Clinical signs: In all combo groups, increased number of animals had reduced number of fecal pellets (frequency/animals incidences were 0/0, 2/1, 12/6, 25/7, 21/8 at 0, atorvastatin 50 mg/kg, and with SCH 58235/atorva 1000/5, 1000/25, 1000/50 mg/kg/day respectively). Size of fecal pellets was reduced (incidences were 0/0, 1/1, 18/2, 12/3, 10/5 respectively). The incidences of pale stool in combo groups (270/20, 291/20, 295/20 respectively) with SCH 58235 was attributed to elimination of unabsorbed SCH 58235 in the feces. However all groups administered the combination (SCH 58235 + atoravastatin) had higher incidences of stool related findings (a reduced number and small fecal pellets).

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

SUMMARY OF CLINICAL OBSERVATIONS DURING GESTATION (frequency / animals)

	GROUP 1 0.4% MC 0 MPK	GROUP 2 0 MPK 0.4% MC 54.5 MPK ATORVA	GROUP 3 1000 MPK SB235 5.5 MPK ATORVA	GROUP 4 1000 MPK SB235 27.3 MPK ATORVA	GROUP 5 1000 MPK SB235 54.5 MPK ATORVA
DAY 2 to 30					
Normal					
NO REMARKABLE CLINICAL OBSERVATIONS	529/20	481/20	262/20	244/20	238/20
Dead					
SCHEDULED SACRIFICE	20/20	20/20	20/20	20/20	20/20
Miscellaneous					
GELATINOUS WHITE MATERIAL IN LITTER PAN	0/ 0	0/ 0	0/ 0	4/ 1	0/ 0
Skin/Fur					
SCABS	0/ 0	8/ 2	5/ 1	19/ 2	17/ 2
WOUNDS, CUT, SCRATCHES	0/ 0	4/ 2	0/ 0	1/ 1	1/ 1
ALOPECIA	22/ 4 a	80/10 a	35/ 6	54/ 3 a	78/ 6
Stool/Urine					
REDUCED NUMBER OF FECAL PELLETS	0/ 0	2/ 1	12/ 6	25/ 7	21/ 8
SMALL FECAL PELLETS	0/ 0	1/ 1	18/ 2	12/ 3	10/ 5
SOFT STOOL	8/ 4	6/ 4	15/ 4	16/ 4	11/ 5
PALE STOOL	0/ 0	0/ 0	270/20	291/20	295/20
FECAL STAINED INGUINAL FUR	28/ 3	4/ 2	14/ 3	18/ 1	2/ 1
FECAL STAINED TAIL FUR	1/ 1	5/ 1	11/ 3	35/ 3	0/ 0
MUCOID STOOL	0/ 0	0/ 0	2/ 1	5/ 4	0/ 0
URINE-STAINED TAIL FUR	0/ 0	1/ 1	3/ 2	5/ 1	0/ 0

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, 0.17, 0.14, 0.15, 0.10 kg respectively). The mean BW change was lower in HD combo group by 38% (360, 378, 325, 386, 308 g respectively). The gravid uterine weights were not different from the control groups.

Food consumption: The decrease in BW gain during GD 7-19 was associated with mild decrease in food consumption. The number of rabbits that ate poorly was 30%, 33%, and 53% respectively in three combo groups vs 0% in control or atorvastatin group. This was also noted with the drug + lovastatin in rabbits. The mean food consumption values in animals were not provided.

Toxicokinetics: The combo administration of SCH 58235/atorva (1000/5, 1000/25, 1000/50 mg/kg/day) did not effect the total SCH 58235 drug AUC exposures in rabbits (124, 132, 149 µg.h/ml respectively). The conjugated drug was not altered with the

combo, but unconjugated drug exposure increased by 1.4-1.9 fold with atorvastatin (100, 136, 140 ng.h/ml respectively), however sponsor states that since this increase was so small and due to inter animal variability in unconjugated drug levels, it was not significant. 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 124-149 µg.h/ml in the current study.

The exposures of atorva free acid were significantly increased by 1.5 fold by the drug in rabbits (32, 199, 323 with combo vs 214 ng.h/ml with atorva alone). Similarly exposure to ortho-hydroxy atorvastatin was significantly increased by 2.5 fold by the drug in rabbits, but no change in para-hydroxy atorvastatin expsosures were observed with the drug. Mean Cmax of atorvastatin (51 vs 34 ng/ml with atorvastatin alone) and ortho-hydroxy atorvastatin (238 vs 76 ng/ml with atorvastatin alone) were also significantly increased in the HD combo groups.

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

	Dose[a]	Toxicokinetic Parameters		
		Cmax (ng/mL)[b]	Tmax (hr)[b]	AUC(0-24 hr) (ng-hr/mL)[b]
Total SCH 58235	1000/5.5	7919	1.75	123728
	1000/27.3	7795	1.75	132084
	1000/54.5	8907	1.00	148510
Conjugated SCH 58235	1000/5.5	7914	1.75	124112
	1000/27.3	7789	1.75	125356
	1000/54.5	8901	1.00	148372
Unconjugated SCH 58235	1000/5.5	7.00	4.50	100
	1000/27.3	9.84	4.50	136[c]
	1000/54.5	10.2	3.75	140[c]

a: mg/kg SCH 58235/mg/kg atorvastatin
b: n=4
c: n=3

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

The mean systemic exposure to atorvastatin free acid and its metabolites on gestation Day 19 is summarized in the following table:

	Dose[a]	Toxicokinetic Parameters		
		Cmax (ng/mL)[b]	Tmax (hr)[b]	AUC(0-24 hr) (ng-hr/mL)[b]
Atorvastatin Free Acid	1000/5.5	5.77	1.00	32.0
	1000/27.3	31.0	1.25	199
	1000/54.5	50.9	1.00	323[c]
	0/54.5	34.1[c]	2.00[c]	214[c]
Ortho-hydroxy Atorvastatin	1000/5.5	17.2	2.50	129
	1000/27.3	99.5	1.25	619
	1000/54.5	238	1.25	1441[c]
	0/54.5	75.7[c]	2.00[c]	584[c]
Para-hydroxy Atorvastatin	1000/5.5	2.49	2.00	10.8
	1000/27.3	16.2	1.75	94.3
	1000/54.5	27.0	2.25	167[c]
	0/54.5	25.2[c]	2.67[c]	152[c]

a: mg/kg SCH 58235/mg/kg atorvastatin
b: n=4
c: n=3

For embryofetal development studies: The percent of early resorptions were slightly higher in the combo group (at low-mid doses). Fetal body weights were not significantly different from controls. Other reproductive data including viable fetuses (sex ratio),

corpora lutea, implantation sites, preimplantation loss, resorptions (late) were generally unremarkable.

In-life observations:

Reproductive data in dams:	Dose SCH58235/Atorvastatin				
	Vehicle control	0/50 mg/kg/day	1000/5 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Mean resorptions, early (%)	3 (1.9%)	5 (3.2%)	16 (9.6%)	12 (7.6%)	6 (3.2%)
Mean fetal body weights (g)	47.7	47.9	46.8	48.0	45.8

External observations in fetuses/litters showed increased external variations (not malformations) in tails (kinked tail) in HD combo group.

Fetal external observations:

	Dose SCH58235/atorvastatin (% fetal incidences/% litter incidences)				
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	19 162	17 154	20 170	17 147	17 150
Shortened Tail	0.6 (5.3)	0 (0)	0.6 (5.0)	0.7 (5.9)	0.7 (5.9)
Kinked tail	0 (0)	0 (0)	0 (0)	0 (0)	1.3 (5.9)

Fetal visceral malformations were increased at MD and /or HD combos. These included gall bladder absent and ectopic or misshapen kidney vs the vehicle control group or atorvastatin group.

Fetal Visceral variations and malformations

Offspring:	Dose SCH58235/Atorvastatin				
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	19 162	17 154	20 170	17 147	17 150
Total malformations: %-fetal incidences (%-litter incidences)					
Incidences	0.6 (5.3)	0 (0)	0 (0)	0.7 (5.9)	1.3 (11.8)
Total variations: %-fetal incidences (%-litter incidences)					
incidences	10.5 (36.8)	9.7 (47)	6.5 (35)	10.2 (47)	12.7 (52.9)
Visceral Malformations: fetal incidences (litter incidences)					
Gallbladder	0 (0)	0 (0)	0 (0)	0.7 (5.9)	0.7 (5.9)

absent					
Ectopic kidney	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (5.9)
Misshapen kidney	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (5.9)

Skeletal malformations (caudal vertebrae fused/mis-aligned, thoracic and lumber vertebrae fused, total litter incidences 18-35% vs 6% in controls and atorvastatin groups) and variations (sternebrae asymmetrical 29% at HD vs 18% with atorvastatin) were increased in fetuses/litters at MD and/or HD combos. caudal and lumber vertebrae fused were also observed in LD combo group

Skeletal variations and malformations in fetuses

Offspring:	Dose SCH58235/Atorvastatin				
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	19 162	17 154	20 170	17 147	17 150
Skeletal Malformations: %-fetal incidences (litter incidences)					
Total Incidences	0.6 (5.3)	0.6 (5.9)	2.4 (5.0)	2.0 (17.6)	9.3 (35.3)
Skeletal Variations: %-fetal incidences (litter incidences)					
Total Incidences	82 (100)	88 (100)	91 (100)	97 (100)	93 (100)
Skeletal Variations:					
Thoracic vertebral centra bipartite	0% (0%)	0% (0%)	0% (0%)	0% (0%)	1.3% (11.8%)
Sternebra asymmetrical	0% (0%)	2.6% (17.6%)	1.8% (15.0%)	2% (11.8%)	4.0% (29.4%)
Sternabrae bipartite	1.2% (10.5%)	0% (0%)	2.4% (15%)	1.4% (5.9%)	3.3% (17.6%)
Skeletal malformations					
Extra thoracic vertebrae	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (5.9)
*thoracic vertebrae centra fused	0.6 (5.3)	0 (0)	0 (0)	0 (0)	1.3 (11.8)
thoracic vertebrae mis-aligned	0 (0)	0 (0)	0 (0)	0 (0)	1.3 (11.8)
Thoracic hemivertebrae	0.6 (5.3)	0 (0)	0 (0)	0.7 (5.9)	1.3 (11.8)

lumber vertebrae mis-aligned	0 (0)	0 (0)	0.6 (5.0)	0 (0)	2.0 (11.8)
lumber hemi-vertebrae	0% (0%)	0% (0%)	0.6% (5.0%)	0% (0%)	0.7% (5.9%)
lumber vertebrae centra fused	0% (0%)	0% (0%)	1.2% (5.0%)	0% (0%)	0.7% (5.9%)
Extra lumbar vertebrae arch	0% (0%)	0% (0%)	0% (0%)	0% (0%)	0.7% (5.9%)
Caudal vertebrae fused	0% (0%)	0% (0%)	0.6% (5.0%)	0.7% (5.9%)	4.0% (5.9%)
Caudal vertebrae mis-aligned	0% (0%)	0% (0%)	0% (0%)	0.7% (5.9%)	1.3% (11.8%)
Sternabrae fused	0% (0%)	0.6% (5.9%)	0% (0%)	0% (0%)	2.7% (23.5%)

Summary of individual study findings:
Co-administration of two drugs did not significantly effect the systemic exposure to SCH 58235 (113 µg.h/ml from a previous study vs 124-149 µg.h/ml in the current combo study). However the combo of SCH 58235 + atorvastatin 50 mg/kg increased the mean systemic exposure of atorvastatin (323 vs 214 ng.h/ml atorvastatin alone 50 mg/kg) and also of ortho-hydroxy atorvastatin (1441 vs 584 ng.h/ml) by 1.5-2.5 fold
All combo groups not only had higher fecal findings (reduced number and small fecal pellets than the atorvastatin/control group, but also in tall combo groups, FC was decreased by 30-53%. At HD combo BW gain was significantly decreased during gestation by 38%.

