)
Skeletal Variations (no malformations observed)					
Thoracic vertebral centra bipartite	0.7% (4.2%)	3.1% (12%)		2.1% (8.7%)	2.7% (12.5%)
Cervical vertebral centra unossified	55.7% (91.7%)	69.6% (96%)	69.5% (100%)	63.7% (95.7%)	70.9% (100%)
Sternebrae reduced ossif.	4% (16.7%)	6.2% (24%)	4.5% (24%)	3.4% (17.4%)	9.5% (29.2%)
Asymmetrical sternebra	0.7% (4.2%)	2.5% (16%)	0.6% (4%)	2.7% (13%)	2.7% (12.5%)
Reduced parietal ossification	1.3% (8.3%)	3.1% (12%)	1.3% (8%)	3.4% (13%)	4.7% (20.8%)
Unossified proximal phalanges Hind paws	28.2% (58.3%)	29.8% (68%)	37.7% (76%)	37% (69.6%)	40.5% (75%)

Skull, cervical/thoracic vertebrae, sternebrae, ribs, metacarpals, metatarsals, phalanges were examined for skeletal findings. The skeletal findings indicated above are above the mean historical background provided by the sponsor but within the historical range by fetus or litter.

Historical Control	Fetuses			Litter	
Skeletal Findings	%	Range	Mean	%	Range
Thoracic vertebral centra bipartite	1	0-4.4	1±4.38	5.8	0-20.8
Cervical vertebral centra unossified	47.8	20.1-74.1	48.6± 33.91	83.9	58.3-100
Sternebrae reduced ossif.	8	1.9-19.8	8±13.3	34.7	8-76
Asymmetrical sternebra	1.4	0-4.1	1.5±5.6	8.3	0-20.8
Reduced parietal bone ossification	2	0-6.4	2±7.73	9.1	0-25
Unossified proximal phalanges Hind paws	25.6	0-44.6	24.7± 30.92	50.8	0-87.5

The sponsor provided historical control data from 242 litters containing ~1708 fetuses for skeletal findings only.

- **Summary of individual study findings:** Exposure to SCH 58235 increased when 500 mg/kg pravastatin was co-administered. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235

- Exposure to pravastatin free acid when co-administered with SCH 58235 increased > dose proportional as the pravastatin dose increased from 125 to 500 mg/kg resulting in pravastatin free acid exposure increasing 1.7X
- The sponsor attributes increased salivation to pravastatin at doses ≥250 mg/kg, decreased body weight gain was attributed to the 500 mg/kg pravastatin dose. Discolored stool was attributed to SCH 58235
- SCH 58235 co-administered with pravastatin 500 mg/kg resulted in a small increased in fetal visceral variation of dilated ureters which the sponsor attributes to pravastatin
- Skeletal variations consisting of reduced ossification of sternebrae, parietal bone, proximal phalanges (hind paws) generally increased in incidence (fetal/litter) with 1000 mg/kg SCH 58235 + 500 mg/kg pravastatin compared to pravastatin alone (500 mg/kg/day). These increases exceed the historical mean but are within the sponsor's historical range.
- Maternal NOAEL=1000 mg/kg SCH 52835 + 250 mg/kg pravastatin based on statistically significant body weight gain decrements in the 1000 SCH + 500 pravastatin mg/kg group
- Developmental NOAEL=1000 mg/kg SCH 52835 + 250 mg/kg pravastatin based on the increased incidence of reduced skeletal ossification observed with 1000 mg/kg SCH 52835 + 500 mg/kg pravastatin compared to pravastatin alone (500 mg/kg/day). The sponsor attributes the reduced ossification to decreased fetal weight. However decreased fetal weight is observed in the pravastatin 500 mg/kg group but not in the combination group with 1000 mg/kg SCH 58235 where the increased incidence of reduced skeletal ossification is observed. The incidence of these variations exceed the sponsor's mean historical data but not the range. Mat ernal toxicity may account for these observed variations since maternal body weight gain is significantly reduced in the combination group of 1000 mg/kg SCH 58235 + pravastatin 500 mg/kg. Exposures of both pravastatin and SCH 58235 increase when co-administered, this may account for the reduced weight gain in these dams.

2. Embryo-Fetal Developmental Toxicity & TK of SCH58235 + pravastatin (SCH 57096) by Oral Gavage to Rabbits

Key study findings:

- **Coadministration of SCH 58235 with pravastatin 50 mg/kg (10 µg.h/ml) had no effect on the mean systemic exposure to pravastatin 50 mg/kg/day (9.7 µg.h/ml). Similarly coadministration of two drugs did not effect the systemic exposure to SCH 58235 (1000 mg/kg/day), values were 113 µg.h/ml from a previous study vs 135-145 µg.h/ml in the current study .**
- **All combo groups had higher fecal findings (fecal stained fur, discolored or soft stool, reduced fecal pellets) than the prava alone or the control group.**
- **In the high dose combo group, BW gain was significantly decreased during GD 7-30 (0.23* vs 0.34 kg, *p<0.01). Food consumption was transiently decreased in all combo groups during GD 8-9.**
- **The post implantation loss was slightly higher in MD and HD combo groups (8-9.1% vs 7% with prava alone)**
- **25 and 50 mg/kg pravastatin + 1000 mg/kg SCH 58235 produced malformations of shortened or kinked tail (1/16 & 2/19 litters with 1-2 fetuses effected) along with fused caudal vertebra.**

- **A HD combo increased the incidences of skeletal variations such as sternbrae bipartite (31.6% vs 22% with prava alone) and extra pair of ribs (110% vs 83% with prava alone)**
- **NOAEL for maternal toxicity was 25 mg/kg pravastatin + 1000 mg/kg SCH 58235, as the higher combo dose (50 mg/kg pravastatin + 1000 mg/kg SCH 58235) produced a significant decrease in BW gain.**
- **Developmental NOAEL= 5 mg/kg pravastatin + 1000 mg/kg SCH 58235, as higher combo doses (MD & HD) produced external malformations (in tail) as well as fused caudal vertebrae compared to prava or control group.**

Study no.: 99496
Volume #, and page #: 1.156-1.157 pg.1
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 5/9/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH ———
SCH 58235 ——— **99-58235-X-02 ; pravastatin 75793-008**
Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: NZW
Doses employed:
52.5 mg/kg/day of pravastatin sodium (or 50 mg/kg of pravastatin free acid) or 5.2, 26.3, 52.5 mg/kg/day of pravastatin sodium (or 0, 5, 25, 50 mg/kg pravastatin free acid) + 1000 mg/kg SCH 58235. Control animals received the vehicle. Females were dosed from gestation days 7 through 19.
Route of administration: oral gavage
Study design:

Table: study design, observations and measurements

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Pravastatin (SCH 57096) Administered Orally by Gavage in Rabbits (SN 99496): **Study Design**					
Group	Total Daily Dose (mg/kg)[a]	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females	
				Toxicity	Toxicokinetic[b]
Control (0.4% methylcellulose)	0	8	0	20	0
Pravastatin Control:				20	4
Methylcellulose	0	5	0		
Pravastatin	52.5	3	17.50		
Low-Dose Combination:				20	4
SCH 58235	1000	5	200		
Pravastatin	5.2	3	1.73		
Mid-Dose Combination:				20	4
SCH 58235	1000	5	200		
Pravastatin	26.3	3	8.77		
High-Dose Combination:				20	4
SCH 58235	1000	5	200		
Pravastatin	52.5	3	17.50		

a: The dose levels of pravastatin sodium at 5.2, 26.3 and 52.5 mg/kg are equal to 5, 25 and 50 mg/kg of the pravastatin free acid, respectively.

b: These animals were used only for determination of plasma concentrations.

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Pravastatin (SCH 57096) Administered Orally by Gavage in Rabbits (SN 99496): **Observations and Measurements**	
Investigation	Performed
Clinical Observations	Daily
Body Weight	Gestation Days 2, 7, 10, 13, 16, 19, 22, 25, 28 and 30
Food Consumption (estimated)	Daily
Necropsy[a]/C-Section	Gestation Day 30 (or after the final bleed for Toxicokinetic animals only)
Plasma Analysis for SCH 58235 and Pravastatin	At 1, 2, 4, 6, 8 and 24 hours after dosing on Gestation Day 19
Reproductive Parameters	Yes
Fetal Body Weight	Yes
Determination of Fetal Sex	Yes
Fetal External/Visceral/ Skeletal Examinations	Yes

a: Toxicokinetic animals were only examined to confirm pregnancy status; no other necropsy examinations were performed.

