Histopathologic findings: Combination SCH 58235/Simvastatin (n=10/sex/dose)
Males: Simvastatin control, **50/10, 250/10, 250/50 mg/kg/day:**
Females: Simvastatin control, **12/10, 50/10, 50/50 mg/kg/day**:

	males	females
Liver:		
Single cell necrosis (min-mild)	**3, 3, 1, 4**	**2, ne, ne, 3**
Hypertrophy, hepatocellular (min)	ne, ne, ne, ne	ne, ne, ne, 5
Bile duct hyperplasia (mini-mild)	2, 1, ne, 2	2, ne, ne, 5
Vacuolation, hepatocellular (min)	ne, 1, ne, 1	1, ne, ne, 1
Karyomegaly, hepatocellular (min)	1, ne, ne, ne	ne, ne, ne 1
Stomach:		
Hyperkeratosis, nonglandular (mini-mild)	1, ne, ne, 2	2, ne, ne, 6
Acanthosis, nonglandular (mini)	1, ne, ne 3	1, ne, ne, 5
Edema,submucosal nonglandular (mini)	ne, ne, ne, 3	1, ne, ne, 6
Cellular infiltration nonglandular (mini)	ne, ne, ne 1	ne, ne, ne, 2

ne=Not examined.

Sponsor Table: Severity of liver histopathologic findings in a 3-month dog toxicity study of SCH 58235 + atorvastatin

Simvastatin-Related Histopathologic Findings								
Group:	SCH 57098 Control		Low-Dose Combination		Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[a]							
Liver								
-single cell necrosis, hepatocellular, periportal								
minimal	2/10	2/10	3/10		1/10		3/10	2/10
mild	1/10						1/10	1/10
-vacuolation, hepatocellular, periportal								
minimal		1/10	1/10				1/10	1/10
-hypertrophy, diffuse, hepatocellular								
minimal								5/10
-karyomegaly, hepatocellular, periportal								
minimal	1/10							1/10
-hyperplasia, bile duct, periportal								
minimal	2/10	2/10	1/10				2/10	3/10
mild								2/10
Stomach								
-hyperkeratosis, nonglandular								
minimal	1/10	2/10					2/10	5/10
mild								1/10
-acanthosis, nonglandular								
minimal	1/10	1/10					3/10	5/10
-edema, submucosal, nonglandular								
minimal		1/10					3/10	6/10
-cellular infiltration, granulocytic, nonglandular								
minimal							1/10	2/10

SCH 57098 = Simvastatin

a: Incidence = Number affected/Number examined

APPEARS THIS WAY
ON ORIGINAL

Toxicokinetics. Systemic exposure to unconjugated and to total SCH 58235 increased in a dose related manner. Both accumulated (by 1 to 5-fold) on day 57 vs day 1. The total and free drug increased by 3-4 fold in males as the simvastatin dose increased from 10 to 50 mg/kg/day in a combo. The total also increased in females with this combo, but free decreased in females.

The simvastatin and hydroxysimvastatin exposures were 2-6 fold higher with high dose combos, than with simva alone. The accumulation ratio of simva and hydroxysimva was 0.3-3.7 fold.

Tables: Expsoures in rats with SCH-58235+ simvastatin

The systemic exposures [AUC(0-24 hr)] to total, unconjugated, and conjugated SCH 58235 on Days 0 and 57 are summarized in the following table:

Day	Male			Female		
	50/10[a]	250/10	250/50	12/10	50/10	50/50
Total SCH 58235 AUC(0-24 hr) (ng·hr/mL)						
0	5613	10776	9485	962	3619	4029
57	6209	13074	48572	1995	19345	14593
Unconjugated SCH 58235 AUC(0-24 hr) (ng·hr/mL)						
0	44.5	87.3	70.5	ND	26.4	14.9
57	71.4	80.6	215	ND	108	21.4
Conjugated SCH 58235 AUC(0-24 hr) (ng·hr/mL)						
0	5560	10703	9412	948	3586	4017
57	6134	12998	48360	1992	19242	14577

a: Dose of SCH 58235/simvastatin.
ND = Not determinable

Table: Simvastatin and hydroxysimvastatin exposures in rats with SCH-58235+simva

STUDY NO. 97124

Day	Male				Female			
	50/10[a]	250/10	250/50	0/50	12/10	50/10	50/50	0/50
Simvastatin AUC(0-24 hr) (ng·hr/mL)								
0	7.11	4.70	65.6	60.9	33.2	27.0	187	289
57	6.57	5.71	145	37.5	12.7	42.3	229	135
Hydroxysimvastatin AUC(0-24 hr) (ng·hr/mL)								
0	301	203	3006	1780	1331	1047	7528	12963
57	400	293	10971	1936	814	1771	8571	4081

a: Dose of SCH 58235/ Simvastatin.