HD combo produced external malformations of kinked tail (1.3 % in fetuses and 5.9% in litters). visceral malformations in fetuses (gallbladder absent, ectopic/misshapen kidneys) were increased at MD and /or HD combos. Skeletal malformations were increased at all combo doses (caudal vertebra & sternebrae fused). The HD combo had increased skeletal variations (sternebra assymmetrical). Sponsor explains that the HD combo produced low incidence of malformations (fused sternbra), but all combo doses produced single incidences of fused caudal vertebra.

NOAEL for maternal toxicity could not be established, as all combo doses (atorvastatin + 1000 mg/kg SCH 58235) produced clinical signs (of reduced number and sizr of fecal pellets), a significant decrease in the FC, and a HD 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 produced skeletal malformations (fused caudal vertebrae and sternabra). MD and/or HD combo produced external (kinked tail) and visceral malformations (gallbladder absent). Sponsor's no effect level (NOEL) for both maternal and in utero effects was < 5 mg/kg/day atorvastatin + 1000 mg/kg SCH 58235.

7. Oral Gavage Rat Embryo Fetal Developmental Toxicity of SCH 58235 and lovastatin (SCH 48176)

***Key study findings:**

- **The sponsor attributes decreased body weight gain to the 50 mg/kg lovastatin dose. Discolored stool was attributed to SCH 58235**
- **SCH 58235 co-administered with lovastatin 50 mg/kg resulted in an increase in the fetal visceral variation of dilated renal pelvis, which was within the facility's historical control range**
- **The HD combo produced significant decreases in BW gain, FC (21 vs 22 g/animal/day during GD 6-17). No viable fetuses in 1/23 females. However decreases in BW gain were attributed to lovastatin 50 mg/kg/day.**
- **HD combo doses produced higher incidences of skeletal variations (such as reduced ossifications of sternebra, and extra single thoracic ribs) compared to lovastatin 50 mg/kg/day alone. All combo doses produced increased incidence of unossified sternebra, focal thickening of ribs and malformations of fused ribs compared to the vehicle or lovastatin control.**
- **Maternal NOAEL=1000 mg/kg SCH 52835 + 25 mg/kg lovastatin based on statistically significant decreases in FC, and no viable fetuses in 1/23 females at 1000 SCH + 50 mg/kg lovastatin group**
- **Developmental NOAEL=1000 mg/kg SCH 52835 + <5 mg/kg lovastatin based on the increased incidence of skeletal variations/malformations observed with 1000 mg/kg SCH 52835 + 5-50 mg/kg lovastatin compared to lovastatin alone (50 mg/kg/day).**

Study no.:99002
Volume #, and page #: 1.142-1.143, pg.1 (reference 68)
Conducting laboratory and location: Safety Evaluation Center; Schering-Plough Research Institute; Lafayette, NJ
Date of study initiation: 6/7/99
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH 58235 97-58235-X-02, purity was not provided. Lovastatin 99-48176-X-03
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: SCH 58235 1000 mg/kg/day + lovastatin 10, 25, 50 mg/kg/day, controls received the vehicle or lovastatin 50 mg/kg/day
Route of administration: oral gavage
Study design: daily dosing GD 6-17
Number/sex/group: 25 F/group
Parameters and endpoints evaluated:

Embryo-Fetal Developmental Toxicity Study of SCH 357015 Administered by Oral Gavage in Rats (SN 99002): **Study Design**				
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females
Group 1 Methylcellulose	0	10[a]	0	25
Group 2 Methylcellulose SCH 48176[b]	0 50	5 5	0 10	25
Group 3 SCH 58235 SCH 48176	1000 10	5 5	200 2	25
Group 4 SCH 58235 SCH 48176	1000 25	5 5	200 5	25
Group 5 SCH 58235 SCH 48176	1000 50	5 5	200 10	25
a: This group was administered two doses in succession at 5 ml/kg per dose for a total daily dose of 10 ml/kg. b: SCH 48176 = lovastatin				

Embryo-Fetal Developmental Toxicity Study of SCH 357015 Administered by Oral Gavage in Rats (SN 99002): **Observations and Measurements**			
Investigation	Performed	Investigation	Performed
Clinical Observations	Daily	Reproductive Parameters	Yes
Body Weight	Gestation Days 0, 6, 9, 12, 15, 17, and 21	Fetal Body Weight	Yes
Food Consumption	Gestation Days 0 to 6, 6 to 12, 12 to 17, and 17 to 21	Fetal External/Visceral/ Skeletal Examinations	Yes
Necropsy/C-Section	Gestation Day 21		

Results:

Mortality: None

Clinical signs: discolored (tan) stool was observed in all combo groups (0/0/, 0/0, 120/23, 78/20, 102/20 respectively), this was attributed to unabsorbed SCH 58235 through biliary route resulting in fecal elimination of unabsorbed drug, as seen in other studies. 3/25 females in LD combo delivered early (on GD 21, prior to C-section), but was not considered drug related as it did not occur at higher doses.

Body Weights: The reduced gain observed with lovastatin 50 mg/kg/day was not associated with decreased food consumption or decreased gravid uterine weight. The reduced weight gain in the combination was attributed to lovastatin (50 mg/kg/day) which caused the decreased BW gain during GD 6-21. Net weight change (minus uterine weight) in HD combo was lower by 12% (30, 24, 27, 27, 21 g respectively).

Food consumption was significantly decreased in a HD combo (21* vs 23 g in controls).

Body weight/ Food consumption (FC)

Body weight:	Dose SCH58235/lovastatin				
	Vehicle	0/50 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Body weight gains					
Gain GD6-17 (g)	66	56*	61	60	55*
Gain GD 6-21 (g)	134	119*	128	130	118*
Food consumption					
FC GD6-17 (g)	23	22	22	22	21*

*p<0.05.

Toxicokinetics: TK were not performed in this combination study

Terminal and necroscopic evaluations: 3/25 females in a LD combo group delivered early (on GD 21, prior to C-section), but was not considered drug related as it did not occur at higher doses.

Reproductive data: In a HD combo group, 1/23 dams had no viable fetuses, sponsor does not consider these significant because these were single incidences. Fetus sex ratio, corpora lutea, implantation sites, preimplantation loss, late resorptions were unremarkable. Mean post-implantation losses (0.4, 0.5, 0.7, 0.2, 1.1) and early resorptions (0.4, 0.5, 0.7, 0.2, 1.1) were higher at HD combination.

For embryofetal development studies:

The fetal body weights were not significantly different

Offspring:	Dose SCH58235/lovastatin				
	Vehicle control	0/50 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
fetal body weights	5.6	5.4	5.6	5.6	5.4

Fetal external observations: In eyes, anophthalmia bilateral at HD combo (fetal incidences 0.3% and litter incidences 4.5% vs none in other groups) or micromelia in limbs at LD combo (fetal incidences 0.3% and litter incidences 4.5% vs none in other groups) were not considered significant because they occurred in a single litter with no dose related relationship.

Visceral malformations: The increased incidence of dilated renal pelvis was noted at the HD combo (fetal incidences 0.6, 0, 0.7, 0.3, 2.1% respectively and litter incidences 8, 0, 9.1, 4, 22.7% respectively), but sponsor states that it was within the facility's historical control data (mean historical litter incidences were 22.6%, range 0-68%).

Skeletal malformations such as thoracic hemivertebrae (at MD & HD combo fetal/litter incidences 0.6/3.3% vs none in other groups), fused ribs (observed at all combo doses, see Table) and missing radius (at LD combo fetal/litter incidences were 0.6%/4.5% vs none in other groups) were not considered drug related because these were single incidences with no dose relationships. **However the thoracic vertebral centra bipartite, thoracic vertebral centra asymmetrical, sternebra bipartite were all attributed to lovastatin group, as there incidences were higher than the historical control data,** and these have been noted previously with lovastatin and its active metabolite at a dose of 60 and 800 mg/kg/day (including skeletal malformations of vertebral column, ribs and sternebra).

Table. Skeletal observations with lovastatin alone and with combo (SCH 58235 +lovastatin). Historical control data are also shown in this table.

Lovastatin-Related Skeletal Observations (and Historical Control Values)						
	Study Incidence[a]					Historical Control[b] Mean Incidence (Per Study Range Maximum)
SCH 48176[c] (mg/kg)	0	50	10	25	50	
SCH 58235 (mg/kg)	0	0	1000	1000	1000	
Litters: N =	25	25	22	25	22	351
Fetuses: N =	185	173	158	180	152	3074
Variation: Thoracic vertebral centra bipartite						
Litter Incidence N	3	6	0	7	7	26
%	12.0	24.0	0.0	28.0	31.8	7.4 (22.7)
Fetal Incidence N	3	7	0	7	16	32
%	1.6	4.0	0.0	3.9	10.5	1.0 (4.3)
Variation: Thoracic vertebral centra asymmetrical						
Litter Incidence N	0	3	0	0	2	12
%	0.0	12.0	0.0	0.0	9.1	3.4 (32.0)
Fetal Incidence N	0	3	0	0	3	18
%	0.0	1.7	0.0	0.0	2.0	0.6 (5.6)
Variation: Sternebra bipartite						
Litter Incidence N	0	2	0	0	1	12
%	0.0	8.0	0.0	0.0	4.5	3.4 (13.6)
Fetal Incidence N	0	2	0	0	1	12
%	0.0	1.2	0.0	0.0	0.7	0.4 (1.2)

a: Values obtained from Data Table 21
b: Values obtained from Appendix 3
c: SCH 48176 = lovastatin

Other skeletal variations such as Sternebra unossified (9-12% vs 4% with lovastatin), sternebra reduced ossification (27% vs 12% with lovastatin), extra single thoracic ribs (41% vs 20% with lovastatin), focal thickening of rib (5-9% vs none in controls or lovastatin groups) were higher with the HD combo, and malformations of fused ribs (5% vs none in controls or lovastatin groups) were seen with all combo doses.

Other Skeletal findings

Offspring:	Dose SCH58235/lovastatin				
Skeletal Malform./ Variations	Vehicle control	0/50 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day	1000/50 mg/kg/day
Litters Fetuses	25 185	25 173	22 158	25 180	22 152
Skeletal Variations					
Sternebra unossified	0% (0%)	0.6% (4%)	4.4% (12%)	4.4% (8.7%)	1.3% (9.1%)
Sternebra reduced ossification	2.2% (20%)	1.7% (12%)	5.1% (23%)	1.7% (12%)	7.2% (27%)
Extra single thoracic rib	4% (20%)	5% (20%)	2.5% (18%)	6% (24%)	7.2% (41%)
Fused ribs	0%	0%	0.6% (4.5%)	0.6% (4%)	1.3% (4.5%)
Focal thickening of rib	0%	0%	1.3% (9%)	1.1% (8%)	0.7% (4.5%)

Skull, cervical/thoracic vertebrae, sternebrae, ribs, metacarpals, metatarsals, phalanges were examined for skeletal findings.