Results:

Mortality: One MD combo dam (group 8) was sacrificed on GD10 due to swollen genitalia and perivaginal cyst. One female in HD combo group aborted on GD24, and was sacrificed. These were not considered drug related by the sponsor.

Clinical signs: Fecal stained fur incidences (frequency/animals) were increased (1/1, 0/0, 41/3, 15/6, 16/4 at 0, prava 50 mg/kg, and in combo studies of SCH

58235/prava of 1000/5, 1000/25, 1000/50 mg/kg/day respectively). Discolored stool incidences were 0/0, 0/0, 257/20, 242/20, 263/20 respectively. Sponsor explains that the discolored stool (pale) was observed previously with SCH 58235 and is attributed to elimination of unabsorbed SCH 58235 in the feces. However all groups administered the combination (SCH 58235 + 5-50 mg/kg pravastatin) had higher incidences of stool related findings (a reduced number of fecal pellets, no stool and small fecal pellets).

Table: clinical signs in rabbits with prav + SCH 58235 combo

ENBRYO-FETAL DEVELOPMENTAL TOXICITY AND TOXICOKINETIC STUDY OF SCH 58235 AND PRAVASTATIN (SCH 57096) ADMINISTERED ORALLY BY GAVAGE IN RABBITS

SUMMARY OF CLINICAL OBSERVATIONS DURING GESTATION (frequency / animals)

	GROUP 1 0.4% MC 0 MPK	GROUP 2 0 MPK 0.4% MC 52.5 MPK PRAVA	GROUP 3 1000 MPK SB235 5.2 MPK PRAVA	GROUP 4 1000 MPK SB235 26.3 MPK PRAVA	GROUP 5 1000 MPK SB235 52.5 MPK PRAVA
DAY 2 to 30					
Normal					
NO REMARKABLE CLINICAL OBSERVATIONS	547/20	530/20	274/20	282/20	233/21
Dead					
ELECTIVE SACRIFICE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
SACRIFICED FOR HUMANE REASONS	0/ 0	0/ 0	0/ 0	1/ 1	8/ 8
ABORTED, SACRIFICED	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
SCHEDULED SACRIFICE	20/20	20/20	20/20	19/19	19/19
Miscellaneous					
RED MATERIAL IN LITTER PAN	7/ 1	2/ 1	0/ 0	3/ 1	4/ 3
EXTERNAL GENITALIA SWOLLEN	0/ 0	0/ 0	0/ 0	1/ 1	6/ 6
PERIVAGINAL CYST	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
RED VAGINAL DISCHARGE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
PUP(s) DELIVERED	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
FETUS(ES) ABORTED	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
Skin/Fur					
ALOPECIA	43/ 4 [a]	64/ 6 [a]	33/ 2 [a]	29/ 5	101/ 9 [a]
WOUNDS, CUT, SCRATCHES	0/ 0	3/ 1	0/ 0	3/ 1	0/ 0
Stool/Urine					
FECAL STAINED INGUINAL FUR	1/ 1	0/ 0	41/ 3	15/ 6	16/ 4
DISCOLORED STOOL, PALE	0/ 0	0/ 0	257/20	242/20	263/20
DIARRHEA	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
REDUCED NUMBER OF FECAL PELLETS	1/ 1	5/ 1	7/ 6	15/11	14/ 5
SMALL FECAL PELLETS	0/ 0	0/ 0	0/ 0	3/ 2	4/ 1
SOFT STOOL	3/ 1	5/ 3	13/ 4	13/ 7	33/ 8
URINE STAINED INGUINAL FUR	0/ 0	6/ 1	16/ 3	0/ 0	10/ 4
MUCOID STOOL	0/ 0	0/ 0	1/ 1	0/ 0	3/ 2

[a] The frequency/animal for alopecia listed here is greater than the number of individual days this clinical observation was noted (Table 3) because alopecia was recorded on some days at more than one site in a specific animal.

Body weight: In the HD combo group, the body weight BW gain was lower during gestation days 7 to 19 (0.16, 0.15, 0.13, 0.12, 0.11 kg respectively). In HD combo group, the BW gain was also lower during GD 19-30 (0.12 vs 0.18 kg in controls), and during day GD 7-30 (0.23* vs 0.34 kg, $p<0.01$). The mean BW was decreased in HD combo group by 31% (339, 302, 339, 341, 233* g respectively). The gravid uterine weights were not different from the control groups.

Food consumption: All the combo drug groups had a transient decrease in food consumption on GD 8-9. On GD 9, the number of rabbits that had poor or moderate food consumption was 8/20, 11/20, 8/20 at SCH 58235/prava doses of 1000/5, 1000/25, 1000/50 mg/kg/day respectively, while controls and prava

group had normal food consumption. The mean food consumption values in animals were not provided.

Toxicokinetics: The combo administration of SCH 58235/prava (1000/5, 1000/25, 1000/50 mg/kg/day on GD19) did not effect the total drug AUC exposures in rabbits (139.1, 135.2, 145.3 µg.h/ml respectively). In a previous study with the drug alone at 1000 mg/kg/day the exposures in rabbits on GD 7-19 were 113.1 µg.h/ml vs 135-145 µg.h/ml in the current study . Similarly the exposures of prav were not altered by the drug in rabbits (1.2, 4.2, 10.0 with combo vs 9.7 µg.h/ml with prava alone). Mean Cmax and Tmax were also not significantly different in the combo groups.
Toxicokinetics:

Table: TK of SCH 58235 in rabbits with prav + SCH 58235 combo

Table 1 Mean Pharmacokinetic Parameters of Total, Conjugated and Unconjugated SCH 58235 on Gestation Day 19 Following Multiple Oral Gavage Administration (Gestation Days 7-19) of 5.2, 26.3 or 52.5 mg/kg Pravastatin Sodium with 1000 mg/kg SCH 58235 to Female Rabbits

		Total SCH 58235					
		1000/5.2[a]		1000/26.3[a]		1000/52.5[a]	
		Mean[b]	%CV	Mean	%CV	Mean	%CV
Cmax	ng/ml	8467	14	7700	8	7705	27
Tmax	hr	2.8	127	4.0	89	2.5	95
AUC(0-24 hr)	ng·hr/ml	139120	24	135223	22	145350	20
		Conjugated SCH 58235[c]					
		1000/5.2[a]		1000/26.3[a]		1000/52.5[a]	
		Mean	%CV	Mean	%CV	Mean	%CV
Cmax	ng/ml	8464	14	7696	8	7698	27
Tmax	hr	2.8	127	4.0	89	2.5	95
AUC(0-24 hr)	ng·hr/ml	139017	24	135136	22	145214	20
		Unconjugated SCH 58235					
		1000/5.2[a]		1000/26.3[a]		1000/52.5[a]	
		Mean	%CV	Mean	%CV	Mean	%CV
Cmax	ng/ml	6.27	41	4.98	18	7.71	9
Tmax	hr	4.3	56	5.5	35	4.3	56
AUC(0-24 hr)	ng·hr/ml	104	47	89.8	18	136	16
% Total[d]	%	0.07	33	0.07	14	0.10	31

a: Dose SCH 58235/Dose Pravastatin Sodium (as mg/kg)
b: N = 4
c: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents.
d: Calculated as [unconjugated SCH 58235 AUC(0-24 hr)/total SCH 58235 AUC(0-24 hr)]*100%

Table: TK of pravastatin in rabbits with prav + SCH 58235 combo

Table 2 Mean Pharmacokinetic Parameters of Pravastatin on Gestation Day 19 Following Multiple Oral Gavage Administration (Gestation Days 7-19) of 52.5 mg/kg Pravastatin Sodium or 5.2, 26.3 or 52.5 mg/kg Pravastatin Sodium with 1000 mg/kg SCH 58235 to Female Rabbits

		0/52.5[a]		1000/5.2[a]		1000/26.3[a]		1000/52.5[a]	
Parameter	Unit	Mean[b]	%CV	Mean	%CV	Mean	%CV	Mean	%CV
Cmax	*ng/ml*	998	19	125	22	502	48	1217	31
Tmax	hr	1.0	0	1.3	40	1.0	0	1.0	0
AUC(0-24 hr)	ng·hr/ml	9711	36	1212	42	4187	45	10027	23

a: Dose SCH 58235/Dose Pravastatin Sodium (as mg/kg)
b: N = 4

For embryofetal development studies: The percent of post implantation loss was slightly higher in MD and HD combo groups. Fetal body weights were not significantly different from controls. Other reproductive data including viable fetuses (sex ratio), corpora lutea, implantation sites, preimplantation loss, resorptions (early, late) were generally unremarkable.