Toxicology summary: In a 3-month combination toxicity study in rats, SCH-58238 doses (of 50, 250, 250 mg/kg/day in males and 12, 50, 50, mg/kg/day in females) were used, in combination with 10, 10, and 50 mg/kg/day of simvastatin. In addition one group of rats received simvastatin alone at 50 mg/kg/day. The plasma drug exposures of simva and hydroxysimva were increased by 2-6 fold vs simva alone. The total and unconjugated drug conc were also increased at high dose combo in males (but not in females). All dose decreased BW in males (by 6-13%, in females at high doses by 13%) and weight gains in both sexes (by 10-22%). All doses increased AP (316-382 vs 168 in controls) and CGT as well as liver weights in females (by 8-25%). Toxicity was observed in the liver (hepatocellular hypertrophy and bile duct hyperplasia), and stomach (hyperkeratosis, acanthosis, submucosal edema, and cellular infiltration). **The tolerated doses of the combination drug in 3-month toxicity study in rats may be 50 mg/kg of drug in males (12 mg/kg/day in females) with 10 mg/kg/day of simvastatin.**

6. Three-Month Oral Toxicity Study of SCH 5823 ~ in combination with simvastatin in dogs, followed by a 4-week recovery period (Study No. 96417):

Sponsor's ID Study #: 96417
Amendment #, Vol. #, and page # : 049, 21.1, page 01.
Conducting laboratory : Schering-Plough Research Institute, Lafayettte, NJ.
Date of study initiation and final report: october 22, 1998 and november 1, 1999
GLP compliance: Yes
QA Report: Yes (X) No ( ), Is the evaluation based on a final, QA report: Yes.
Methods: This study examined the effects of SCH-5823 ~ (at 0.3, 3, 30 and 30 mg/kg/day) in combination with simvastatin (1, 1, 1, and 10 mg/kg respectively) for 3-months in dogs, followed by a 1-month drug-free recovery period in control and high dose animals.

Dosing information:
species: Beagle dogs.
#/sex/group or time point: 4-6/sex/group
age: ≈ 6-8 months old
weight: 5-14 kgrams.
satellite groups used for toxicokinetics: N/A
Dosage groups in administered units: Five groups (4-6 dogs/sex/group) were given oral SCH-5823 ~ by gavage (once daily) at doses of 0, 0.3, 3, 30 and 30 mg/kg/day) in combination with simvastatin (0, 1, 1, 1, and 10 mg/kg respectively) for 3-months. Control animals received the vehicle only. One additional group of dogs received simvastatin alone at 10 mg/kg/day. Also additional 2 dogs/sex/group were added to the control and high dose group. At the end of treatment period all groups were sacrificed, except the additional control and the high dose groups (n=2/sex/dose), which were kept for additional 4 week of drug free recovery period.

Route, form, volume, and infusion rate (if i.v.): Oral (via gavage).
Drug, lot #: 8-MCS-3 for SCH 5823 ~ 8-MCS-8 for simvastatin.
Formulation/vehicle: both formulated in 0.4% (w/v) in aqueous methylcellulose

Times at which Observations are made:

Clinical signs/Physical exams: Daily
Body weights: prior to dosing, and weekly thereafter.
Food consumption: Daily.
Hematology/Coagulation: prior to dosing, during weeks 5, 8, 13 and week 16 for Reversal animals.
Clinical chemistry: prior to dosing, during weeks 5, 8, 13 and week 16 for reversal animals.
Urine analysis: prior to dosing, during weeks 5, 13 and week 16 for reversal animals.
Ophthalmic Examination: Prior to the treatment, once during week 4, 12 and week 17 (of reversal period)
Electrocardiograph Examinations: ECG were taken prior to dosing, once during week 4, 6, 11 and week 17
Gross pathology: At sacrifice in week 14 and week 18 (reversal).
Organs weighed: *Marked organs in the appended Table were weighed.
Histopathology: At sacrifice from vehicle control, simvastatin control and high dose combination groups. Liver, muscle, and small intestine tissues were obtained from vehicle control, and high dose combination group dogs, because of high ALT levels in these treated animals. These tissues were frozen at –80°C.