Summary of individual study findings: The HD combo produced significant decreases in FC (during GD 6-17), BW gain was decreased with both the lovastatin alone and with the HD combo. HD combo produced no viable fetuses in 1/23 females. All combo doses produced higher incidences of skeletal variations (consisting of sternebra unossified, and focal thickening of ribs compared to vehicle or lovastatin 50 mg/kg/day alone). Skeletal mlaformations of fused ribs were noted at all combo doses vs none with the vehicle or lovastatin control, and sponsor considers these not drug related, because these were single incidences. However these single incidences were seen at all doses vs none in both control groups. Maternal NOAEL=1000 mg/kg SCH 52835 + 25 mg/kg lovastatin based on statistically significant decreases in FC, and no viable fetuses in one female at 1000 SCH + 50 lovastatin mg/kg group. Developmental NOAEL=1000 mg/kg SCH 52835 + <10 mg/kg lovastatin based on the increased incidence of skeletal variations/malformations observed with all combo doses (1000 mg/kg SCH 52835 + 10 to 50 mg/kg lovastatin compared to lovastatin alone 50 mg/kg/day). No exposures were available in this study, but based on exposures from an oral 3-month toxicity/TK study in rats (with ezetimibe doses of 50, 250, 250, 750 mg/kg/day in males, and 12, 50, 50, 250 mg/kg/day in females + lovastatin doses of 10, 10, 100, 100, AUC values of total ezetimibe of 4.5-81 µg.h/ml in males and 1.7-69 µg.h/ml in females), these values were ≈ 3-120X the exposure multiples in humans.

8. Oral Gavage Rabbit Embryo Fetal Developmental Toxicity of SCH 58235 and lovastatin (SCH 48176)

***Key study findings:**

- **The sponsor attributes abortions at LD combo in rabbits not significant because these were not seen in previous studies, however since these were also seen at a HD combo, they can not be ruled out as not significant**
- **Discolored stool was attributed to SCH 58235**
- **All combo doses (SCH 58235 co-administered with lovastatin 2.5-25 mg/kg/day) resulted in increased incidences of post implantation losses due to increased early resorptions**
- **MD & HD combo doses produced higher incidences of skeletal variations (such as sternebra fused, and forelimb distal humeral epiphysis unossified) compared the vehicle or lovastatin control.**
- **Maternal NOAEL=1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on increases in abortions at LD and HD combo (1000 mg/kg SCH 52835 + 2.5 & 25 mg/kg lovastatin compared to lovastatin alone)**
- **Developmental NOAEL=1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on the increased incidence of post implantation losses and early resorptions, observed with 1000 mg/kg SCH 52835 + 2.5-25 mg/kg lovastatin compared to vehicle or lovastatin 25 mg/kg/day).**

Study no.:99004
Volume #, and page #: 1.153-1.154, pg.1 (reference 77)
Conducting laboratory and location: Safety Evaluation Center; Schering-Plough Research Institute; Lafayette, NJ
Date of study initiation: 6/28 /99
GLP compliance: yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SCH 58235 97-58235-X-02, Lovastatin 99-48176-X-03
Formulation/vehicle: 0.4% aqueous methylcellulose; 6 ml/kg vehicle control, others 5 ml/kg of SCH 58235 + 1 ml/kg of lovastatin.

Methods:

Species/strain: NZW Rabbits

Doses employed: SCH 58235 1000 mg/kg/day + lovastatin 2.5, 10, 25 mg/kg/day, controls received the vehicle or lovastatin 25 mg/kg/day
Route of administration: oral gavage
Study design: daily dosing GD 7-19
Number/sex/group: 20 F/group
Parameters and endpoints evaluated:

Embryo-Fetal Developmental Toxicity Study of SCH 357015 Administered by Oral Gavage in Rabbits (SN 99004): **Study Design**				
Group	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	Number of Females
Group 1 (Control) 0.4% Methylcellulose	0	6	0	20
Group 2 0.4% Methylcellulose SCH 48176	0 25	5 1	0 25	20
Group 3 SCH 58235 SCH 48176	1000 2.5	5 1	200 2.5	20
Group 4 SCH 58235 SCH 48176	1000 10	5 1	200 10	20
Group 5 SCH 58235 SCH 48176	1000 25	5 1	200 25	20

Embryo-Fetal Developmental Toxicity Study of SCH 357015 Administered by Oral Gavage in Rabbits (SN 99004): **Observations and Measurements**			
Investigation	*Performed*	*Investigation*	*Performed*
Clinical Observations	Daily	Reproductive Parameters	Yes
Body Weight	Gestation Days 0, 7, 10, 13, 16, 19, 22, 25, 28 and 30	Fetal Body Weight	Yes
Food Consumption (estimated)	Gestation Days 1 through 30	Fetal Sex Determination	Yes
Necropsy/C-Section	Gestation Day 30	Fetal External/Visceral/ Skeletal Examinations	Yes

Results:

Mortality: None, however, 1/20 rabbits aborted in LD combo group (on GD 28) and 2/20 in HD combo group (on GD 21 and 23), these were sacrificed pre-terminally. The sponsor does not consider the LD combo abortions significant or drug related, as these were not seen in previous studies with the drug alone, or in a pilot study with the combo.

Clinical signs: discolored (light green) stool was observed in all combo groups (frequency/animal incidences 0/0, 0/0, 270/20, 270/20, 274/20 respectively), this was attributed to unabsorbed SCH 58235 through biliary route resulting in fecal elimination of unabsorbed drug, as seen in the rat study with this combination. Soft stool incidences were higher at MD/HD combos (0/0, 0/0, 0/0, 2/1, 4/1 respectively)

Body Weights/Food consumption: No treatment related effects were observed. However gravid uterine weight was lower (but not significant) in MD and HD combo groups

Gestation Body weight/ Food consumption (FC)

Body weight:	Dose SCH58235/Pravastatin				
	Vehicle	0/25 mg/kg/day	1000/2.5 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day
Body weight gains					
Gain GD7-19 (kg)	0.19	0.21	0.24	0.20	0.20
Gravid uterine weight					
grams	485	499	522	443	469

Toxicokinetics: TK were not performed in this combination study

Maternal necroscopic evaluations: In a HD combo group, placentas were found in a litter pan (1/20 rabbits), and this dose produced mottled lungs (1/20 rabbits), and small uterus (1/20) in rabbits

Reproductive data: The percent of mean post implantation loss and early resorptions were higher in all 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 (late) were generally unremarkable.

Reproductive data: Summary of C-section results

Reproductive data in dams:	Dose SCH58235/lovastatin %-fetal/litter incidences				
	Vehicle control	0/25 mg/kg/day	1000/2.5 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day
Mean post implantation loss (%)	8 (4%)	5 (2.6)	15 (8.7)	35 (21)	20 (13.6%)
Mean early resorptions (%)	5 (2.6%)	2 (1%)	13 (7.7%)	33 (19.6%)	18 (12.3%)
Live fetuses (%)	159	176	162	140	126
Mean fetal body weights (g)	43.3	42.6	42.5	45.2	43.9

Fetal external observations: At a HD combo, omphalocele (fetal incidences 0.8% and litter incidences 6.3% vs none in other groups) and at MD/HD combo shortened tails (fetal incidences 0.6-0.8% and litter incidences 5.6-6.3% vs none in other groups) were observed but were not considered significant because they occurred in a single litter.

Visceral malformations: At a HD combo dilated renal pelvis was observed (fetal incidences 3.2% and litter incidences 6.3% vs none in other groups), but sponsor states that it was within the facility's historical control data (mean historical litter incidences were 22.6%, range 0-68%).

Skeletal variations such as Sternebra unossified were increased with lovastatin alone (litter incidences 60% vs 42% in controls). However there were higher incidences of sternebra fused (litter incidences 5-6% vs none in controls) and forelimb distal humeral epiphysis unossified (11-25% vs 5% in controls) in MD/HD combo groups.

Table. Skeletal observations with lovastatin alone and with combo (SCH 58235 +lovastatin).

Skeletal findings

Offspring:	Dose SCH58235/lovastatin				
Skeletal Malform./ Variations	Vehicle control	0/25 mg/kg/day	1000/2.5 mg/kg/day	1000/10 mg/kg/day	1000/25 mg/kg/day
Litters Fetuses	19 159	20 176	18 162	19 140	16 126
Skeletal Variations: %-fetal/litter incidences					
Sternebra unossified	15/42	14/60	14/39	8/26	6/38
Sternebra feused	0/0	0/0	0/0	0.7/5.3	0.8/6.3
Forelimbs-Distal humeral epiphysis unossified	0.6/5.3	0.6/5	0/0	2.1/10.5	5.6/25
Hindlimbs-Distal humeral epiphysis unossified	5/26	5/30	10/50	7/21	15/50

Summary of individual study findings: In LD and HD combo groups 1/20 and 2/20 rabbits aborted vs none in other groups. The HD combo produced mottled lungs and small uterus in 1/20 females. All combo doses increased mean post implantation losses (15-35% vs 5-8% in controls) and early resorptions (13-33% vs 2-5% in vehicle and lovastatin control) which may have led to abortions at LD/HD combos. MD and/or HD combo produced higher incidences of skeletal variations such as sternebra fused (litter incidences 5-6% vs none in controls) and forelimb distal humeral epiphysis unossified (11-25% vs 5% in controls). Maternal NOAEL=1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on abortions noted at low and high combo doses. Developmental NOAEL=1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on the increased incidences of post implantation losses and early resorptions with all combo doses (1000

mg/kg SCH 52835 + 2.5 to 25 mg/kg lovastatin compared to vehicle or lovastatin alone 25 mg/kg/day).

Reproductive and developmental toxicology summary with SCH 58235 + statins

1. In a segment II teratology study with **drug+pravastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + pravastatin (125, 250, 500 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or pravastatin alone (500 mg/kg/day). Maternal exposures of the total drug on GD 17 were 9.8, 11.2, 30 µg.h/ml at 1000/20, 1000/250, 1000/500 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, exposures to total drug were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by 2-6 fold when SCH 58235 was co-administered with pravastatin. 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 exposure of 1.7X (18.2 with SCH 58235 + pravastatin vs 10.9 µg.h/ml with pravastatin alone). The sponsor attributes increased salivation to pravastatin at doses ≥250 mg/kg/day. The HD combo (SCH 58235 with pravastatin 500 mg/kg/day) significantly decreased the body weight gain vs the controls (61* g vs 64-72 g in the vehicle/pravastatin controls) and produced a small increase in fetal visceral variation of dilated ureters (which the sponsor attributes to pravastatin). The HD combo generally increased the incidences (fetal/litter) of skeletal variations consisting of reduced ossification of sternebrae, parietal bone, proximal phalanges (hind paws) compared to pravastatin alone (500 mg/kg/day). These increases are within historical control range, but exceed the mean. **Maternal and developmental NOAELs were both 1000 mg/kg SCH 52835 + 250 mg/kg pravastatin** based on statistically significant decreases in body weight gain at HD (1000 SCH + 500 pravastatin mg/kg/day) in mothers, and general increases in incidences in reduced skeletal ossifications in fetuses at HD combination compared to pravastatin alone (500 mg/kg/day).