In-life observations:

Reproductive data in dams:	Dose SCH58235/Pravastatin				
	Vehicle control	0/50 mg/kg/day	1000/5 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Mean post implantation loss (%)	10 (5.2%)	12 (7%)	13 (6.7%)	16 (9.2%)	14 (8.1%)
Mean fetal body weights (g)	44.3	45.6	45.2	47.4	46.2

Total skeletal malformations and variations were increased in fetuses and litters in MD and HD combo groups.

Total Skekeletal malformations and variations:

Dams	Dose SCH58235/Pravastatin				
	Vehicle control	0/50 mg/kg/day	1000/5 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Total malformations fetal incidences	0/170 (0%)	1/156 (0.6%)	1/177 (0.6%)	1/142 (0.7%)	6/158 (3.8%)
Total malformations litter incidences	0/18 (0%)	1/18 (5.6%)	1/19 (5.3%)	1/16 (6.3%)	3/19 (15.8%)
Total variations	79.4	86.5	84.2	95.1	93

fetal Incidences (%)					

External observations in fetuses/litters showed increased hemorrhages and malformations in tails (shortened or kinked tail) in MD and/or HD combo groups

Fetal external observations:

	Dose SCH58235/Pravastatin				
Offspring:	Vehicle control	0/50 mg/kg/day	1000/5 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Litters Fetuses	18 171	18 156	19 177	16 142	19 158
Hemorrhage Fetal incidences Litter incidences	0 0	0 0	0 0	0 0	1 (or 0.6%) 1 (or 5.3%)
Tail Fetal incidences Litter incidences	0 0	0 0	0 0	1 1	1 2
Shortened Tail Malformation Fetal incidences Litter incidences	0 0	0 0	0 0	1 (or 0.7% 1 (or 6.3%)	2 (or 1.3% 1 (or 5.3%)
Kinked Tail Malformation Fetal incidences Litter incidences	0 0	0 0	0 0	1 1(or 0.7% 1 (or 6.3%)	0 0

Skeletal variations (thoracic/cervical vertebral centra bipartite etc.) and malformations (thoracic/lumber/sacral hemivertebrae and fused caudal vertebrae) in fetuses/litters were increased in MD and HD combo groups. The malformations were attributed to the combination drug treatment of SCH 58235 + pravastatin, as fused caudal vertebra and shortened tail malformations were seen previously in rabbits with the present drug + simvastatin and were not seen in the facility's historical control data

Skeletal variations and malformations in fetuses

Offspring:	Dose SCH58235/Pravastatin				
Skeletal Malform./ Variations	Vehicle control	0/50 mg/kg/day	1000/5 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Litters Fetuses	18 170	18 156	19 177	16 142	19 158
Skeletal Variations: fetal incidences (litter incidences)					
Thoracic vertebral centra bipartite	0% (0%)	0% (0%)	0% (0%)	0% (0%)	0.6% (5.3%)
Cervical vertebral centra bipartite	0% (0%)	0% (0%)	0% (0%)	0% (0%)	0.6% (5.3%)
Extra lumber vertebrae	2.4% (16.7%)	1.3% (11.1%)	18.6% (42.1%)	7.7% (6.3%)	7% (26.3%)
Sternabrae bipartite	2.9% (16.7%)	2.6% (22.2%)	4.0% (26.3%)	2.1% (18.8%)	3.8% (31.6%)
Extra pair of Thoracic ribs	48.2% (88.9%)	44.2% (83.3%)	66.7% (89.5%)	85.9% (93.8%)	73.4% (100%)

Skeletal malformations					
Thoracic hemivertebrae	0% (0%)	0.6% (5.6%)	0% (0%)	0% (0%)	1.3% (10.5%)
lumber hemivertebrae	0% (0%)	0% (0%)	0% (0%)	0% (0%)	0.6% (5.3%)
Sacral hemivertebrae	0% (0%)	0% (0%)	0% (0%)	0% (0%)	0.6% (5.3%)
Caudal vertebrae fused	0% (0%)	0% (0%)	0% (0%)	0.7% (6.3%)	1.9% (5.3%)

No visceral (abdominal cavity) malformations were observed, except omphalocele (fetal/litter incidences of 0.6%/5.65) in the vehicle control group.

Summary of individual study findings:
Exposure to SCH 58235 or to pravastatin was not altered when two drugs were co-administered. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Discolored stool was attributed to elimination of unabsorbed SCH 58235 in the feces. However, all combo groups (SCH 58235 + 5-50 mg/kg pravastatin) had higher incidences of stool related findings (a reduced number of fecal pellets, no stool and small fecal pellets) compared to controls. In a HD combo group BW gains were lower during GD 7-30, sponsor considers this toxicologically relevant to HD combination. Food consumption decreased transiently in all the combo groups on GD 8-9. Skeletal variations consisting of increased incidences of sternebrae bipartite (litters 16.7, 22.2, 26.3, 18.8, 31.6%), and extra pair of thoracic ribs (88.9, 83.3, 89.5, 93.8, 100%) were observed in the HD combo group. Malformation of caudal vertebrae fused (0, 0, 0, 6.3, 5.3%) were observed in the MD & HD combo group. Shortened tail, kinked tail were observed in the MD and HD combo group. Sponsor states that this was limited to a single litter. However these findings were attributed to the drug, because this was also observed in study of drug + simva in rabbits, and not seen in historical control data. Maternal NOAEL=1000 mg/kg SCH 52835 + 25 mg/kg pravastatin based on statistically significant body weight gain decrements in the 1000 SCH + 50 mg/kg pravastatin group. Developmental NOAEL=1000 mg/kg SCH 52835 + 5 mg/kg pravastatin based on the increased incidence of malformations in the tail, and fused caudal vertebrae (observed with 1000 mg/kg SCH 52835 + 25/50 mg/kg pravastatin compared to pravastatin alone 50 mg/kg/day). Maternal toxicity may account for some of these observed malformations/variations since maternal body weight gain was significantly reduced in the combination group of 1000 mg/kg SCH 58235 + pravastatin 50 mg/kg/day, but does not explain the malformations at a lower combination dose of SCH 58235 + pravastatin (1000 + 25 mg/kg/day respectively). Exposures of both pravastatin and SCH 58235 did not increase when co-administered, which does not explain the malformations in the combo group. Sponsor's no effect levels doses for both maternal (<1000 SCH 58235 + 50 mg/kg/day pravastatin) and embryo-fetal (1000 SCH 58235 + 50 mg/kg/day pravastatin) toxicity were higher, despite the fact they acknowledge that HD combo

produced decreases in BW gain in rabbits, and MD/HD combo produced malformations in fetuses.

3. Rat Oral Gavage Embryo-Fetal Developmental Toxicity and TK of SCH 58235 and Simvastatin (SCH 57098)

Key study findings:

- **Systemic exposure to unconjugated SCH 58235 was <2% total drug, indicating extensive conjugation in the pregnant rat**
- **Co-administration of 1000 mg/kg SCH 58235 with ≥5 mg/kg simvastatin increased mean systemic exposure to total and conjugated SCH 58235. Mean systemic exposure to, hydroxysimvastatin increased in a greater than dose proportional manner. Co-administration of 1000 mg SCH 58235 with simvastatin (25 mg/kg) increased exposure to simvastatin relative to administration of simvastatin (25 mg/kg) alone**
- **Increase in skeletal variations (reduced ossification) was observed in the HD combination and simvastatin control group. Bipartite thoracic vertebral centra and sternebrae; shortened ribs and unossified proximal hind paws exceeded the historical mean provided but were within the sponsor's reported range except for the bipartite sternebrae which exceeded historical mean and range. An increased incidence of a skeletal malformation (hemivertebrae) is also observed in the HD combination group.**
- **Visceral exam found an increased incidence of right carotid and subclavian arteries arising from the aortic arch in all combination drug groups and in the HD combination group the right subclavian artery was absent. External exams were unremarkable.**
- **Developmental NOAEL= 1000 mg/kg SCH58235 + 10 mg/kg simvastatin based on reduced fetal weights, visceral findings involving blood vessels and skeletal malformations/variations in combination with 25 mg/kg simvastatin. The maternal NOAEL is at least 1000 mg/kg SCH 58235 + 25 mg/kg simvastatin**