Toxicokinetics: Blood was collected from all dogs (in tubes containing sodium heparin, and on ice) on day 0, and day 56, at 1, 2, 3, 4, 6, 12 and 24 hrs. Plasma concs. of the free form of the drug and glucuronide, as well as simvastatin and the metabolite were determined using [redacted]

Results:
Mortality: None

Clinical Signs: Two males in high dose combination had excessive salivation from day 91. One female in the above group also had hyperactivity.

Body weight/Food consumption: In two high dose combo of simvastatin +drug, decreased body weight gain was observed in both sexes (on day 91 body weight in males were 9.5, 9.1 vs 10.1 gin controls, in females these were 7.8, and 7.6 g in two above groups vs 8 g in controls). No drug related effects were observed on food consumption.

Ophthalmic Examination: No drug related effects were observed.

Electrocardiograms: No drug related effects were observed.

Hematology: In the high dose combination, hemoglobin and/or RBC counts and hematocrit were slightly decreased and reticulocyte counts (males 1.1 vs 0.8, females 1.3 vs 0.8 in simva controls) and PT were slightly increased. Reticulocyte counts remained high during recovery period (males 1.8 vs 0.7%, females 1.5 vs 0.6% vehicle controls). Also mild-moderate increases in platelets counts were seen with simva + high dose combination (males 385 vs 361, females 349 vs 300 k/ul in vehicle controls during recovery).

Biochemistry: In all treated dogs, minimal to marked increases in ALT were observed compared to vehicle controls during weeks 5, 8, and 13. In high dose combination

group, mean ALT values were 50-fold higher compared to controls (1875 vs 34 in controls, in week 13). During recovery, half of dogs in high dose combination still had increased ALT values (130-171 vs 26-54 IU/L in controls). However intestinal ALT values were not significantly higher in high dose combination group. AST increases (week 13: 72-230 vs 21-44 IU/L in controls) were mostly seen with high dose combination during weeks 5, 8, and 13 and were reversible after discontinuation of the drug. AP increases were mostly seen at mid-high dose combination during weeks 5, 8, and 13 (week 13 with high combinaion: 166-729 vs 47-185 IU/L in controls) and were reversible after discontinuation of the drug. Also with high combination there were decreases in serum glucose, total protein, and albumin values vs controls. The drug + simvastatin at all doses decreased the serum chol and TG values. No effects on urine analysis were observed. The Table below shows the changes in liver enzymes.

Incidence and Range of Values (Weeks 5-13) for Increased Serum ALT, AST and AP Activities Compared to Pretest

Group	↑ ALT (IU/L)		↑ AST (IU/L)		↑ AP (IU/L)	
	Males	Females	Males	Females	Males	Females
Overall Pretest Range						
SCH 57098 Control						
Incidence[a]	3/4	2/4			1/4	
Range						
Low-Dose Combination						
Incidence	3/4	3/4	1/4			
Range						
Low-Mid-Dose Combination						
Incidence	4/4	4/4			2/4	1/4
Range						
High-Mid-Dose Combination						
Incidence	4/4	4/4			1/4	
Range						
High-Dose Combination						
Incidence	6/6	6/6	5/6	6/6	6/6	6/6
Range						

SCH 57098 = Simvastatin
a: Incidence = Number affected/Number examined

Sponsor's Table: Changes in serum glucose, TP, albumin, cholesterol and TG levels in a 3-month dog toxicity study of SCH 58235 + simvastatin

Studies in dogs with drug + simvastatin

Incidence and Range of Values (Week 13 unless otherwise noted) for Decreased Serum Glucose, Total Protein and Albumin Concentrations Compared to Pretest