2. In a segment II teratology study with **drug+pravastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + pravastatin (5, 25, 50 mg/k/day) from day 6-19 of gestation. Control animals received the vehicle or pravastatin alone (50 mg/kg/day). Maternal exposures of the total drug on GD 19 were 139, 135, 145 µg.h/ml at 1000/5, 1000/25, 1000/50 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with pravastatin. Systemic exposure to unconjugated SCH 58235 was <0.2% of exposure to total SCH 58235. Coadministration of SCH 58235 with pravastatin 50 mg/kg (AUC= 10 µg.h/ml) had no effect on the mean systemic exposure to pravastatin 50 mg/kg/day (9.7 µg.h/ml). All combo doses produced higher fecal findings (fecal stained fur, discolored or soft stool, reduced fecal pellets) than the vehicle or pravastatin control. High dose combo group had significantly decreased BW gain 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. 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 (5.3-6.9% vs 0% in controls or pravastatin group). A HD combo increased the incidences of skeletal variations such as sternbrae bipartite (31.6% vs 22% with prava alone) and extra pair of thoracic ribs (100% vs 83% with prava alone). **NOAEL for maternal toxicity was 25 mg/kg pravastatin + 1000 mg/kg SCH 58235**, as the higher doses (50 mg/kg pravastatin + 1000 mg/kg SCH 58235) produced significant decreases in BW gain in rabbits. **Developmental NOAEL was 5 mg/kg pravastatin + 1000 mg/kg SCH 58235**, as mid and higher combination doses produced external malformations (in tail) as well as fused caudal vertebrae compared to pravastatin or control group. Sponsor's no effect level doses for both maternal and embryo-fetal toxicity were higher (i.e. 1000 mg/kg/day SCH 58235 and ≤ 50 mg/kg/day pravastatin), despite the fact they acknowledge that HD combo produced decreases in BW gain in rabbits, and Mid-high dose combination of SCH 58235 +pravastatin produced malformations in rabbit fetuses.

3. In a segment II teratology study with **drug+simvastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + simvastatin (5, 10, 25 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or simvastatin alone (25 mg/kg/day). Maternal exposures of the total drug on GD 17 were 3.6, 4.7, 8.1 µg.h/ml at 1000/5, 1000/10, 1000/25 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, total drug exposures were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by ≈2 fold when SCH 58235 was co-administered with 25 mg/kg/day of simvastatin. Systemic exposure to unconjugated SCH 58235 was <2% of exposure to total SCH 58235. Exposures to simvastatin and hydroxy-simvastatin increased by 1.4 to 2 fold at the HD combination (with SCH 58235 + 25 mg/kg/day simvastatin). Fetal body weights were decreased with simva alone (5.3* vs 5.6 g in controls). All combination groups had an increased incidence of right carotid and subclavian arteries arising from the aortic arch in the visceral exam, and in the HD combination group the right subclavian artery was absent. HD combination had increased incidences of bipartite sternebrae which exceeded historical mean and range and increased incidences of a skeletal malformation (hemivertebrae). The maternal NOAEL was 1000 mg/kg SCH 58235 + 25 mg/kg simvastatin. Developmental NOAEL was 1000 mg/kg SCH58235 + 10 mg/kg simvastatin based on visceral findings (involving blood vessels and skeletal malformations and variations in combination with 25 mg/kg simvastatin).

4. In a segment II teratology study with **drug+simvastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + simvastatin (1, 5, 10 mg/k/day) from day 6-19 of gestation. Control animals received the vehicle or simvastatin alone (10 mg/kg/day). Maternal exposures of the total drug on GD 19 were 99, 104, 114 µg.h/ml at 1000/1, 1000/5, 1000/10 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with simvastatin. Systemic exposure to unconjugated SCH 58235 was <2% of exposure to total SCH 58235. Coadministration of SCH 58235 with simvastatin 10 mg/kg (AUC= 20 ng.h/ml) had no effect on the mean systemic exposure to simvastatin 10 mg/kg/day (21 ng.h/ml) or to hydroxysimvastatin. Two of 20 rabbits at HD combination died or were sacrificed, one death was attributed to mechanical injury. ALT was mildly increased for all 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. MD & HD combination (5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235) not only produced short, filamentous tails with fused caudal vertebra and reduced numbers of caudal vertebra (2/18 litters in both groups, with 5 & 2 fetuses effected respectively), but also heart malformations (in two fetuses each with MD/HD combo groups). In the MD combo one fetus had ventricular septum defect (membraneous and muscular) and thickened ventricular valve with one small and one enlarged atrial chamber. In the HD combo 2 fetuses had multiple heart malformations, 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. At HD combination, skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fused and/or bifurcated ribs were present. NOAEL not established for maternal toxicity based on transient food consumption and fecal changes, according to the sponsor. However, maternal NOAEL was1000 mg/kg SCH 5832 +1 mg/kg simvastatin due to premature deaths of 2 dams in the HD combination. Developmental NOAEL was <1 mg/kg simvastatin + 1000 mg/kg SCH 58235 based on increased external and visceral malformation compared to controls.

5. In a segment II teratology study with **drug+atorvastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (25, 50, 100 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or atorvastatin alone (100 mg/kg/day). Maternal exposures of the total drug on GD 17 were 8.6, 21.3, 66.2 µg.h/ml at 1000/25, 1000/50, 1000/100 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, exposures were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by 2-10 fold when SCH 58235 was co-administered with atorvastatin. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Exposures to atorvastatin free acid, para-hydroxy atorvastatin, and ortho hydroxy atorvastatin were all slightly decreased when high dose of atorvastatin (100 mg/kg/day) was co-administered with SCH 58235. The HD combination significantly decreased the gestation body weight gain (by 10%, 62* g vs 69 g in the control), food consumption (by 8%, 22 vs 24 g/rat/day in controls) in rats, decreased the mean body weights of fetuses, and produced increased incidences of skeletal variations (reduced ossification of sternebrae which sponsor attributes to decreased fetal body weight). Maternal/Developmental NOAEL was 1000 mg/kg SCH 58235 + 50 mg/kg atorvastatin, based on decreased maternal/fetal body weights, maternal FC and increased incidence of reduced ossification of sternebrae.

6. In a segment II teratology study with **drug+atorvastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (5, 25, 50 mg/k/day) from day 7-19 of gestation. Control animals received the vehicle or atorvastatin alone (50 mg/kg/day). Maternal exposures of the total drug on GD 19 were 124, 132, 149 µg.h/ml at 1000/5, 1000/25, 1000/50 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures to total drug were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with atorvastatin (124-149 in the present study vs 113 µg.h/ml with the drug alone in a previous study). Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Coadministration of SCH 58235 with atorvastatin 50 mg/kg increased the exposure to atorvastatin free acid by 1.5 fold (323 vs 214 ng.h/ml) and to ortho-hydroxy atorvastatin

by 2.5 fold (1441 vs 584 ng.h/ml), but had no effect on the mean systemic exposure to para-hydroxy atorvastatin (167 vs 152 ng.h/ml). All combination doses produced increased clinical signs (a reduced number and small fecal pellets in 6-8/20 rabbits vs 0-1/20 in vehicle or atorvastatin controls), decreased food consumption in rabbits (by 30, 33 & 53% respectively), and HD combo produced decreased BW gains by 38%. All combination doses produced skeletal malformations (fused caudal vertebra and sternebrae), MD & HD combination (25 and 50 mg/kg atorvastatin + 1000 mg/kg SCH 58235) produced visceral malformations (gallbladder absent, ectopic/misshapen kidneys), and HD combination produced external malformations of kinked tail (1.3% in fetuses and 5.9% in litters) and increased skeletal variations (sternebrae assymetrical). NOAEL could not be established for the maternal toxicity as all combination doses decreased food consumption and produced fecal changes, and HD combo decreased BW gain. Maternal NOAEL and developmental NOAEL were both 1000 mg/kg SCH 58235 + < 5 mg/kg/day atorvastatin. Developmental NOAEL was based on increased skeletal malformations (fused caudal vertebra and sternabra) with all doses of combination, MD and/or HD combo produced external (kinked tail) and visceral malformations (gallbladder absent). Sponsor's no effect level (NOEL) for both maternal and in utero effects was also < 5 mg/kg/day atorvastatin + 1000 mg/kg SCH 58235.

7. In a segment II teratology study with **drug+lovastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + lovastatin (10, 25, 50 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or lovastatin alone (50 mg/kg/day). Maternal exposures of the total drug or lovastatin were not evaluated in this study. The HD combination group had significantly decreased food consumption (by 9%), and a net body weight change (by 12%). No viable fetuses in 1/23 rats. All combination doses of SCH 58235 + lovastatin produced higher incidences of skeletal variations (sternebra unossified, and focal thickening of ribs compared to vehicle or lovastatin 50 mg/kg/day alone), and skeletal malformations (fused ribs vs none with the vehicle or lovastatin control), sponsor considers these not drug related, because these were single incidences. However these single incidences were seen at all doses vs none in both control groups. Extra thoracic ribs were observed at MD & HD combinations vs lovastatin controls. There is maternal toxicity at all lovastatin doses, which probably accounts for the skeletal variations seen in the combination groups, although there are increased incidences at HD combination. Maternal NOAEL=1000 mg/kg SCH 52835 + 25 mg/kg lovastatin based on statistically significant decreases in FC, and no viable fetuses in one female at 1000 SCH + 50 lovastatin mg/kg group. Developmental NOAEL was1000 mg/kg SCH 52835 + <10 mg/kg lovastatin based on the increased incidence of skeletal variations/malformations observed with all combo doses (1000 mg/kg SCH 52835 + 25 to 50 mg/kg lovastatin compared to lovastatin alone 50 mg/kg/day).

8. In a segment II teratology study with **drug+lovastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + lovastatin (2.5, 10, 25 mg/k/day) from day 7-19 of gestation. Control animals received the vehicle or lovastatin alone (25 mg/kg/day). Maternal exposures of the total drug or lovastatin were not eval uated in this study. In the LD and HD combination groups, 1/20 and 2/20 rabbits aborted vs none in other groups. All combination doses produced increased early resorptions (8-20% vs 1-3 in vehicle and lovastatin control), and increased incidences of post implantation losses (9-21% vs 3-4% in controls). Also decreases in live fetuses at MD and HD combo (126-140 vs 160-176 with vehicle & lovastatin controls), none of these repro data reached the statistical significance. Mid and high dose combination

groups had higher incidences of skeletal variations such as sternebra fused (litter incidences 5-6% vs none in controls) and forelimb distal humeral epiphysis unossified (11-25% vs 5% in controls). Omphalocele at MD and shortened tails at ≥ MD occurred in a single litter. Maternal and developmental NOAEL was 1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on the increased incidences of abortions in mothers at low and high doses, and post implantation losses and early resorptions observed with all combination doses (1000 mg/kg SCH 52835 + 2.5 to 25 mg/kg lovastatin compared to vehicle or lovastatin alone 25 mg/kg/day). Sponsor's NOAEL for maternal and developmental toxicity is 1000 mg/kg SCH 52835 + 2.5 mg/kg lovastatin, based on soft stool findings in 1/25 rabbits at MD and HD combination, and developmental is based on increased resorptions (fetal incidences 5, 2, 13, 33, 18% respectively, litter incidences 2.6, 1, 7.7, 19.6, 12.3% respectively) and abortions at mid and/or high combination doses

In conclusion in **segnment II study in rats** the combination of SCH 58235 + statins generally increased the exposure of both the drug and statins (or their acive hydroxy acids) by ≈ 2X and produced skeletal variations and other effects (see individual study). In the rat reduced skeletal ossification seen in pravastatin and atorvastatin combination may be related to the maternal toxicity with the combination. However other statin combinations including those in rabbits show additional malformations, some of which appear independent of concurrent maternal toxicity. In the **segment II study in rabbits** the combination of SCH 58235 + statins did not generally increase the exposure of both the drug or the statins (except for atorvastatin) and produced malformations in tails, caused fused caudal vertebra, and produced visceral malformations of gallbladder, kidney and heart (see individual study)