Study no.: 97131
Volume #, and page #: 1.140; pg.1
Conducting laboratory and location: Schering-Plough Safety Evaluation Center; Lafayette, NJ
Date of study initiation: 3/2/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot # and % purity: SCH 58235 98-58235-X-04
Simvastatin 99-57098-X-02
Formulation/vehicle: 0.4% aqueous methylcellulose

Methods:

Species/strain: Crl:CD(SD)IGS BR VAF/plus Sprague-Dawley
Doses employed: 1000 mg/kg SCH 58235 + 5, 10 or 25 mg/kg simvastatin; controls include vehicle and 25 mg/kg simvastatin alone
Basis of dose selection: according to the sponsor a prior pilot study suggested that≥ 50 mg/kg simvastatin in combination with 1000 mg/kg SCH 58235 resulted in pronounced, dose related maternal toxicity. The combination dose with 100

mg/kg simvastatin was sacrificed early due to excessive toxicity. Fetal body weights were reduced in combination groups of ≥25 mg/kg simvastatin.
Route of administration: oral gavage
Study design: daily GD 6-17
Number/sex/group: 25 F for main and 12 F/group for TK
Parameters and endpoints evaluated:

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Simvastatin (SCH 57098) Administered by Oral Gavage in Rats (SN 97131): **Observations and Measurements**	
Investigation	Performed
Clinical Observations	Daily
Body Weight	Gestation Days 0, 6, 9, 12, 15, 17 (and gestation day 21 for toxicity portion animals)
Food Consumption	Recorded for the intervals of gestation Days 0-6, 6-12, 12-17, and 17-21.
Plasma Analysis for SCH 58235, Simvastatin and Hydroxysimvastatin	Gestation Day 17 (1, 2, 4, 6, 8 and 24 hours postdose)
Necropsy[a]/C-Section	Gestation Day 21
Reproductive Parameters	Yes
Fetal Body Weight	Yes
Determination of Fetal Sex	Yes
Fetal External/Visceral/Skeletal Examinations	Yes
a: Toxicokinetic animals were only examined to confirm pregnancy status; no other necropsy examinations were performed.	

Results:

Mortality: Two females in the vehicle control (GD 9,18) and SCH 58235 + simvastatin 25 mg/kg groups (GD 12) were moribund following misdosing. All three had perforation of the esophagus consistent with this diagnosis. Two rats from the MD combination TK group died pre-termination one prior to the 24h sample collection and one prior to the 6h collection. It does not appear that these animals were examined. The sponsor reports no treatment related deaths suggesting that a handling error may have caused the mortality in the TK group.

Clinical signs: discolored tan stool occurred in all females from groups 3-5 from GD7 which was attributed to excretion of unabsorbed SCH 58235, consistent with other studies
Body weight: unremarkable
Food consumption: unremarkable

Toxicokinetics GD17:

Dose Group	Dose (mg/Simvastatin/kg)	Dose (mg SCH 58235/kg)	Mean SCH 58235 AUC (0-8 hr) (ng.hr/mL)		
			Total	Conjugated	Unconjugated
Low-Dose	5	1000	3561	3504	56.7
Mid-Dose	10	1000	4729	4678	52.7
High-Dose	25	1000	8078	8017	61.5

Dose Group	Dose (mg/Simvastatin/kg)	Dose (mg SCH 58235/kg)	Mean AUC (0-8 hr) (ng.hr/mL)	
			Simvastatin	Hydroxysimvastatin
Simvastatin Control	25	0	120	3947
Low-Dose	5	1000	17.5	936
Mid-Dose	10	1000	43.4	2424
High-Dose	25	1000	163	9249

SCH 58235 was extensively conjugated in the pregnant rat. Systemic exposure to unconjugated drug was <2% of total. Systemic exposure to conjugated and unconjugated drug increased with co-exposure to simvastatin ≥5 mg/kg. Systemic exposure to simvastatin and hydroxysimvastatin increased more than dose proportionally. Co-administration of 1000 mg/kg SCH 58235 with 25 mg/kg simvastatin increased mean exposure to simvastatin and hydroxysimvastatin. A simvastatin control and LD combination rat were not pregnant at the time of blood sampling and were therefore not used to assess plasma levels.

For embyrofetal development studies:

In-life observations:

	Vehicle	1000 mg/kg SCH 58235 +Simvastatin @			
		5 mg/kg	10 mg/kg	25 mg/kg	Simvastatin 25 mg/kg
Body Weight (g)	5.6±0.2	5.4±0.31	5.6±0.24	5.5±0.21	5.3*±0.33

Terminal and necroscopic evaluations:

Dams: One female in the LD, two in the HD combination groups and one in the simvastatin control groups had a pair of fused placentas. One vehicle control female had two sets of fused placentas and therefore the sponsor considers this finding incidental. Cesarean evaluations included viable fetuses, sex ratio, corpora lutea, implantation sites, preimplantation loss, postimplantation loss, early/late resorptions and all are unremarkable.

Offspring: External fetal examinations were unremarkable.

Visceral Exam	Vehicle	25 mg/kg simvastatin	1000 mg/kg SCH+ 5 mg/kg simva	1000 mg/kg SCH + 10 mg/kg simva	1000 mg/kg SCH + 25 mg/kg simva

Litters	23	25	24	23	25
fetuses	158	166	157	159	170
R carotid and subclavian arise from aortic arch			1.3% (8.3%)	0.6% (4.3%)	1.8% (8%)
R subclavian absent					0.6% (4%)

Skeletal Exam	Vehicle	25 mg/kg simvastatin	1000 mg/kg SCH+ 5 mg/kg simva	1000 mg/kg SCH + 10 mg/kg simva	1000 mg/kg SCH + 25 mg/kg simva
Litter	23	25	24	23	25
Fetuses	147	156	144	148	155
Malformation: Hemivertebrae					0.6% (4%)
Variations					
Cervical vertebral centra asymmetrical		0.6% (4%)			1.9% (4%)
Thoracic vertebral centra bipartite	0.7% (4.3%)	0.6% (4%)		0.7% (4.3%)	3.2% (20%)
Thoracic vertebral centra reduced ossif.					0.6% (4%)
Sternebra bipartite		0.6% (4%)			1.90% (12%)
Extra single thoracic rib	2% (8.7%)	3.8% (20%)	0.7% (4.2%)	3.4% (13%)	2.6% (16%)
Shortened ribs			0.7% (4.2%)	0.7% (4.3%)	1.3% (4%)
Unossified proximal phalanges hind paws	18.4% (52.2%)	26.9% (68%)	29.9% (58.3%)	34.5% (65.2%)	43.9% (72%)

( )= litter incidence

Skeletal variations occurred in all groups e.g. unossified phalanges (hind paws), vertebral centra and bipartite thoracic centra were indicative of delayed ossification. The

incidence was increased in the HD combo group and with simvastatin alone and correlates with the slightly lower fetal weights in these groups according to the sponsor.

Five pups had soft tissue malformations. Two pups in the simvastatin control had moderate dilation of the ventricles in the brain and one pup form the MD combo had slight constriction of the innominate artery, two control pups had had similar findings. One of the two pups had a dilated third ventricle (litter 1) and the other (litter 14) had other malformations which included a small (1/3 size) pale and rounded heart and a left carotid artery which was in juxtaposition to the innominate artery at its origin. One pup for the HD combo had a skeletal malformation , a hemivertebrae (7th thoracic). The sponsor does not consider these malformations drug related.

Sponsor Historical Control	Fetuses			Litter	
Skeletal Findings	%	Range	Mean	%	Range
Thoracic vertebral centra bipartite	1	0-4.4	1±4.38	5.8	0-20.8
Sternebrae bipartite	0.5	0-1.2	0.4±2.34	3.3	0-8.3
Extra single thoracic rib	2.9	0-5.9	2.8±7.67	14.9	0-33.3
Shortened ribs	0.3	0-1.3	0.3±2.25	1.7	0-8
Unossified proximal phalanges Hind paws	25.6	0-44.6	24.7±30.92	50.8	0-87.5

The sponsor provided historical control data from 242 litters containing ~1708 fetuses for skeletal findings only.