Group	↓ Glucose (mg/dL)		↓ Total Protein (g/dL)		↓ Albumin (g/dL)	
	Males	Females	Males	Females	Males	Females
Overall Pretest Range						
SCH 57098 Control						
Incidence[a]	1/4	2/4				
Range						
Low-Dose Combination						
Incidence	1/4	2/4				
Range						
Low-Mid-Dose Combination						
Incidence		1/4				
Range						
High-Mid-Dose Combination						
Incidence		1/4				
Range						
High-Dose Combination						
Incidence	2/6	4/6	5/6	5/6	6/6	3/6
Range						

SCH 57098 = Simvastatin

a: Incidence = Number affected/Number examined

b: Week 5 value used for animal No. 17F

c: Week 5 and/or 8 values used for animal Nos. 51F and 65F

Mean Serum Cholesterol and Triglyceride Concentrations during Week 13

Group	Cholesterol (mg/dL)		Triglycerides (mg/dL)	
	Males	Females	Males	Females
Vehicle Control	136	138	19	21
SCH 57098 Control	69	65	≤13[a]	≤12[a]
Low-Dose Combination	70	60	≤11[a]	≤12[a]
Low-Mid-Dose Combination	62	54	≤11[a]	<10[b]
High-Mid-Dose Combination	50	49	<10[b]	≤13[a]
High-Dose Combination	14	16	<10[b]	<10[b]

SCH 57098 = Simvastatin

a:

b:

Organ Weights: No drug related effects were observed.

Gross pathology: No drug related changes were observed.

Histopathology: In liver, minimal hepatocytic cytoplasmic eosinophilia was observed in 1-2 dogs at low-mid doses and in 5-6/6 dogs in high dose combination in both sexes (vs none in controls groups, recovery animals are included here). Sponsor stated that these are class statin effects on liver and have been reported previously. Biliary hyperplasia was observed in all 4-5 of 6 dogs at high dose combination (including in recovery animals in both sexes). These data suggest that liver findings in dogs were not reversible after drug free recovery period.

Toxicokinetics. The plasma AUC values are shown in the Table below. Exposure to simvastatin and hydroxysimvastatin generally increased by 2.5 and 1.7 fold respectively (on day 56 vs day 1) vs that with simvastatin alone. Exposure to hydroxysimvastatin was up to 5-fold greater than simvastatin. There was a dose related increase in exposure to simvastatin and hydroxysimvastatin when 1 or 10 mg of simvastatin was given with the 30 mg of the drug. As for exposure to total SCH 58235 is concerned, sponsor claims that there was no accumulation of the drug, however total drug seem to accumulate at 30 mg/1 mg combination, but not at 30 mg/10 mg combination . The combination treatment did not alter the total drug exposure (drug+glucuronide), and the mean AUC values (0-24 hr) in M+F at high dose combination on day 56 were 4010 vs 5350 ng/ml.hr on day 1.

Studies in dogs: Simvastatin and hydroxysimvastatin exposures with drug+simvastatin vs simvastatin alone in dogs

Dose[a] (mg/kg)	Gender	AUC(tf) (ng·hr/mL)			
		Simvastatin		Hydroxysimvastatin	
		Day 0	Day 56	Day 0	Day 56
0/10	M[e]	137	122	308[i]	300
	F[e]	562	103	201[i]	269
	M & F[f]	350	113	254[c]	285
0.3/1	M[e]	10.5[b]	9.75	16.7[b]	23.2
	F[e]	8.11	9.25	15.5	38.3
	M & F[f]	9.13	9.50	16.0	30.7
3/1	M[e]	7.42	7.93	12.1	17.7
	F[e]	5.69	8.75	15.8	25.9
	M & F[f]	6.55	8.34	13.9	21.8
30/1	M[e]	11.1	12.0	23.1	58.3
	F[e]	11.5	12.4	25.3	26.9
	M & F[f]	11.3	12.2	24.2	42.6
30/10	M[g]	147	280	250[j]	448
	F[g]	567	294	276[d]	545
	M & F[h]	357	287	263[j]	497

a: Dose of SCH 58235/dose of simvastatin
b: n = 3
c: n = 2
d: n = 5
e: n = 4
f: n = 8
g: n = 6
h: n = 12
i: n = 1
j: n = 10

Studies in dogs: SCH 58235 exposures in 3-month dogs study with drug+simvastatin.