NOAEL* values in segment II developmental toxicity studies with SCH 58235+ statins:

Repro-tox study	**NOAEL's**	
Segment II study in rats and rabbits with SCH 58235 + pravastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +250 mg/kg/day of pravastatin (or ≈ 16 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone in non-pregnant adult humans).	1000 mg/kg/day of SCH 58235 +250 mg/kg/day of pravastatin (or ≈ 16 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone in adult non-pregnant humans).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of pravastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +5 mg/kg/day of pravastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + simvastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of simvastatin (or ≈ 12 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +10 mg/kg/day of simvastatin (or ≈ 7 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 +1 mg/kg/day of simvastatin (or ≈ 150 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +<1 mg/kg/day of simvastatin (or ≈ 150 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + atorvastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +50 mg/kg/day of atorvastatin (or ≈ 30 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +50 mg/kg/day of atorvastatin (or ≈ 30fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 + <5 mg/kg/day of atorvastatin (or ≈ 200 fold the human AUC exposures,	1000 mg/kg/day of SCH 58235 + <5 mg/kg/day of atorvastatin (or ≈ 200 fold the human AUC exposures,

	based on 10 mg/day dose of SCH 58235 alone).	based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + lovastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of lovastatin (no exposures were available).	1000 mg/kg/day of SCH 58235 +<5 mg/kg/day of lovastatin.
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 + <2.5 mg/kg/day of lovastatin (no exposures were available).	1000 mg/kg/day of SCH 58235 + <2.5 mg/kg/day of lovastatin.

- =NOAEL's are based on exposures in non-pregnant human adult. Also note that exposures of statins (and hydroxy statin metabolites) are also increased with the combination of SCH 58235 +statin, which are not calculated here.

- Note that in humans, the combination of SCH 58235 + statin does not alter the PK of either the statin or the SCH 58235 in a 2-week study, but not so in animal studies. In rats and dogs, the combination of drug + statin increases not only the SCH 58235 exposures, but also the statin (as well as hydroxy-statin) exposures. Some toxicity can be explained on this PK interaction, but in dogs sometimes the PK does not change with the combination, but synergistic toxicity is seen in the target organ (such as liver) with two drugs

In conclusion in segment II studies with ezetimibe monotherapy, the maternal/developmental NOAELs were 4-8 folds in rats, and 100-140 folds in rabbits, based on the human exposures. With combination therapy, these NOAEL's were higher based on human exposures (i.e. with ezetimibe + pravastatin, the maternal/developmental NOAELs were 16 folds in rats and ≈200 folds in rabbits, with ezetimibe + simvastatin 7-12 folds in rats and ≈150 folds in rabbits, and with ezetimibe + atorvastatin 30 folds in rats and ≈200 folds in rabbits). The reason these were higher because the exposures with the combination were increased in animals but not in humans. However, as indicated earlier the combination produced higher reproductive and general toxicity in animals.

VIII. Special toxicology Studies with monotherapy

1. Effects of ezetimibe on biliary cholesterol levels in dogs and mice:
The sponsor has examined if the drug ezetimibe has an effect on the concentration of cholesterol in the gallblader, and has determined this in a 1-month exploratory toxicity study in dogs and a 14 day study in mice

1A. In the 1-month exploratory gavage toxicity study in dogs (vol 1.172, SN 00640, 0.03, 0.3, 30 300 mg/kg/day), bile fluid was collected from the gall bladder and liver in dogs at necropsy. Bile fluid was evaluated for cholesterol ——→ method), phospholipids, and for bile salt content in these dogs. A positive group was included in this study, which received 30 mg/kg/day of ezetimibe + 10 mg/kg/day of simvastatin similarly. Ezetimibe did not effect the bile salts ——————→ mmol/L in controls)

or phospholipid concentrations [redacted] mmol/L in controls) in the bile in dogs, but significantly increased the cholesterol content in the bile in both sexes by 2 to 4 fold (males 5.4, 8.6, 13.6, 13, 13.9, 19.3 mmol/L in the vehicle control, positive control, 0.03, 0.3, 30 300 mg/kg/day respectively, femeales 8.3, 8.0, 13.6, 16.1, 18.1, 19.5 mmol/L respectively). There was a trend of increased basal values in females (in cholesterol content in bile), but these were not significant, so the data are pooled for males+females, see the Table below. Sponsor states that there was no dose related effect in these levels, and a plateau was noted at the lowest dose.

Bile salt, phospholipid and cholesterol content changes in exploratory 1-month toxicity study in dogs (study # SN 00640)

Changes[a] in Bile Salt, Phospholipid and Cholesterol Content, and % of Sex-Appropriate Vehicle Control Mean Value

Group Name	Bile Salt (M/F[b] %)	Phospholipid (M/F %)	Cholesterol (M/F %)
Positive Control	↓ (86/70%)	↓ (50/70%)	NC (96/159%)
Low-Dose	NC (100/101%)	NC (102/107%)	↑ (163/251%)
Low Mid-Dose	NC (99/101%)	NC (97/104%)	↑ (193/241%)
High Mid-Dose	NC (98/101%)	NC (94/110%)	↑ (218/256%)
High-Dose	NC (90/104%)	NC (91/114%)	↑ (234/357%)

a: ↑ = increase, ↓ = decrease, NC = no change
b: M = male, F = female

1B. Two-week studies (study 1 & 2) in female mice (F2 hybrid strain designated B6129SF2, volume 1.173, study # 39913, reference 129) were conducted to determine the biliary cholesterol levels. In the first study, mice were fed a low fat diet with and without 5 mg/kg/day of ezetimibe in the diet for 14 days. In the second study mice were fed a high fat diet (0.15% cholesterol, 40% kcal butter fat) for 14 days with ezetimibe (by gavage 0, 0.3, 1, 3 mg/kg/day in 0.4% methyl cellulose). Bile samples were collected from the gall bladder at the end of the study and cholesterol levels were determined. The results showed that the low (114 vs 107 mg/dl in controls) or high fat diet (177, 176, 202 mg/dl at 0.3, 1, 3 mg/kg/day respectively vs 173/268 mg/dl in the low fat/high fat diet controls) had no effect on the biliary cholesterol levels in mice at any dose.

Note that no histopathological changes in liver were observed in the 6- or 12 month standard toxicity studies in dogs. Also no gallstone formation was observed in these toxicity studies, **but bile smples were not collected in the 6-12 month studies in dogs and analyzed for the cholesterol concentrations.**

2. Studies of the drug (SCH 58235) spiked with impurities and justification using the doses:

[redacted] impurities that could potentially be present in the clinical batches of drug were examined by conducting 1-month dietary and gavage toxicity study of the drug (Zeita) with impurities in rats and dogs respectively. **The impurities are**

[redacted]

The drug tablet is an [redacted]

but this process does not change the drug's dissolution, particle size or stability. Also, Initial clinical studies were conducted with a [redacted] but all subsequent clinical studies including the phase III were conducted using a tablet dosage form, [redacted] As indicated earlier SCH 58235 (or ezetimibe) has 3 chiral centers, and therefore 8 possible isomers can be formed. All 8 isomers have been synthesized and assigned stereochemical configuration [redacted]

The impurity profile of SCH 58235 is shown below.

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[redacted] of impurity profile of the drug substance (SCH 58235)

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The [redacted] impurities were present in the drug substance in amounts greater than [redacted], see Table below, which shows the acceptance limits of these impurities.

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Following are the acceptance limits for impurities in the [redacted] drug substance (SCH 58235), shown by the sponsor:

However, according to ICH Q3A guidance document, from pharm/tox aspect these need to be qualified if present in amounts > 0.1% in the drug substance. In the drug product, the degredants and impurities should be <1%, or these would need to be qualified. The total [redacted] impurities in the drug product were not more than [redacted] Therefore, above impurities were qualified in 1-month toxicity studies in rats and dogs and in genotox studies. Note that some impurities (see Table below) have been added at slightly higher amounts then they are found in the drug substance.

Also, note that another impurity. [redacted] was not added to the list of impurities to be qualified for the tox studies, this compound could be present in amounts greater than [redacted] in the final drug substance (depending on the humidity and temperature), but for some reason this compound was not specifically evaluated.

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Following impurity (present in the drug substance) was not qualified in the tox studies:

NDA Shelf-Life Specifications for Ezetimibe (SCH 58235) Tablets, 10 mg
Specification Number: 058235- [redacted]
Formula Number: 3346
Effective Date: October 31, 2001

Test	Specification	Procedure
Description:	White to off-white capsule-shaped, embossed tablets	[redacted]
Moisture: (Average of n=2)	[redacted]	[redacted]
Assay:	[redacted]	[redacted]
Degradation Products:	Each specified [redacted]	[redacted]
Dissolution:	Consistent with USP criteria where Q= [redacted] dissolved in 30 minutes	[redacted]

2A. Study title: A 1-month Dietary Toxicity Study of SCH-58235 With Impurities in Rats (Study No. SN 00037)

Key study findings: SCH 58235 + impurities following a 1-month dietary administration in rats (males 250, 750, 1500 mg/kg/day, females 50, 250, 500 mg/kg/day) produced toxicity at a high dose in the male hearts (mononuclear cellular infiltration in 2/10 rats vs 0/10 controls), and lymph nodes (hyperplasia in 1/10 vs 0/10 in controls). In females, it produced toxicity in the pituitary gland (diffuse hyperplasia adenoma in 0/10, 1/1, ne, 2/10 respectively) and spleen (increased extramedullary hematopoiesis in 0/10, 1/1, ne, 1/10 respectively). The NOAEL in the 6-month rat study may be 750 mg/kg/day in males and 250 mg/kg/day in females. Thus this study had similar target organs of toxicity as without impurities, except additional toxicity was identified in the pituitary. This qualifies the "impurities", and therefore toxicities identified are similar to SCH 58235 previously tested.

Study no: 00037
Volume #, and page #: 1.84, page 1
Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 4/18/2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #: 00-58235-X-101
Formulation/vehicle: [redacted] (meal)

Methods (unique aspects):
Dosing:
Species/strain: Sprague-Dawley rats/Crl:CD (SD)IGS BR VAF/PLUS
#/sex/group or time point (main study):10/sex/dose

Satellite groups used for toxicokinetics or recovery: Additional 12/sex/dose for TK study.
Age: Approximately 6 weeks of age
Weight: Males 165-241 g, females 130-186 g.
Doses in administered units: Males 0, 150, 750, 1500 mg/kg/day, females 0, 50, 250, 500 mg/kg/day. **This study examined the toxicity of SCH 58235 with added impurities in the diet** (see Table). The impurities are

[ ] Dose selection was based on the previous gender related differences in exposure at above doses in a 3-month dietary (study # P-6290) and 2-week PK (study # P-6666) study in rats, where plasma levels plateaued at 1500 and 500 mg/kg/day in males and females respectively. Therefore high doses of 1500 and 500 mg/kg/day were chosen for males and females respectively in the current study.

Table 32: SCH 58235 with added impurities in the diet. The justification for these doses being tested is that these impurities were present in doses not more than —— Therefore less than —— or in some instances slightly higher doses were used (see the justification above).

Added Impurities		
Compound	SCH Number	Percentage
[ ]		

Route of administration: Dietary for 28-29 consecutive days.