- **Summary of individual study findings:** Systemic exposure to unconjugated SCH 58235 was <2% total drug, indicating extensive conjugation in the pregnant rat
- Co-administration of 1000 mg/kg SCH 58235 with ≥5 mg/kg simvastatin increased mean systemic exposure to total and conjugated SCH 58235. Mean systemic exposure to, hydroxysimvastatin increased in a greater than dose proportional manner. Co-administration of 1000 mg SCH 58235 with simvastatin (25 mg/kg) increased exposure to simvastatin relative to administration of simvastatin (25 mg/kg) alone
- Increase in skeletal variations (reduced ossification) was observed in the HD combination and simvastatin control group. Bipartite thoracic vertebral centra and sternebrae; shortened ribs and unossified proximal hind paws exceeded the historical mean provided but were within the sponsor's reported range except for the bipartite sternebrae which exceeded historical mean and range. An increased incidence of a skeletal malformation (hemivertebrae) is also observed in the HD combination group.
- Visceral exam found an increased incidence of right carotid and subclavian arteries arising from the aortic arch in all combination drug groups and in the HD combination group the right subclavian artery was absent. External exams were unremarkable.
- Developmental NOAEL= 1000 mg/kg SCH58235 + 10 mg/kg simvastatin based on reduced fetal weights, visceral findings involving blood vessels and skeletal malformations/variations in combination with 25 mg/kg simvastatin. The maternal NOAEL is at least 1000 mg/kg SCH 58235 + 25 mg/kg simvastatin

4. Embryo-Fetal Developmental Toxicity & TK of SCH58235 + Simvastatin (SCH 57098) by Oral Gavage to Rabbits

Key study findings:

- **Mean systemic exposure to hydroxysimvastatin was 87-147 X greater than to simvastatin. Coadministration of SCH 58235 with simvastatin 10 mg/kg had no effect on the exposure to simvastatin or hydroxysimvastatin.**
- **ALT was minimally increased with 1 mg/kg simvastatin + 1000 mg/kg SCH (68 vs. 32 IU/L baseline) and mildly increased for all other groups including simvastatin alone on GD 20 (92-172 IU/L vs. baseline 28-35 IU/L). Some animals reached moderate levels (max. 489 IU/L) approximately 10X increase from baseline. Similarly AST was minimally increased for all groups (47-56 IU/L) including the simvastatin alone group compared to baselines of 26.3-26.6 IU/L.**
- **5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235 (2/18 litters in both groups with 5 fetuses and 2 fetuses effected in the 5 and 10 mg/kg groups respectively) had short, filamentous tails with fused caudal vertebra and reduced numbers of caudal vertebra.**
- **Heart malformations were observed in two fetuses each of the 5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235 combo groups. In the HD combo one fetus had a small heart with a ventricular septum defect (membranous) and a small atrial chamber and the other fetus had a small atrial chamber. In the MD combo one fetus had a ventricular septum defect (membranous and muscular) and thickened ventricular wall, with one small and one enlarged atrial chamber, and another fetus had an enlarged atrial chamber. Skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fuse and/or bifurcated ribs were present in the HD combo.**
- **NOAEL not established for maternal toxicity based on transient food consumption and fecal changes according to the sponsor. However these effects were transient. Greater concern with premature deaths of 2 dams in the HD combination, suggesting that the maternal NOAEL=1000 mg/kg SCH 5832 +1 mg/kg simvastatin.**
- **Developmental NOAEL=10 mg/kg for simvastatin and <1 mg/kg simvastatin + 1000 mg/kg SCH 58235 based on increased external and visceral malformation compared to controls.**

Study no.: 97133
Volume #, and page #: 1.149-1.151 pg.1
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 1/16/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH ——
SCH 58235 98-58235-X-04 ; simvastatin 99-57098-X-02
Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: NZW

Doses employed: 10 mg/kg simvastatin or 1, 5, 10 mg/kg simvastatin + 1000 mg/kg SCH 58235
Route of administration: oral gavage
Studydesign:

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Simvastatin (SCH 57098) Administered by Oral Gavage in Rabbits (SN 97133): **Study Design**

Group	Test/Control Article	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)[a]	Dose Conc. (mg/ml)	Number of Rabbits	
					Toxicity	Toxicokinetic[b]
1	Vehicle Control:				20	0
	0.4% Methylcellulose	0	6	0		
2[c]	Simvastatin Control:				20	4
	0.4% Methylcellulose	0	5	0		
	SCH 57098	10	1	10		
3	Low-Dose Combination:				20	4
	SCH 58235	1000	5	200		
	SCH 57098	1	1	1		
4	Mid-Dose Combination:				20	4
	SCH 58235	1000	5	200		
	SCH 57098	5	1	5		
5[c]	High-Dose Combination:				20	4
	SCH 58235	1000	5	200		
	SCH 57098	10	1	10		
6[d]	Vehicle Control:				20	0
	0.4% Methylcellulose	0	6	0		
7[d]	Simvastatin Control:				20	0
	0.4% Methylcellulose	0	5	0		
	SCH 57098	10	1	10		
8[d]	High-Dose Combination:				20	0
	SCH 58235	1000	5	200		
	SCH 57098	10	1	10		

a: Groups 2 through 5, and 7 and 8 were administered two doses in succession at 5 and 1 ml/kg per dose for a total daily dose of 6 ml/kg.

b: These animals were used only for blood collection to determine plasma concentrations of test articles.

c: From January 22 through 25, 2000, Toxicity and Toxicokinetic animals in Groups 2 (simvastatin control) and 5 (high-dose combination) were dosed with 7.14 mg/kg instead of 10 mg/kg simvastatin. Thereafter, the animals were dosed with their correct dose of 10 mg/kg simvastatin. Toxicity and Toxicokinetic animals in Groups 2 and 5 were bled at their scheduled blood collections for serum chemistry analysis on gestation Day 20 (January 31, February 1/7/8, 2000) and plasma analysis of test article(s) on gestation Days 19/20 (February 1/2, 2000), respectively. All animals in Groups 2 and 5 were then sacrificed with no collection of reproduction or fetal data.

d: The simvastatin control (Group 7) and high-dose combination (Group 8) groups were repeated along with a corresponding vehicle control (Group 6).

Due to incorrect dose of simvastatin Groups 2& 5 were terminated without exam and replaced by Group 7&8.

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Simvastatin (SCH 57098) Administered by Oral Gavage in Rabbits (SN 97133): **Observations and Measurements**			
Investigation	Performed	Investigation	Performed
Clinical Observations	Daily	Plasma SCH 58235/Simvastatin (SCH 57098) Analysis	1, 2, 4, 6, 12 and 24 hours postdose on Gestation Day 19 (Toxicokinetic animals only)
Body Weight	Gestation Days 2, 7, 10, 13, 16, 19, 22, 25, 28 and 30 (up to Gestation Day 19 for Toxicokinetic animals)	Reproductive Parameters	Yes (Toxicity animals with the exception of Groups 2 and 5)
Food Consumption (estimated)	Daily (Toxicity animals only)	Fetal Body Weight	Yes (Toxicity animals with the exception of Groups 2 and 5)
Serum Chemistry (ALT, AST, Hemolysis, Lipemia, Icterus)	Gestation Days 6 and 20 (Toxicity animals in Groups 1 through 5 only)	Determination of Fetal Sex	Yes (Toxicity animals with the exception of Groups 2 and 5)
Necropsy/C-Section	Gestation Day 30 (19 or 20 for Toxicokinetic animals)	Fetal External/Visceral/ Skeletal Examinations	Yes (Toxicity animals with the exception of Groups 2 and 5)

Results:

Mortality: One HD combo dam (group 8) was found dead on GD29. Death was preceded by a scab on dewlap on GD 27 and no food consumed GD27-29 (body weight –3% GD25-29). The sponsor attributes the scab to mechanical injury and notes that cause of death could not be determined by necropsy. Another HD combo dam (group 5) lost 22% of its GD2 body weight and was sacrificed GD 13. Clinical signs included fecal stained fur and no stool GD 9-13, pale eyes GD 13. Cause of health decline is uncertain according to the sponsor.

Clinical signs: Discolored (light green) stool with SCH 58235 + simvastatin, but not simvastatin alone. This was previously reported in rabbits in combination studies and is attributed to elimination of unabsorbed SCH 58235 as with rats. All groups administered SCH 58235 + 5/10 mg/kg simvastatin had a reduced number of fecal pellets, soft stool and small fecal pellets.

Body weight: unremarkable except for pre-terminated dams (see mortality)

Food consumption: Within 1-2 days post dose, co-administration of 5 and 10 mg/kg simvastatin + SCH 58235 had reduced food consumption thereafter no effect on food.