Dose[b] (mg/kg)	Gender	SCH 58235 AUC(tf) (ng·hr/mL)					
		Total		Unconjugated		Conjugated	
		Day 0	Day 56	Day 0	Day 56	Day 0	Day 56
0.3/1	M[c]	48.0[g]	184[h]	2.70[g]	8.05[h]	44.5[g]	176[h]
	F[c]	NA[e]	NA	NA	1.84[h]	NA	NA
	M & F[d]	NA[e]	NA[e]	NA[e]	4.94[e]	NA[e]	NA[e]
3/1	M[c]	1340	544	22.5	14.7[i]	1310	528
	F[c]	307	580	7.72[i]	22.5[i]	262	562
	M & F[d]	825	562	16.2[j]	18.6[e]	788	545
30/1	M[c]	6400	9350	283	259	6110	9100
	F[c]	2280	3900	126	101	2150	3760
	M & F[d]	4340	6630	204	180	4130	6420
30/10	M[e]	3780	3080	309	141	3470	2940
	F[e]	6910	4930	2090[k]	267	4760[k]	4670
	M & F[f]	5350	4010	1120[l]	204	4060[l]	3800

a: NA: Data not amenable to pharmacokinetic analysis
b: SCH 58235/simvastatin doses
c: n = 4
d: n = 8
e: n = 6
f: n = 12
g: n = 2
h: n = 1
i: n = 3
j: n = 7
k: n = 5
l: n = 11

Toxicology summary: In summary, in a 3-month combination toxicity study in dogs, with 1-month recovery period, SCH-58238 doses of 0.3, 3, 30 and 30 mg/kg/day were used, in combination with 1, 1, 1, and 10 mg/kg/day of simvastatin. In addition one group of dogs received simvastatin alone at 10 mg/kg/day. The combination treatment did not alter the total drug exposure ($AUC_{0\text{-}24h}$ values in M+F of drug + glucuronide at high dose combo were 4010 vs 5350 ng/ml.hr). However, exposure to simvastatin and hydroxysimvastatin increased by 2.5 and 1.7 fold, when compared to simvastatin alone. Exposure to hydroxysimvastatin was up to 5-fold greater than simvastatin. In all treated dogs, serum ALT increased (minimal to marked) compared to vehicle controls during weeks 5, 8, and 13. In high dose combination group, mean serum ALT was 50-fold higher compared to controls (1875 vs 34 in controls, in week 13). **During recovery, half of dogs in high dose combination still had increased ALT values (130-171 vs 26-54 IU/L in controls)**. In high dose combination, AST (week 13: 72-230 vs 21-44 IU/L in controls) and AP (166-729 vs 47-185 IU/L in controls) also increased but these were reversible after discontinuation of the drug, whereas glucose (83-89 vs 90-124 mg/dl in controls), total protein (4.2-5.0 vs 5.2-6.2 g/dl) and albumin (1.8-2.7 vs 2.9-3.3 g/dl in controls) were decreased. In liver, minimal hepatocytic cytoplasmic eosinophilia was observed in 1-2 dogs (in both sexes) at low-mid doses, and in all 5-6/6 dogs at high dose combination (vs 0/8 in simva control dogs). Biliary hyperplasia was observed in all 4-5/6 dogs (in both sexes) at high dose combination (vs 0/8 in simva control dogs). Sponsor states that these histopathology changes in liver are class statin effects, and have been reported previously. **The tolerated doses of the combination drug in 3-month toxicity study in dogs could not be established since liver histopathology was observed even in low dose combo groups in dogs.**

In an NDA 21-445 submission of 4/26/02, sponsor has provided a brief summary of the ongoing 6-month study of simvastatin (2 mg/kg/day) + SCH 58235 (0.3, 1, 3 mg/kg/day) in dogs. Sponsor states that biliary hyperplasia was noted in some dogs (in 3-month tox study) who had very low cholesterol levels (<28% of baseline). The lowest observable effect dose level (LOEL) for bile duct hyperplasia was 3 mg/kg/day for ezetimibe + 1 mg/kg/day of simvastatin. In the current ongoing 6-month tox study in dogs with this combination, histopath exams confirm above liver findings, but the 6-month study now shows that this biliary hyperplasia is associated with mononuclear cell infiltration, which was not seen at 3-months. LOEL in 6-month toxicity study was 1 mg/kg/day of drug +2 mg/kg/day of simvastatin in dogs (i.e the mid dose). Sponsor states that this biliary hyperplasia with mononuclear cell infiltration has been well documented with atorvastatin alone in the literature.