Observations and times:
Clinical signs: Once daily
Body weights: Weekly
Food consumption: Weekly.
Test Article Intake: Weekly
Ophthalmoscopy: Prior to treatment, and once during week 4.
Hematology: During week 4.
Clinical chemistry/Coagulation: During week 4/week 5.
Urinalysis: During week 4.
Gross pathology: At sacrifice.
Organs weighed: Organs weighed are listed in the Table
Table 33. Tissues collected for organ weights in the 6-month dietary rat tox study

Organs Weighed	
Adrenal Glands	Pituitary Gland
Brain	Prostate Gland (ventral)
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid Gland/Parathyroid Glands[a]
Lungs	Uterus (plus Cervix)
Ovaries	

a: Thyroid gland/parathyroid glands were weighed post-fixation.

Histopathology: This was performed at sacrifice in control and high dose animals, listed in the histopathology Table 34.

Table 34. Tissues collected for histopath evaluation in the1-month dietary rat tox study with the drug+impurities.

Tissues Collected[a]	
Adrenal Glands	Peripheral Nerve – Sciatic
Aorta – Thoracic	Pituitary Gland
Bone (Femur and Sternum)	Prostate Gland
Bone Marrow Section – Sternum	Salivary Glands – Mandibular
Bone Marrow for Cytology – Sternum[b]	Seminal Vesicles
Brain	Skeletal Muscle - Biceps Femoris
Epididymides	Skin
Esophagus	Small Intestine – Duodenum, Jejunum, Ileum
Eyes[c]	Spinal Cord – Thoracolumbar
Harderian Glands[c]	Spleen
Head[d]	Stomach
Heart	Testes
Kidneys	Thymus
Large Intestine – Cecum and Colon	Thyroid Gland
Liver	Tongue[d]
Lungs	Trachea
Lymph Nodes (Mandibular and Mesenteric)	Urinary Bladder
Mammary Gland[e]	Uterus (plus Cervix)
Ovaries	Vagina
Pancreas	Animal Identification[d]
Parathyroid Gland(s)[e]	

a: Collected in 10% neutral buffered formalin unless otherwise indicated
b: Bone marrow smears were prepared for all toxicity portion rats except those found dead during the morning viability check but were not evaluated because it was not warranted by changes in the peripheral blood.
c: Collected in 3% glutaraldehyde
d: Collected but not processed
e: Examined histopathologically when present in routine section

Toxicokinetics: During weeks 4 at 1, 4, 8 and 24 hrs. Conjugated and unconjugated drug was measured by [redacted] by [redacted]

Results:
Mortality: One female in the control group (on day 22) was found dead, and the cause of death was not determined.

Clinical signs: No drug related effects were observed.

Body weights: No significant treatment related effects on body weights or weight gains in males or females were observed compared to controls.

Food consumption: No treatment related effects on food consumption were observed.

Test Article Intake: Drug consumption was consistent in each group throughout the study and was within 4% of the intended intake.

Ophthalmoscopic Exam: No summary data were provided. At mid dose one female animal had focal retinopathy in the left eye in week 4, however Sponsor states that it was not attributed to the drug due to lack of a dose response relationship. No drug related histopath findings in eyes were observed

Hematology/coagulation: No drug related effects were observed.

Clinical chemistry: No significant drug related effects were observed.

Urinalysis: No drug related effects were observed.
Organ weights: No drug related effects were observed.

Gross pathology: Drug related findings were observed in brain (altered shape/hemorrhage, meningeal, males 0/10, 0/10, 1/10, 0/10, females 0/10, 1/10, 0/10, 0/10 respectively, but no histopath findings were observed), as well as in pituitary gland in females (enlarged 0/10, 1/10, 0/10, 1/10 respectively), and thymus in males (small or discoloration & mottled 1/10, 1/10, 0/10, 2/10 respectively)

Histopathology: These were performed only in control and high dosed animals. In males histopath findings were observed in the heart (mononuclear cellular infiltration of minimal severity in 2/10 rats vs 0/10 controls), and in lymph nodes (minimal reactive hyperplasia in 1/10 vs 0/10 in controls). In females, in the pituitary gland (diffuse hyperplasia in pars aterior of minimal severity in 0/10, 0/1, ne, 2/10 rats respectively, at low dose dose 1/1 female rat had pituitary adenoma) and in spleen (increased extramedullary hematopoiesis of minimal severity in 0/10, 1/1, ne, 1/10 respectively). Sponsor states that no histopath findings were observed in the 1-month study with the drug + impurities in rats, these are all common and incidental.

Toxicokinetics: The plasma AUC values are shown in the Table. The AUC values did not proportionally increase with doses. The exposure were similar in both sexes despite a 3-fold higher drug doses used in males. The mean (total drug+glucuronide) systemic exposures to the drug in a 6-month study (The AUC of parent + metabolite were Males 4.5, 7.8, 8.8 µg.h/ml, females 3.5, 6.5, 10.3 µg.h/ml respectively) in rats were comparable to the exposures in this 4-week study in rats (Males 7.9, 12.0, 11.6 µg.h/ml, females 5.3, 9.7, 11.6 µg.h/ml respectively). There was extensive glucuronidation of the drug in rats. Sponsor states that the gender dependent systemic exposure was similar as in a 6-month study. Cmax were observed from 1 to 24 hrs, and steady state plasma conc. were achived by day 26 of dosing. Tmax was observed at 24 hr. The AUC of parent + metabolite at 750 mg/kg/day in male (12 µg.h/ml) and at 250 mg/kg/day in female (9.7 µg.h/ml) rats were 8-9 fold, the human AUC at 20 mg/day (1.314 µg.h/ml).

Table 35: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in week 4 in a 1-month dieatry rat toxicity study of the drug + impurities:

Group	Gender	Dose (mg SCH 58235/kg)	Mean SCH 58235 AUC(0-24 hr) (ng-hr/mL)		
			Total	Conjugated	Unconjugated
Low-Dose	M	150	7928	7812	115
	F	50	5312	5237	76.6
Mid-Dose	M	750	11996	11799	207
	F	250	9671	9605	68.7
High-Dose	M	1500	8814	8734	80.5
	F	500	11610	11504	108

Toxicology summary: In a 1-month dietary toxicity study in rats with the drug containing impurities (from manufacturing and/or degradation of the drug in a drug substance), doses of 0, 250, 750, 1500 mg/kg/day in males, and 0, 50, 250, 500 mg/kg/day in females were used. The increases in AUC values of the total drug were not dose proportional in week 4 (males 7.9, 12.0, 11.6 µg.h/ml, females 5.3, 9.7, 11.6 µg.h/ml respectively), as was noted in almost all tox studies. The target organs of toxicity at a high dose in males are heart (mononuclear cellular infiltration in 2/10 rats vs 0/10 controls), and lymph nodes (hyperplasia in 1/10 vs 0/10 in controls). In females, the target organs are pituitary gland (diffuse hyperplasia in pars aterior in 0/10, 0/1, ne, 2/10 respectively, at low dose dose 1/1 female rat had pituitary adenoma) and spleen (increased extramedullary hematopoiesis in 0/10, 1/1, ne, 1/10 respectively). Note that these histopath changes were not examined at lower doses. The NOAEL in the 6-month rat study may be 750 mg/kg/day in males and 250 mg/kg/day in females. Pituitary hyperplasia was not previously observed with the drug without impurities.

2B. Study title: A 1-month Oral Toxicity Study of SCH-58235 With Impurities in Dogs (submission 3/4/2002, Study No. 00053)-

Key study findings: SCH 58235 + impurities following a 1-month gavage administration in dogs (0, 30, 100, 300 mg/kg/day) produced toxicity at a high dose in the heart (**males** 1/4 dogs had hypertrophy and degeneration of myxomatous aortic valve, 1/4 **females** had mononuclear cellular infiltration vs 0/4 controls), lymph nodes (brown pigment accumulation in 2/4 M+ 2/4 F vs 0/4 in controls, and lymphoid hyperplasia in ¼ M+ ¼ F vs 0/4 controls), liver (centrilobular vacuolation in ¼ males vs 0/4 controls), lungs (cellular infiltration with neutrophilic focus in ¼ females, metaplasia in ¼ males vs 0/4 controls, histiocytic/lymphohisiocytic multifocal inflammation in ¼ M +1/4 F vs 0/4 controls), ovaries (paraovarian cyst in ¼ females vs 0/4 controls), stomach (mineralization, mucosal focal in 2/4 males vs 0/4 controls), small intestine nematodes luminal (**males** 1/4, 2/2, 4/4, 4/4, **females** 4/4, 2/2, 4/4, 2/2 respectively), spleen (was not remarkable in both **males** 0/4, 3/3, ne,1/4 and **females** 2/4, 2/2, 3/3, ¼) and findings included siderofibrosis in 1 male at a low dose, hyperplasia at mid/high doses in females, while focal congestion was observed in controls and drug treated, with mini-mild severity in controls while of moderate severity in drug treated dogs). The NOAEL in the 1-month dog study with impurities may be 100 mg/kg/day. This study had similar target organs of toxicity (heart, spleen, & lungs) as without impurities (in 3-6 month dog

studies), but additional toxicity was identified in lymph nodes, liver, ovaries, small intestine, not previously identified without impurities. Sponsor considers these common findings in dogs and unrelated to impurities.

Study no: 00053
Volume #, and page #: ilnitial study was submitted in volume **1.86**, reference **37**, page 1, but since only plasma conc, were and validation of assay were provided in that report, an amendment was submitted with the complete report of this study in dogs on 3/4/2002 in 3 volumes
Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 4/25/2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #: 00-58235-X-101
Formulation/vehicle: 0.4% (w/v) aqueous methylcellulose

Methods (unique aspects):
Dosing:
Species/strain: Beagle dogs
#/sex/group or time point (main study): 4/sex/dose
Satellite groups used for toxicokinetics or recovery: N/A.
Age: Approximately 8-10 months of age
Weight: Males 6.6-11.3 kg, females 7.1-10.8 kg.

Doses in administered units: 0, 30, 100, 300 mg/kg/day. **This study examined the toxicity of SCH 58235 with added impurities by gavage** (see Table). The following impurities are present in the drug substance:

[ ]. The justification for these doses is the fact these impurities were present in amounts less than —, therefore similar or in some instances slightly higher doses were used (see justification above in section VIII)

Table 36: SCH 58235 with added impurities by gavage

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Added Impurities		
Compound	SCH Number	Percentage

Route of administration: Oral by gavage for 28-29 consecutive days.

Observations and times:
Clinical signs: Once daily
Body weights: Weekly
Food consumption: Daily.
Ophthalmoscopy: Prior to treatment, and once during week 3.
Hematology: Pretest and during week 4.
Clinical chemistry/Coagulation: Pretest and during week 4.
Urinalysis: Pretest and during week 4.
Gross pathology: At sacrifice.
Organs weighed: Organs weighed are listed in the Table

Table 37. Tissues collected for organ weights in the 1-month oral (gavage) dog tox study

Organs Weighed	
Adrenal Glands	Pituitary Gland
Brain	Prostate Gland
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid Gland/Parathyroid Glands
Lungs	Uterus (plus Cervix)
Ovaries	

Histopathology: This was performed at sacrifice in control and high dose animals, listed in the histopathology Table 38.

Table 38. Tissues collected for histopath evaluation in the 1-month oral gavage dog tox study with the drug+impurities.