Toxicokinetics:

Dose (mg/kg) SCH 58235/Simvastatin	SCH 58235 AUC(0-24 hr) (ng·hr/ml)					
	Total		Conjugated		Unconjugated	
	Mean	%CV	Mean	%CV	Mean	%CV
1000/1	99325[a]	9	99213[a]	9	114[a]	36
1000/5	103768[b]	7	102572[b]	7	103[b]	74
1000/10	114200[b]	25	114015[b]	25	181[b]	45

a: n=4
b: n=3 (one rabbit from Group 4 and another rabbit from Group 5 were not pregnant at the time of plasma sample collection, so their plasma samples were not assayed).

Dose (mg/kg) SCH 58235/Simvastatin	AUC(tf) (ng·hr/ml)			
	Simvastatin		Hydroxysimvastatin	
	Mean	%CV	Mean	%CV
1000/1	0.889[a]	166	131[a]	42
1000/5	11.1[b]	20	1368[b]	28
1000/10	20.3[b]	65	1808[b]	24
0/10	21.0[a]	52	1837[a]	45

a: n=4
b: n=3 (one rabbit from Group 4 and another rabbit from Group 5 were not pregnant at the time of plasma sample collection, so their plasma samples were not assayed).

Terminal and necroscopic evaluations:

Dams: HD combo 1/20 dams with mottled lungs and 1/20 absent left uterine horn, different dams.

Dose (mg/kg/day)	Control (gp 1)	1000 SCH + 1 Simvastatin (gp 3)	1000 SCH+ 5 Simvastatin (gp 4)	Control (group 6)	10 mg/kg/d Simvastatin (group 7)	1000 SCH + 10 Simvastatin (group 8)
Male live fetuses	37.3%	47.7%	54.8%**	56.9%	49.3%	51.2%
Female live fetuses	62.7%	52.3%	45.2%**	43.1%	50.7%	48.8%

Offspring:

Doses (mg/kg/day)	Control (gp1)	1000 SCH + 1 Simvastatin (gp 3)	1000 SCH + 5 Simvastatin (gp 4)	Control (gp6)	10 Simvastatin (gp7)	1000 SCH + 10 Simvastatin (gp 8)
External		0.6%	3.2%			2.3%

Malformations Fetal incidence Litter incidence		5.6%	11.1%			11.8%
Short tail			2.5% 11.1%			
Short filamentous Tail			0.6% 5.6%			0.8% 5.9%
Umbilical hernia						0.8% 5.9%
Kinked tail						0.8% 5.9%
Visceral Malformations Fetal incidence Litter incidence		0.6% 5.6%	1.9% 11.1%			1.5% 11.8%
For heart malformations see text-multiple fetal findings						
Enlarged atria			1.3% 11.1%			
Thick ventricular wall			0.6% 5.6%			
Ventricular septum defect			0.6% 5.6%			0.8% 5.9%
Small atrial chamber			0.6% 5.6%			1.5% 11.8%
Small heart						0.8% 5.9%
Hydronephrosis			0.6% 5.6%			
Dilated renal pelvis			0.6% 5.6%			
Convoluted ureter			0.6% 5.6%			
Visceral Variations	16.9% 40%	11.3% 27.8%	12.7% 33.3%	63.9% 95%	42.1% 83.3%	45.1% 82.4%

Fetal incidence Litter incidence						
Skeletal Malformations Fetal incidence Litter incidence	0.7% 6.7%	0.6% 5.6%	4.5% 16.7%	0.6% 5%		2.3% 11.8%
Caudal vertebrae reduced #			0.6% 5.6%			
Caudal vertebrae fused			3.2% 11.1%			1.5% 11.8%
hemivertebrae				0.6% 5%		1.5% 5.9%
Scoliosis						0.8% 5.9%
Lumbar vertebrae scrambled						0.8% 5.9%
Bifurcated ribs						0.8% 5.9%
Skeletal Variations Fetal incidence Litter incidence	81.6% 100%	93.5% 100%	91.1% 100%	80.5% 100%	86% 100%	97% 100%

The sponsor does not provide an explanation for the large increase in total visceral variations in control group 6 compared to control group 1 however the respective treatment group incidences are below that of their respective controls. Heart malformations were observed in two fetuses each of the MD, HD combo groups. In the HD combo one fetus had a small heart with a ventricular septum defect (membranous) and a small atrial chamber and the other fetus had a small atrial chamber. In the MD combo one fetus had a ventricular septum defect (membranous and muscular) and thickened ventricular wall, with one small and one enlarged atrial chamber, and another fetus had an enlarged atrial chamber. Heart findings have not been observed previously with SCH 58235 or simvastatin alone or with SCH 58235 co-administered with lova, prava or atorva in rabbits according to the sponsor. The sponsor does not attribute these findings to the test articles. Single observances of extreme hydronephrosis, dilated pelvis and a dilated and convoluted ureter for one fetus was not considered test article related. Skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fuse and/or bifurcated ribs were of low incidence, not dose related and

considered unrelated to test article according to the sponsor. Historical control data has not been provided.

Summary of individual study findings: Mean systemic exposure to hydroxysimvastatin was 87-147 X greater than to simvastatin. Co-administration of SCH 58235 with simvastatin 10 mg/kg had no effect on the exposure to simvastatin or hydroxysimvastatin.
ALT was minimally increased with 1 mg/kg simvastatin + 1000 mg/kg SCH (68 vs. 32 IU/L baseline) and mildly increased for all other groups including simvastatin alone on GD 20 (92-172 IU/L vs. baseline 28-35 IU/L). Some animals reached moderate levels (max. 489 IU/L) approximately 10X increase from baseline. Similarly AST was minimally increased for all groups (47-56 IU/L) including the simvastatin alone group compared to baselines of 26.3-26.6 IU/L.
In combination groups of 5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235 (2/18 litters in each group; 5 fetuses and 2 fetuses effected in the 5 and 10 mg/kg groups respectively) short, filamentous tails with fused caudal vertebra and reduced numbers of caudal vertebra were observed and considered treatment related.
Multiple heart malformations were observed in two fetuses each of the 5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235 combo groups. In the HD combo one fetus had a small heart with a ventricular septum defect (membranous) and a small atrial chamber and the other fetus had a small atrial chamber. In the MD combo one fetus had a ventricular septum defect (membranous and muscular) and thickened ventricular wall, with one small and one enlarged atrial chamber, and another fetus had an enlarged atrial chamber. Heart findings have not been observed with in prior studies with SCH 5832 or in combination with statins. Skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fused and/or bifurcated ribs were present in the HD combo. Single observances of extreme hydronephrosis, dilated pelvis and a dilated and convoluted ureter for one fetus was not considered test article related. Skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fuse and/or bifurcated ribs were of low incidence, not dose related and considered unrelated to test article according to the sponsor. Historical control data has not been provided. Transient decreases in food consumption along with fecal changes were observed in dams. Premature death was observed in 2 HD combo treated dams, suggesting that the maternal NOAEL=1000 mg/kg SCH 5832 +1 mg/kg simvastatin. The developmental NOAEL=10 mg/kg for simvastatin and <1 mg/kg simvastatin + 1000 mg/kg SCH 58235 based on increased external and visceral malformation compared to controls.

5. Embryo Fetal Developmental Toxicity and TK of SCH 58235 and Atorvastatin (SCH 412387) by Oral Gavage in Rats

Key study findings:

- **Co-administration of atorvastatin increased conjugate (major exposure), unconjugated and total SCH 58235. The extent of SCH 58235 conjugation was not influenced by co-administration with atorvastatin**
- **Systemic exposure to atorvastatin free acid did not increase with co-administration with SCH 58235. Exposure to the free acid and ortho-hydroxy atorvastatin was decreased by co-administration of SCH 58235. Exposure to ortho-hydroxy atorvastatin = free acid exposure whereas exposure to para-hydroxy atorvastatin < exposure to free acid, ortho-hydroxy at all dose levels.**

The extent of free acid metabolite formation was not influenced by co-administration of atorvastatin with SCH 58235.