In an NDA 21-445 submission of 5/16/02, a 6-month study of simvastatin (2 mg/kg/day) + SCH 58235 (0.3, 1, 3 mg/kg/day) in dogs sponsor provides the summary which states that cholesterol was decreased by 60-94% vs up to 44% with simvastatin alone in dogs. At mid-high dose combination, biliary hyperplasia was noted in some dogs (as seen in a 3-month tox study in dogs), and marked increases in ALT were not accompanied by liver necrosis. Liver weights were decreased at all combination doses, and decreased liver weights were associated with bile duct hyperplasia, and low cholesterol levels. High dose combination group had decreased prostate weights (which sponsor claims have been seen previously with cerivastatin and simvastatin in dogs). Histopath exams at MD and HD combination showed bile duct hyperplasia (2/8 & 3/8 respectively) with mononuclear cell infiltration (in 2/8 & 5/8 dogs), and pigment accumulation (3/4 females, and ¼ M + 2/4 F dogs). The NOEL in 6-month tox study was 0.3 mg/kg/day of SCH 58235 + 2 mg/kg/day of simvastatin in dogs (i.e the lowest dose). The sponsor claim that this biliary hyperplasia with mononuclear cell infiltration has been well documented with atorvastatin alone in the literature in dogs and is not new, and would not pose any risk in humans because we already have experience with atorvastatin in humans. Basically 6-month toxicity study confirms what was seen in the 3-month tox study in dogs.

7. Three-Month Oral dietary Toxicity Study of SCH 5823 - in combination with lovastatin (gavage) in rats (Study No. 99012):

Sponsor's ID Study #: 99012
Amendment #, Vol. #, and page # : 049, 21.7, page 5.
Conducting laboratory : Schering-Plough Research Institute, Lafayettte, NJ.
Date of study initiation and final report: Final report: November 1, 1999
GLP compliance: Yes
QA Report: Yes () No (X), Is the evaluation based on a final, QA report: No.
Methods: This study examined the effects of SCH-5823 -(males at 50, 250, 250 and 750 mg/kg/day, females at 12, 50, 50, 250 mg/kg/day) in combination with lovastatin (10, 10, 100, and 100 mg/kg respectively) for 3-months in rats.

Dosing information:
species: Rats.
#/sex/group or time point: 10/sex/group
age: ≈ Not provided

weight: Not provided
satellite groups used for toxicokinetics: n=36 rats/sex/group
Dosage groups in administered units: Five groups (10-rats/sex/group) were given oral SCH-5823- by diet (once daily) at doses of 0, 50, 250, 250 and 750 mg/kg/day for males, and doses of 0, 12, 50, 50, 250 mg/kg/day for females, in combination with lovastatin (0, 10, 10, 100, and 100 mg/kg respectively) for 3-months. Control animals received the vehicle only. One additional group of rats received lovastatin alone at 100 mg/kg/day.

Route, form, volume, and infusion rate (if i.v.): Oral (via diet).
Drug, lot #: Not provided.
Formulation/vehicle: Not provided

Times at which Observations are made: These are approximations, as no details were provided:

Clinical signs/Physical exams: Daily
Body weights: prior to dosing, and weekly thereafter.
Food consumption: Daily.
Hematology/Coagulation: prior to dosing, during weeks 5, 12.
Clinical chemistry: prior to dosing, during weeks 5, 12.
Urine analysis: prior to dosing, during weeks 5, 12.

Gross pathology: At sacrifice in week 13.
Organs weighed: *Marked organs in the appended Table were weighed.
Histopathology: At sacrifice
Toxicokinetics: Blood was collected, but no data are provided, it is supposedly in progress.
Results:
Mortality: At mid-high dose (250 or 50 mg/kg/day of drug in males or females/100 mg/kg/day lovastatin), one male and one female died. At high-high dose (250/100 mg/kg/day), two females died. All four were drug related (three of these deaths were attributed to skeletal muscle degeneration and the fourth one due to hepatocellular single cell necrosis). Six other deaths in animals were considered incidental, one was in the control group, five in treated animals, and one of these animals also had skeletal muscle degeneration.