Tissues Collected[a]	
Adrenal Glands	Parathyroid Gland(s)
Aorta - Thoracic	Peripheral Nerve – Sciatic
Bone (Femur, Rib[b] and Sternum)	Pituitary Gland
Bone Marrow Section - Rib[b] and Sternum	Prostate Gland
Bone Marrow for Cytology - Rib[c]	Salivary Glands - Mandibular
Brain	Skeletal Muscle - Biceps Femoris
Epididymides	Skin
Esophagus	Small Intestine - Duodenum, Jejunum and Ileum
Eyes[d]	Spinal Cord - Thoracolumbar
Gallbladder	Spleen
Heart	Stomach
Kidneys	Testes
Lacrimal Glands	Thymus
Large Intestine - Cecum and Colon	Thyroid Gland
Liver	Tongue[b]
Lungs	Trachea
Lymph Nodes (Mandibular and Mesenteric)	Urinary Bladder
Mammary Gland	Uterus (plus Cervix)
Ovaries	Vagina
Pancreas	Animal Identification[b]

a: Collected in 10% neutral buffered formalin unless otherwise indicated
b: Collected but not processed
c: Bone marrow smears were prepared for all dogs but were not evaluated because it was not warranted by changes in the peripheral blood.
d: Collected in 3% glutaraldehyde

Toxicokinetics: During weeks 4 at 1, 4, 8 and 24 hrs. Conjugated and unconjugated drug was measured by ——— by ———

Results:
Mortality: None

Clinical signs: No drug related effects were observed. Sponsor states that abnormal stool (soft, loose, red-streaked and /or mucoid) was observed in all groups and was considered incidental. No summary Table was provided.

Body weights: Sponsor states that no significant treatment related effects on body weights in males or females were observed compared to controls. However no weigh gain data were provided. The starting weights in males on day-0 were 9.3, 8.7, 9.0, 10.1 respectively, on day-28 the weights were 9.5, 8.8, 9.4, 10.5 kg respectively, it appears that at a low dose the weight gain was reduced. Same was observed in females, on day-0 BW were 8.6, 8.6, 8.1, 8.7 kg respectively, on day-28 the weights were 9.1, 8.8, 8.5, 9.2 kg respectively.

Food consumption: Similarly it is stated that no treatment related effects on food consumption were observed, occasional lower food consumption (<50% food consumed) was not persistent and was considered incidental. No summary data were presented, only individual animal food consumption values were provided.

Ophthalmoscopic Exam: No drug related effects were observed

Physical exam and electrocardiograms: One male high dose dog (#28) had an enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve. However sponsor considers this isolated case, since the above dog in a pretest had a minor ECG anomaly (right axis QRS). Sponsor states that no significant changes were observed in heart rates, BP, body temperatures etc., however heart rates seem to be increased at a high dose (in males increased from pretest of 114-119 to 136 beats/min after the drug treatment) vs control (increased from pretest of 125-128 to 119 beats/min after the drug treatment). This was also observed at a high dose in females (135 vs 111-117 beats/min pretest) vs controls (120 vs 119-125 beats/min pretest)

Hematology/coagulation: No significant drug related effects were observed.

Clinical chemistry: Sponsor states that no significant drug related effects were observed. However, ALT was increased at all doses by 1.5-2 fold in both sexes (males baseline values pretest in -1 week were 41, 37, 30, 33 IU/L and after drug treatment increased to 48, 64, 56, 49 IU/L at 0, 30, 100, 300 mg/kg/day respectively in week 4). Similar observations were observed in females (pretest 39, 36, 47, 31, after drug treatment were 45, 81, 76, 40 IU/l respectively). Cholesterol was decreased at all doses (males pretest 144, 168, 162, 200, after drug treatment 130, 102, 87, 127 mg/dl respectively). Similar trend in cholesterol levels were seen in females. However no significant effects on TG levels were observed.

Urinalysis: No drug related effects were observed.
Organ weights: In males at a high dose, mean heart weights (absolute 79.2, 76.5, 79.4, 99.1 g respectively, relative 0.82, 0.87, 0.85, 0.93 % respectively, at a high dose 2/4 male dogs had a significant increase in heart weight,108-125 g in two males vs 71-91 g in 4 control dogs), liver weights (280, 278, 261,318 g respectively), and testes weights (12, 10, 13, 16 g respectively) were increased, while prostate weights were increased at a mid dose (3.5, 2.5, 6.0, 3.3 g respectively). In females at a high dose, mean ovary weights (absolute 1.3, 1.0, 0.7, 1.5 g respectively) were decreased, relative ovary weights were also similarly decreased

Gross pathology: Heart was enlarged in one male at a high dose. In lungs, the surface was altered in 0/4, 0/4, ¼, ¼ males, and ¼ females at a mid dose vs none in other groups. Changes in spleen were observed (discolored, focal dark-red **males** 0/4, ¾, 0/4, ¼, **females** ¼, ¼, 2/4, ¼ respectively). Uterus was enlarged in two females at a low dose vs none in other groups.

Histopathology: Performed only in control and high dosed animals. Histopath findings were observed in one male heart (# 28 male dog had minimal to mild medial hypertrophy of arterioles in the myocardium and lungs, minimal focal minieralization in the right myocardium and mild myxomatous degeneration of aortic valve, which correlated with altered shape of the valve, i.e in ¼ vs 0/4 in controls). Sponsor explains that these

changes were probably present prior to the start of the study, as similar findings have been seen historically, and this may have been a developmental vascular anomaly in dog #28. The toxicity was also observed in one female heart (cellular infiltration of mononuclear cells, perivascular, of moderate severity in ¼ vs 0/4 in controls), in kidneys (mineralization in ¼ males vs 0/4 controls), lymph nodes (brown pigment accumulation of minimal to mild severity in 2/4 males and 2/4 females vs 0/4 in controls, and lymphoid hyperplasia of mild degree in ¼ males and ¼ females vs 0/4 controls), liver (centrilobular vacuolation of minimal degree in ¼ males vs 0/4 controls), lungs (cellular infiltration with neutrophilic focus of minimal degree was in ¼ females, metaplasia (minimal) in ¼ males vs 0/4 controls, histiocytic /lymphohisiocytic multifocal inflammation in ¼ males +1/4 females at a high dose vs 0/4 controls). Also findings were observed in ovaries (paraovarian cyst in ¼ females vs 0/4 controls), stomach (minimal mineralization, mucosal focal in 2/4 males vs 0/4 controls), small intestine nematodes luminal (males 1/4, 2/2, 4/4, 4/4, females 4/4, 2/2, 4/4, 2/2 respectively), and in spleen (which was not remarkable in males 0/4, 3/3, ne,1/4 and females 2/4, 2/2, 3/3, ¼, and findings included siderofibrosis in 1 male at a low dose, hyperplasia at mid/high doses in females, while focal congestion was observed both in control and drug treated dogs, all were of mini-moderate severity, with moderate severity in drug treated dogs). Sponsor states that no histopath findings were observed in the 1-month study with the drug + impurities in dogs, these are all common and incidental. The NOAEL in the 1-month dog tox study with impurities may be 100 mg/kg/day. Note that histopath findings were not generally determined at low-mid doses.

Toxicokinetics: The plasma AUC values are shown in the Table. The AUC values did not proportionally increase with doses. The exposure were similar in both sexes. The mean (total drug+glucuronide) systemic exposures to the drug in a 6-month dog study were (The AUC of parent + metabolite were 2.8, 5.0, 6.4 µg.h/ml respectively) were comparable to the exposures in this 4-week study in dogs (3.8, 5.9, 7.4 µg.h/ml respectively). There was extensive glucuronidation of the drug in rats. Variability in mean AUC values and plasma conc. ranged from 18-86% and 13-200% respectively. Cmax were observed from 1 to 24 hrs, and steady state plasma conc. were achieved by day 26 of dosing. Tmax was observed at 1-8 hrs, 1 hr, 1-4 hrs at low mid and high doses. Because of the wide variability in the mean AUC values, the TK of ezetimibe with impurities do not appear to be significantly different from the TK without impurities in dogs

The AUC of parent + metabolite at 300 mg/kg/day in dogs (7.4 µg.h/ml) was 10 fold, the human AUC at 10 mg/day (0.71 µg.h/ml).

Table 39 a and b: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in a 1-month gavage dog toxicity study of the drug + impurities:

Table 39a

Table 1 Mean (%CV) Plasma Concentrations and Pharmacokinetic Parameters of Total SCH 58235 During Week 4 of a One-Month Oral (Gavage) Administration of 30, 100 and 300 mg SCH 58235/kg to Male and Female Dogs

		Total SCH 58235 (ng/mL)[a]															
		30 mg/kg						100 mg/kg						300 mg/kg			
	Units	M		F		M & F		M		F		M & F		M		F		M & F	
1	hr	351	(51)	322	(41)	337	(44)	403	(28)	794	(44)	599	(53)	1009	(15)	730	(33)	869	(28)
4		314	(105)	188	(47)	251	(93)	299	(89)	530	(54)	414	(69)	788	(42)	516	(89)	652	(61)
8		301	(61)	132	(101)	216	(81)	149	(13)	400	(85)	274	(94)	191	(38)	390	(67)	290	(71)
24		8.48	(123)	8.36	(44)	8.42	(86)	7.41	(73)	14.4	(68)	10.9	(75)	79.1	(81)	20.4	(37)	49.7	(106)
Cmax	ng/mL	409	(65)	322	(41)	366	(55)	446	(41)	794	(44)	620	(51)	1053	(10)	831	(39)	942	(27)
AUC(0-8 hr)	ng·hr/mL	2408	(77)	1569	(52)	1988	(70)	2154	(51)	4248	(53)	3201	(62)	5195	(23)	4054	(55)	4625	(38)
AUC(0-24 hr)	ng·hr/mL	6041[b]	NC[c]	2688	(70)	3806[d]	(86)	3749[e]	(28)	7559	(65)	5926[f]	(69)	7354	(18)	7337	(55)	7345	(38)

a: N = 4 for male or female, N=8 for male and female combined.
b: N = 2
c: NC = Not calculated; %CV not calculated if N < 3.
d: N = 6
e: N = 3
f: N = 7

Table 39b

APPEARS THIS WAY
ON ORIGINAL

Dose (mg SCH 58235/kg)	Mean (%CV) SCH 58235 AUC(0-24 hr) (ng·hr/mL)		
	Total	Conjugated	Unconjugated
30[a]	3806 (86)	3555 (85)	347[b] (115)
100[c]	5926 (69)	5587 (72)	341 (40)
300[d]	7345 (38)	6751 (38)	594 (49)

a: N = 6; b: N = 3
c: N = 7; d: N = 8

Toxicology summary: In a 1-month oral gavage toxicity study in dogs with the drug containing impurities, doses of 0, 30, 100, 300 mg/kg/day were used. The increases in AUC values of the total drug were not dose proportional in week 4 (Males +females 3.8, 5.9, 7.4 µg.h/ml respectively), as was noted in almost all tox studies. The target organs of toxicity at a high dose may be heart (1/4 males had an enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve, ¼ females had mononuclear cellular vs 0/4 controls), lymph nodes (brown pigment accumulation in 2/4 M+ 2/4 F vs 0/4 in controls, and lymphoid hyperplasia in ¼ M+ ¼ F vs 0/4 controls), liver (centrilobular vacuolation in ¼ males vs 0/4 controls), lungs (cellular infiltration with neutrophilic focus in ¼ females, metaplasia in ¼ males vs 0/4 controls, histiocytic/lymphohisiocytic multifocal inflammation in ¼ M +1/4 F vs 0/4 controls), ovaries (paraovarian cyst in ¼ females vs 0/4 controls), stomach (mineralization, mucosal focal in 2/4 males vs 0/4 controls), small intestine nematodes luminal (**males** 1/4, 2/2, 4/4, 4/4, **females** 4/4, 2/2, 4/4, 2/2 respectively), spleen (siderofibrosis in 1/4 males at a low dose, hyperplasia at mid/high doses in females). The NOAEL in the 1-month dog study with impurities may be <30 mg/kg/day. The TK of ezetimibe with impurities were slightly higher than without impurities, but there was a

wide variability in plasma and AUC values, which may explain the differences. The toxicity seen here with impurities in heart, spleen, lungs have been observed previously in 3-6 month studies in dogs without impurities (with ezetimibe). However, toxicity seen in lymph nodes, liver, ovaries, small intestine with ezetimibe + impurities in dogs were not previously observed without impurities. Sponsor considers all histopath findings in dogs as common findings, and unrelated to impurities.