- **Decreases in maternal food consumption and body weight gain with HD combination**
- **Decreased mean body weight of fetuses in the HD combination group**
- **Increased incidence of skeletal variation- reduced ossification of sternebrae in the HD combination group which sponsor attributes to decreased fetal body weight**
- **Maternal/Developmental NOAEL=1000 mg/kg SCH 58235 + 54.3 mg/kg atorvastatin based on decreased maternal/fetal body weight and increased incidence of reduced ossification of sternebrae**

Study no.: 99506
Volume #, and page #: 1.146, pg.1
Conducting laboratory and location: Schering-Plough Research Institute Safety Evaluation Center, Lafayette, NJ
Date of study initiation: 6/20/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot # and % purity: 99-58235-X-02; — (SCH58235)
Atorvastatin 76590-003
Formulation/vehicle: 0.4% methylcellulose; 10 ml/kg to control and others 5 ml/kg

Methods:

Species/strain:Crl:CD(SD_IGSBR VAF/Plus rat
Doses employed: 1000 mg/kg of SCH 58235 + 27.1/54.3/108.6 mg/kg atorvastatin calcium trihydrate an additional control of 108.6 mg/kg atorvastatin was included
Route of administration: oral gavage
Study design: daily GD 6-17
Number/sex/group: 25 and 12/sex/group for TK
Parameters and endpoints evaluated:

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Atorvastatin (SCH 412387) Administered Orally by Gavage in Rats (SN 99506): **Observations and Measurements**

Investigation	Performed
Viability	Daily
Clinical Observations	Daily, beginning gestation Day 0
Body Weight	Gestation Days 0, 6, 9, 12, 15, 17 and 21
Food Consumption	Gestation Days 0-6, 6-12, 12-17 and 17-21
Plasma Analysis for SCH 58235 and Atorvastatin	Gestation Day 17 (1, 2, 4, 6, 8 and 24 hours postdose)
Necropsy[a]/C-Section	Gestation Day 21
Reproductive Parameters	Yes
Fetal Body Weight	Yes
Fetal Sex Determination	Yes
Fetal External/Visceral/Skeletal Examinations	Yes

a: Toxicokinetic animals were examined after the final blood sampling only to confirm pregnancy status; no other necropsy examinations were performed.

Results:

Mortality: 1 HD combination group dam due to gavage error (perforated neck muscles/esophagus, congested lungs and white substance under pectoral muscles and in the thoracic cavity).

Clinical signs: Tan stool was observed in all animals of all groups given SCH58235 and has been previously reported and is considered related to the excretion of SCH58235

Body weight: A 10% decrease in mean gestation body weight gain in the 1000/108.6 mg/kg SCH58235+ atorvastatin group (69 vs. 62 g control) GD6-17

Food consumption: An 8.3% decrease in daily food consumption in the 1000/108.6 mg/kg SCH58235+atorvastatin group during GD 6-17 (22 vs. 24 g/rat/day control).

Toxicokinetics:

Table 1 Pharmacokinetic Parameters of Total, Conjugated and Unconjugated SCH 58235 on Gestation Day 17 Following Multiple Oral (Gavage) Administration (Gestation Days 6-17) of 27.1, 54.3 or 108.6 mg/kg Atorvastatin Calcium with 1000 mg/kg SCH 58235 to Female Rats

		Total SCH 58235		
Parameter	Unit	1000/27.1[a]	1000/54.3	1000/108.6
Cmax	ng/mL	1675	1984	6009
Tmax	hr	1	1	2
AUC(0-24 hr)	ng·hr/mL	8597	21324	66198
		Conjugated SCH 58235[b]		
Parameter	Unit	1000/27.1	1000/54.3	1000/108.6
Cmax	ng/mL	1662	1972	5989
Tmax	hr	1	1	2
AUC(0-24 hr)	ng·hr/mL	8520	21144	65929
% Total[c]	%	99.1	99.2	99.6
		Unconjugated SCH 58235		
Parameter	Unit	1000/27.1	1000/54.3	1000/108.6
Cmax	ng/mL	12.7	15.3	25.1
Tmax	hr	1	2	6
AUC(0-24 hr)	ng·hr/mL	75.8[d]	180	271
% Total[e]	%	0.9	0.8	0.4

a: Dose SCH 58235/Dose Atorvastatin Calcium (as mg/kg). Doses of 27.1, 54.3 and 108.6 mg/kg atorvastatin calcium are equivalent to 25, 50 and 100 mg/kg atorvastatin free acid, respectively.

b: Calculated as plasma total SCH 58235 minus plasma unconjugated SCH 58235, and reported as SCH 58235 equivalents.

c: Calculated as [conjugated SCH 58235 AUC(0-24 hr)/total SCH 58235 AUC(0-24 hr)]*100

d: %ext = 27%.

e: Calculated as [unconjugated SCH 58235 AUC(0-24 hr)/total SCH 58235 AUC(0-24 hr)]*100

Table 2 Pharmacokinetic Parameters of Atorvastatin, Para-hydroxy atorvastatin and Ortho-hydroxy atorvastatin on Gestation Day 17 Following Multiple Oral (Gavage) Administration (Gestation Days 6-17) of 108.6 mg/kg Atorvastatin Calcium Alone or 27.1, 54.3 or 108.6 mg/kg Atorvastatin Calcium with 1000 mg/kg SCH 58235 to Female Rats

		Atorvastatin			
Parameter	Unit	0/108.6[a]	1000/27.1	1000/54.3	1000/108.6
Cmax	ng/mL	3466	419	1012	1232
Tmax	hr	2	1	2	2
tf	hr	24	8	24	24
AUC(0-8 hr)	ng·hr/mL	9650	746	3583	5511
AUC(0-24 hr)	ng·hr/mL	13007	849	6150	7910
AUC (0-24 hr)/D	(ng·hr/mL)/(mg/kg)	130	34.0	123	79.1
		Para-hydroxy atorvastatin			
Parameter	Unit	0/108.6	1000/27.1	1000/54.3	1000/108.6
Cmax	ng/mL	455	58.7	198	302
Tmax	hr	2	1	2	6
tf	hr	8	8	8	24
AUC(0-8 hr)	ng·hr/mL	2005	184	894	1452
AUC(0-24 hr)	ng·hr/mL	3291[b]	257[c]	1579[d]	2825
AUC (0-24 hr)/D	(ng·hr/mL)/(mg/kg)	32.9	10.3	31.6	28.2
Metabolite Ratio[e]		0.25	0.30	0.26	0.36
		Ortho-hydroxy atorvastatin			
Parameter	Unit	0/108.6	1000/27.1	1000/54.3	1000/108.6
Cmax	ng/mL	1674	237	918	1069
Tmax	hr	2	1	2	6
tf	hr	24	24	24	24
AUC(0-8 hr)	ng·hr/mL	7675	945	3489	4516
AUC(0-24 hr)	ng·hr/mL	12175	1172	6950	7674
AUC (0-24 hr)/D	(ng·hr/mL)/(mg/kg)	122	46.9	139	76.7
Metabolite Ratio[f]		0.94	1.38	1.13	0.97

a: Dose SCH 58235/Dose Atorvastatin Calcium (as mg/kg). Doses of 27.1, 54.3 and 108.6 mg/kg atorvastatin calcium are equivalent to 25, 50 and 100 mg/kg atorvastatin free acid, respectively.

b: %ext = 39%

c: %ext = 28%

d: %ext = 43%

e: Calculated as [Para-hydroxy atorvastatin AUC(0-24 hr)]/[Atorvastatin AUC(0-24 hr)]

f: Calculated as [Ortho-hydroxy atorvastatin AUC(0-24 hr)]/[Atorvastatin AUC(0-24 hr)]

For fertility studies:

In-life observations: Reproductive parameters (corpora lutea, implantations, pre-/post-implantation loss, live fetuses, fetal sex, early/late resorptions) were unremarkable

Terminal and necroscopic evaluations:

For embyrofetal development studies:

In-life observations: Mean fetal body weights were decreased 3.6% in female and 3.4% in male fetuses in the 1000/108.6 mg/kg SCH58235+atorvastatin group.

Terminal and necroscopic evaluations:

Dams:
Offspring: External malformations/variations were unremarkable. Visceral malformations were unremarkable.