Clinical Signs: At mid-high, and high-high doses, clinical signs were observed such as alopecia of the hindquaters, urogenital staining, abnormal thinness, coolness to touch, abnormal gait, stool and haircoat, chromorhinorrhea, scabs, hypoactivity .

Body weight/Food consumption: At low-mid, mid-high and high dose combination decreased body weight was observed in males/females (on day 91 body weight in females was 244, 226, 235 g respectively in the above three groups vs 254-255 g in vehicle and lovastatin controls, in males these were 394, 370, 361 vs 419-425 g in controls). No significant drug related effects were observed on food consumption.

Hematology: In the mid and high dose combination, minimally to mild lower platelet counts were observed (males 1002, 975 vs 1104 k/ul in lovastatin controls, females 1156, 948 vs 1102 k/ul in lovastatin controls).
Biochemistry: In the two high dose group rats, minimal to moderate increases in

transaminases and AP were observed compared to vehicle controls during weeks 3, 5, and 12. In the two high groups, the mean ALT values in males in week 12 were 41, 88 vs 32-51 IU/L in vehicle-lova controls, in females ALT values were not increased. AST (males : 154, 242 vs 100-109 IU/L in both controls, females 117, 490 vs 105-113 IU/L in controls), and AP (males 351, 394 vs 189-206 IU/L in both controls, females 295, 287 vs 126-130 IU/L in controls) were increased in above two high dose combo groups. Also in above two combinations, there were minimal decreases in globulin (2.7-3.2 vs 1.6 g/dl in controls), and slight increases in albumin/globulin ratios vs controls (1.7-1.8 vs 1.3-1.4 in both controls). In all treated groups a higher incidence of bilirubin (1+) was observed with lova alone or with combination in week 3, but this was not seen in week 12. The drug + lovastatin at all doses decreased the serum chol and TG values. No effects on urine analysis were observed.

Organ Weights: In the two high dose group rats, significant increase in liver weights were observed in females, and these were also observed at lower doses or with lovastatin alone in females, but were not dose related.

Combination SCH 58235/lovastatin (n=10/sex/dose). Lova cont (100 mg/kg).

	Absolute liver weights (g)	Relative liver weights(% total BW)
Males		
Vehicle control	11.4	2.68
Lovastatin control	11.2	2.72
SCH 58235/lovastatin:		
50/10	10.3	2.58
250/10	10.1	2.58
250/100	9.7	2.72
750/100	10.5	3.01
Females		
Vehicle control	6.6	2.66
Lovastatin control	7.4	2.97
SCH 58235/lovastatin:		
12/10	8.7	3.54
50/10	8.0	3.37
50/100	9.0	4.18
250/100	8.5	3.86

Gross pathology: yellow or pale discoloration in liver was noted in 2 animals that died, and one had small spleen.

Histopathology: Toxicity was observed in the liver, skeletal muscle, tongue (skeltal muscle) and stomach. In the liver of rats, aside from the listed toxicity, at two high dose combinations, hepatocellular vacuolation (5/20, 4/20, vs 0/40 in controls), pigment accumulation (1, 1, vs 0 in controls), mid zonal/focal necrosis (4, 5, vs 0 in controls), Kupffer cell hypertrophy, centrolobular hypertrophy (14, 14 vs 1 in lova controls), and biliary hyperplasia (19, 19, vs 14 in lova control) was observed. In the skeletal muscle, at two high dose combinations toxicity was observed in myofiber degeneration/regeneration, mixed cellular infiltration, interstitial-edema/fibrosis, and were seen with higher incidence and severity in females. In the glandular stomach (with low

incidence and severity) these doses produced hyperkeratosis, acanthosis, submucoal edema and/or ulceration. Some skeletal muscle findings in tongue were reported in 1-2 animals with combination vs none in controls, but details were not provided.

Sponsor stated that these are class statin effects on liver/muscle, and therefore predicatable.