Sponsor states that no toxicity emerged in rat or dogs due to impurities, and TK in rats/dog was also not different with drug containing impurities.

Special Genetic-toxicity Studies

2C. Study title: Effects of SCH 58235 with impurities on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: SCH 58235 with impurities was negative in AMES test

Study no: 00081
Volume #, and page #: Volume 86, page 1 (reference 38)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 6/7/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: 00-58235-X-101
Impurities: ——— drug substance was spiked with followin impurities are

[ ]

Formulation/vehicle: Dimethylsulfoxide (DMSO)
Sterile distilled water, at a conc. of 500 mg/ml

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA98, TA100, TA1535, TA102, TA07a, and E. coli tester strain WP2 urvA

Dose selection criteria:
Basis of dose selection: The dose selection was based on the first mutagenicity study in which doses of 50, 158, 500, `581 and 5000 µg/plate were used in Salmonella and E. coli strains in the plate incorporation method in the presence and absence of metabolic activation. In the above assay in the absence of activation, no cytotoxicity was noted, even at the top dose in all strains, except in TA102, where it was observed at 500 µg/plate. Precipitate was noted at 158 µg/plate in TA 1535 and at 500 µg/plate in strains TA97a, TA100, & TA102, and at 1581 µg/plate in strains TA98, and WP2urvA. In the presence of activation, cytotoxicity was observed at 158-5000 µg/plate in different strains, and precipitate was noted from 500 µg/plate. However in this assay the drug did not induce an increase in revertant colony counts in any bacterial starin tested. Based

on this assay, two mutagenicity assays (trial 2 and 3) were conducted, and doses chosen are sown below.

Range finding studies: Doses of 50-5000 µg/plate were used in all strains in the presence and absence of metabolic activation in trial 1, and based on cytotoxicity and precipitate following doses were selected.

Table 40:

Bacterial Strain	Doses (µg/plate)	
	Nonactivation Phase	Activation Phase
TA1535	313, 625, 1250, 2500, 5000	313, 625, 1250, 2500, 5000
TA97a	39, 78, 156, 313, 625	19.5, 39, 78, 156, 313
TA98	313, 625, 1250, 2500, 5000	313, 625, 1250, 2500, 5000
TA100	156, 313, 625, 1250, 2500	156, 313, 625, 1250, 2500
TA102	39, 78, 156, 313, 625	39, 78, 156, 313, 625
WP2uvrA	313, 625, 1250, 2500, 5000	313, 625, 1250, 2500, 5000

3.3. Mutagenicity Assay - Trial 3

Trial 3 was conducted with the same procedures as Trial 1. The doses and bacterial strains for Trial 3 were selected based on the results of Trial 2 and are tabulated as follows:

Bacterial Strain	Doses (µg/plate)	
	Nonactivation Phase	Activation Phase
TA1535	39, 78, 156, 313, 625, 1250, 2500, 5000	Not Tested
TA97a (1)	Not Tested	19.5, 39, 78, 156, 313, 625
TA97a (2)	39, 78, 156, 313, 625	19.5, 39, 78, 156, 313, 625
TA100	Not Tested	156, 313, 625, 1250, 2500
TA102	78, 156, 313, 625, 1250	39, 78, 156, 313, 625

Test agent stability: The prepared drug was stable in DMSO for at least 4 hours under the ambient temperature and light at conc. of 0.15-49.8 mg/ml.

Metabolic activation system: Rat liver microsome S9 fraction.

Controls:
Vehicle or negative controls: DMSO
Positive controls: These were as follows

Table 41:

Bacterial Strains	Nonactivation Phase (µg/plate)	Activation Phase (µg/plate)
Salmonella typhimurium		
TA1535 and TA100	Sodium azide (5)	2-Aminoanthracene (2.5)
TA97a	9-Aminoacridine (75)	2-Aminoanthracene (2.5)
TA98	2-Nitrofluorene (5)	2-Aminoanthracene (2.5)
TA102	Cumene hydroperoxide (200)	2-Aminoanthracene (5)
Escherichia coli		
WP2uvrA	N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG)(2)	2-Aminoanthracene (20)

Comments:
Exposure conditions/Study design: The plate incorporation method was used. The tester strains in the plate (in triplicate cultures) were exposed to the vehicle, drug, or positive controls. The cells were incubated for 40-56 hrs at 37^{0}C on selective top agar, in both the presence and absence of S9 fraction. Colonies were counted manually or with automated colony counter.

Doses used in definitive study: 39-5000 µg/plate.

Analysis:
No. of replicates: Duplicates cultures/dose
Counting method: Revertant colonies for a given tester strain were counted manually or with automated colony counter.

Criteria for positive results: If the drug induces an increase in revertant colonies in a dose dependent manner, and the increase is at least 2 times for strains TA97a, TA98, TA100, TA102, WP2 urvA, and 3 times for strain TA1535 and TA1537, compared to vehicle controls, the drug would be considered positive.

Summary of individual study findings:
Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable

Study outcome: In the initial dose range study, cytotoxicity or drug precipitation was observed at doses of 158-5000 µg/plate in the presence or absence of S-9 in any tester strains. In the two confirmatory mutagenicity assays, the drug (SCH 58235) was not mutagenic in any of the tester strains at 156 to 313 µg/plate in the presence or absence of metabolic activation, as precipitate was observed above these doses. A significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). In conclusion, the AMES test was negative.

Genetic toxicology summary: SCH 58235 with impurities was negative in AMES test in all tester strains.

2D. **Study Title: Effects of SCH 58235 spiked with impurities on in Vivo Micronuclei in Mice.**

Key findings: SCH 58235 with impurities was negative in vivo micronucleus test in mice

Study no: 00084
Volume #, and page #: Volume 86, page 1 (Reference 39)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 7/27/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: Lot #: 00-58235-X-101

Formulation/vehicle: 0.4% aqueous methylcellulose, at a conc of 50-200 mg/ml

Methods:
Test strain and Cells: Mice (Crl:CD-1(CR)BR VAF/PLUS, 6-8 weeks of age, males 26-35.8 g, females 20.7-26.5 g.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a previous in vivo micronucleus study in mice with the drug without impurities, where doses of 200, 400, 800 mg/kg/day were used. Therefore in the current assay doses of 200, 400, 800 and 1000 mg/kg/day were used. Mice were observed for clinical signs. Bone marrow toxicity (decrease in mean PCE/NCE was observed at 1000 mg/kg/day (males 1.07, 0.88, 0.75. 0.53, 0.42 or by 83 to 40% at 0, 200, 400, 6000, 800, 1000 mg/kg/day). Similarly toxicity at 1000 mg/kg/day in females was 50%. Based on the bone marrow toxicity, doses of 200, 400, 800 mg/kg/day were chosen for the main micronucleus test. In the main micronucleus test, no mortality was observed in males and females up to 800 mg/kg/day . Clinical signs included rough hair coat at 800 mg/kg/day in both sexes, 24 hrs after second dosing

Range finding studies: 200-1000 mg/kg/day for two consecutive days
Test agent stability: The prepared drug at 0.1-200 mg/ml was stable for up to 7 days at the ambient laboratory conditions of temperature and light, and is homogenous.

Controls:
Vehicle or negative controls: 0.4% aqueous methylcellulose
Positive controls: Cyclophosphamide (CP), 50 mg/kg.

Exposure conditions/Study design: This assay determines clastogenesis, or the chromosome damaging activity in vivo. Erythroblasts in the bone marrow undergoing their last chromosome replication are the target cells here. Mice (6/sex/group/sacrifice time) were given an IP dose of the drug (SCH 58235) at 200, 400, 800 mg/kg/day for two consecutive days. A group of mice were similarly treated with the vehicle (0.4%

aqueous methylcellulose) or CP (positive control). Animals (5/sex/group based on homogeneity of BW) were sacrificed at 24 hrs and 48 hrs after the administration of the drug, and bone marrow cells from femur of each mice were prepared. Cells were stained with acridine orange, and total of at least 2000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei. Bone marrow toxicity was evaluated by the ratio of PCE/NCE from 200 PCE in each mouse

Doses used in definitive study: IP doses of 200, 400, and 800 mg/kg/day were used.

Analysis:
No. of animals used: 5/sex/group/sacrifice time
Counting method: Total of 2000 polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were analyzed, and examined for the presence of micronuclei.

Criteria for positive results: If two consecutive doses produced a significant increase in micronucleated PCE (MN-PCE) compared to the vehicle control (in mean MN-PCE), and if the incidence of MN-PCE for the positive control was statistically significant, the drug would be considered positive.

Summary of individual study findings:
Study validity: Appropriate dose selection was made for this study, and positive control responses were acceptable.
Study outcome: The drug did not induce an increase in micronucleated PCE up to doses of 800 mg/kg/day. In contrast cyclophosphamide (CP) induced a significant increase in the PCE in both male/female mice, compared to vehicle control ([redacted] vs [redacted] in controls, $p<0.001$). The drug produced clinical signs at 800 mg/kg/day (rough hair coat in both sexes), no deaths occurred up to doses of 800 mg/kg/day in animals. The drug at all doses produced dose dependent increase of bone marrow toxicity (mean PCE/NCE was 0.53-0.88 in males and 0.56-0.75 in females at 200-800 mg/kg/day, 0.33-0.49 in positive controls, and 1.06-1.15 in negative controls). The exposures of the drug or mean plasma concentration (TK analysis) in mice were not examined in this study. In conclusion, the drug was not cytogenic in this assay.

Genetic toxicology summary: SCH 58235 with impurities was not cytogenic at doses up to 800 mg/kg/day in an in vivo micronucleus test in mice.

<u>Special toxicology Studies with combination therapy of ezetimibe + statins</u>:

Sponsor has conducted a series of studies in dogs with mevalonate (a product of HMG-CoA reductase) to determine if increases in liver enzymes with combination of ezetimibe + statin is related to inhibition by HMG-CoA reductase. Mevalonate supplementation attenuated the serum increases in ALT by 71-78% when coadministered with ezetimibe/simvastatin (at 3/10 and 30/10 mg/kg/day) for one week in dogs. These changes in serum ALT returned to pre-test values at cessation of mevalonate dosing, while serum cholesterol levels remained unaffected by mevalonate. Thus sponsor suggests that increased ALT is not a direct consequence of decreased serum cholesterol, but it is related to inhibition by HMG-CoA reductase. Similarly mevalonate supplementation (100 mg/kg) for one month in dogs, while being dosed with