	Vehicle	SCH 58235 + Atorvastatin (mg/kg/day)			
	0+ 0	0+108.6	1000+ 27.1	1000 +54.3	1000+ 108.6
%Visceral variations Fetal, (litter)	5.2 (24)	7.5 (32)	5.8 (25)	7 (25)	6.2 (23)
Convoluted ureter	5.2 (24)	5.6 (32)	5.8 (25)	6.4 (25)	6.2 (23)
Dilated ureter	0.7 (4)	3.1 (8)	1.9 (12.5)	1.3 (8.3)	2.8 (9.1)
% Skeletal variations fetal (litter)	69 (100)	69.6 (100)	68.2 (95.8)	65.2 (100)	78.5 (100)
Parietals ↓ossif.	1.3 (8)	1.9 (8)	4.5 (12.5)	3.9 (16.7)	3.5 (18.2)
Cervical vertebral centra Unossif.	61.3 (100)	59.5 (96)	52.9 (91.7)	58.7 (100)	69.4 (100)
Cervical vertebral centra bipartite	1.9 (12)	5.7 (32)	5.7 (29.2)	4.5 (29.2)	5.6 (31.8)
Sternebra ↓ossif.	1.9 (8)	4.4 (20)	4.5 (25)	1.9 (8.3)	9 (45.5)
Extra thoracic rib	2.6 (16)	1.9 (8)	1.3 (8.3)	2.6 (16.7)	4.2 (13.6)
Proximal phalanges hind paws Unossificatio n	1.3 (8)	5.1 (20)	10.2 (37.5)	4.5 (20.8)	16.7 (40.9)

Increased percentage of litters with reduced ossification of the sternebrae (45.5% vs. 8% in controls) in the 1000/108.6 mg/kg/day SCH58235+atoravastatin group. The sponsor attributes the reduced ossification with the decreased fetal body weight. The incidence of other skeletal variations (cervical vertebral centra bipartite, unossified hind paw proximal phalanges) were not considered dose related since the incidence lacked a dose response and fell within the historical control range according to the sponsor. Decreased or lack of ossification of: parietals, cervical vertebral centra and proximal phalanges of hind paws in addition to extra thoracic ribs and cervical vertebral centra bipartite are increased in combination treatment groups compared to vehicle and show higher incidence in the HD combination than atorvastatin alone. The increase looks dose related and may be attributed to a threshold effect rather than a true dose response. Except for the extra thoracic rib and unossified proximal phalanges of the hind paws, the other skeletal variations appear at incidences greater than the historical mean but within the range. The incidence of extra thoracic rib is greater than the

historical mean for the HD combination and the incidence of unossified proximal phalanges of the hind paws is within the historical mean and range.

Sponsor's Historical Control	Fetuses			Litter	
	%	Range	Mean	%	Range
Parietals, reduced ossfication	2	0-6.4	2±7.73	9.1	0-25
Cervical vertebrae centra unossif.	47.8	20.1-74.1	48.6± 33.9	83.9	58.3-100
Cervical vertebrae centra bipartite	4.4	0-11.9	4.6±9.15	24	0-60
Sternebra reduced ossification	137	1.9-19.8	8±13.3	34.7	8-76
Extra thoracic rib	2.9	0-5.9	2.8±7.67	14.9	0-33.3
Unossified proximal phalanges hindpaws	25.6	0-44.6	24.7± 30.9	50.8	0-87.5

Summary of individual study findings: The 10% decrease in mean body weight gain during dosing in the HD combination group correlates with the 8.3% decline in food consumption over the same period.

6. Embryo Fetal Developmental Toxicity and TK of SCH 58235 and Atorvastatin (SCH 412387) by Oral Gavage in Rabbits

Key study findings:

- **Coadministration of SCH 58235 with atorvastatin 50 mg/kg (323 ng.h/ml) increased the mean systemic exposure vs atorvastatin alone 50 mg/kg/day (214 ng.h/ml) and also to ortho-hydroxy atorvastatin by 2.5 fold.**
- **Coadministration of two drugs did not significantly effect the systemic exposure to SCH 58235 (1000 mg/kg/day), values were 113 µg.h/ml from a previous study with SCH 58235 alone vs 124-149 µg.h/ml in the current study with combos.**
- **All combo groups had higher fecal findings (reduced number and small fecal pellets) than the atorvastatin alone or the control group.**
- **In the high dose combo group, BW gain was significantly decreased during gestation by 38%. Food consumption was decreased in all combo groups by 30%, 33%, and 53% respectively.**
- **50 mg/kg atorvastatin + 1000 mg/kg SCH 58235 produced external malformations of kinked tail (1.3 % in fetuses and 5.9% in litters.**
- **A MD and/or HD combo increased the incidences of visceral malformations in fetal rabbits such as gallbladder absent, or ectopic/misshapen kidney (31.6% vs 22% with prava alone)**
- **Skeletal malformations were increased at all combo doses vs the control or atorvastatin group. These included caudal vertebra fused, sternebra fused. These were also observed with combo of drug +simva, and drug + prava in rabbits, and were not present in the historical control data. The HD combo also increased skeletal variations (sternebra assymmetrical)**

- **No NOAEL for maternal toxicity could be establshed with atorvastatin + 1000 mg/kg SCH 58235, as all combo doses produced a significant decrease in FC and high dose combo decreased BW gain. Materanl NOAEL was <5 mg/kg/day of atorvastatin +1000 mg/kg/day SCH 58235 in rabbits.**
- **Developmental NOAEL= <5 mg/kg atorvastatin + 1000 mg/kg SCH 58235, as all combo doses (LD, MD & HD) produced skeletal malformations (fused caudal vertebrae and sternabra). HD combo produced external malformations (kinked tail), and visceral malformations compared to atorvastatin or control group.**

Study no.: 99507
Volume #, and page #: 1.159-1.160 pg.1
Conducting laboratory and location: Safety Evaluation Center/Schering-Plough Research Center; Layfayette, NJ
Date of study initiation: 5/31/00
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH 58235 ——— 99-58235-X-02 ; atorvastatin calcium (SCH 412387, contains —— free acid) 76590-003
Formulation/vehicle: 0.4% aq. methylcellulose

Methods:

Species/strain: NZW
Doses employed:
54.5 mg/kg/day of atorvastatin calcium trihydrate (or 50 mg/kg of atoravastatin free acid) or 5.5, 27.3, 54.5 mg/kg/day of atorvastatin calcium (or 5, 25, 50 mg/kg atorvastatin free acid) + 1000 mg/kg SCH 58235. Control animals received the vehicle. Females were dosed from gestation days 7 through 19.
Route of administration: oral gavage
Study design:

Table: study design, observations and measurements

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Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Atorvastatin (SCH 412387) Administered Orally by Gavage in Rabbits (SN 99507): **Study Design**

Group	Test/Control Article	Total Daily Dose (mg/kg)[a]	Dose Volume (ml/kg)[b]	Dose Conc. (mg/ml)	Number of Females: Toxicity Portion	Number of Females: Toxicokinetic Portion[c]
1	Control: 0.4% Methylcellulose	0	8	0	20	0
2	Atorvastatin Control: 0.4% Methylcellulose Atorvastatin	0 54.5	5 3	0 18.2	20	4
3	Low-Dose Combination: SCH 58235 Atorvastatin	1000 5.5	5 3	200 1.8	20	4
4	Mid-Dose Combination: SCH 58235 Atorvastatin	1000 27.3	5 3	200 9.1	20	4
5	High-Dose Combination: SCH 58235 Atorvastatin	1000 54.5	5 3	200 18.2	20	4

a: Expressed as atorvastatin calcium (trihydrate). Atorvastatin calcium (trihydrate) contains ⅛ of atorvastatin free acid. The dose levels of atorvastatin free acid in Groups 2, 3, 4 and 5 are 50, 5, 25 and 50 mg/kg.

b: All animals were administered two doses in succession for a total daily dose of 8 ml/kg.

c: Pregnant animals were evaluated for toxicokinetic parameters only.

Embryo-Fetal Developmental Toxicity and Toxicokinetic Study of SCH 58235 and Atorvastatin (SCH 412387) Administered Orally by Gavage in Rabbits (SN 99507): **Observations and Measurements**

Investigation	Performed
Viability	Daily
Clinical Observations	Daily, beginning gestation Day 2
Body Weight	Gestation Days 2, 7, 10, 13, 16, 19, 22, 25, 28 and 30
Food Consumption (estimated)	Daily, gestation Days 3 to 30
Necropsy[a]/C-Section	Gestation Day 30
Plasma Analysis for SCH 58235 and Atorvastatin	Gestation Day 19 (1, 2, 4, 6, 8 and 24 hrs. postdose)
Reproductive Parameters	Yes
Fetal Body Weight	Yes
Fetal Sex Determination	Yes
Fetal External/Visceral/Skeletal Examinations	Yes

a: Toxicokinetic animals were examined after the final blood sampling only to confirm pregnancy status; no other necropsy examinations were performed.

Results:

Mortality: None