Histopathologic findings: Combination SCH 58235/lovastatin (n=10/sex/dose)

Males: vehicle cont, Lova cont, **50/10, 250/10, 250/100, 750/100 mg/kg/day:**
Females: vehicle cont, Lova cont, **12/10, 50/10, 50/100, 250/100 mg/kg/day:**

	males	females
Liver:		
Mitotic figures (min-mild)	**0, 1, 3, 1, 3, 7**	**0, 3, 3, 1, 8, 5**
Single cell necrosis (min-mild)	**0, 2, 3, 2, 8, 8**	**0, 3, 0, 2, 9, 7**
Hypertrophy, periportal, hepatocellular[a]	0, 3, 2, 2, 10, 10	0, 5, 4, 3, 9, 9
Biliary hyperplasia (mini-mild)	0, 7, 3, 7, 9, 10	0, 7, 5, 8, 10, 9
Hypertrophy Kupffer cells (min)	0, 1, 1, 0, 2, 2	0, 1, 0, 0, 3, 8
Stomach:		
Single cell necrosis glandular(mini)	0, 0, ne, 1, 1, 0	7, 9, ne, 8, 6, 6
Cellular infiltration (min)	0,, 0, ne, 0, 2, 0	0, 0, ne, 0, 3, 1
Skeletal muscle:		
Regeneration, myofiber (mini)	0, 0, 0, 0, 1, 1	
Pigment accumulation, macrophage	0, 0, 0, 0, 1, 0	
Edema, interstitial, biceps femoris[b]	0, 0, 0, 0, 1, 0	0, 0, 0, 0, 0, 1
Degeneration, myofiber	0, 0, 0, 0, 0, 1	0, 0, 0, 0, 0, 3
Single cell necrosis (min)	0, 0, 0, 1, 1,0	

[a]- Minimal to moderate
[b]= Minimal to severe

Toxicokinetics. This was submitted under a separate study (Study No. 99012).
Both total drug and conjugated drug accumulated by up to 8 fold in week 5 vs day 1. Systemic exposure to conjugated and to total SCH 58235 increased by 4-15 fold in week 5 as the lovastatin dose was increased from 10 to 100 mg/kg/day + drug (50 mg/kg/day in female and 250 mg/kg/day in male rats). The free drug (unconjugated) also increased by 2-3 fold, as the lovastatin dose was increased from 10 to 100 mg/kg/day in rats.

The lovastatin and hydroxylovastatin exposures were 2-6 fold higher with high dose combos (from 100 mg/kg/day lova with increasing doses of SCH), than with lova alone. See the Tables.

Table 1: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH-58235 on day 0 and week 5, in 3-month Tk study in rats:

	Female				Male			
	12/10[a]	50/10	50/100	250/100	50/10	250/10	250/100	750/100
	Total SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	566	3526	2856	9021	3085	7191	10505	10975
Week 5	1724	8099	32099	69154	4510	7535	115476	81188
	Conjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	566	3508	2850	8949	3061	7140	10392	10850
Week 5	1718	8058	32062	69019	4465	7487	115342	81088
	Unconjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	ND	ND	ND	72.1	28.5	55.9	118	124
Week 5	ND	46.1	40.4	134	47.0	51.6	134	101

a: Dose mg SCH 58235/kg/ dose mg lovastatin/kg.
ND = Not determinable

Table 2: Systemic exposures (AUC 0-24 hr) to lovastatin and hydroxylovastatin on day 0 and week 5 in 3-month Tk study in rats:

	Female					Male				
	12/10	50/10	0/100	50/100	250/100	50/10	250/10	0/100	250/100	750/100
	Lovastatin AUC(tf) (ng-hr/mL)									
Day 0	4.12	3.95	80.3	171	219	3.46	3.33	39.1	63.3	73.9
Week 5	1.93	5.23	52.4	483	319	2.17	3.36	21.5	381	155
	Hydroxylovastatin AUC(tf) (ng-hr/mL)									
Day 0	197	230	2970	5787	7340	165	152	1432	2019	2238
Week 5	218	388	1949	21612	20311	132	199	999	11193	5111

a: Dose of SCH 58235/lovastatin (mg/kg).

Toxicology summary: In summary, in a 3-month combination toxicity study in rats, SCH-5823 - doses (of 50, 250, 250 and 750 mg/kg/day in males and 12, 50, 50, and 250 mg/kg/day in females) were used, in combination with 10, 10, 100, and 100 mg/kg/day of lovastatin. In addition one group of rats received lovastatin alone at 100 mg/kg/day. The total drug (drug + glucuronide) exposure was increased in rats in week 5 (AUC_{0-24h} values in week 5 were 4.5, 7.5, 116, 81 µg/ml.hr) vs on day 0 (3.1, 7.1, 10.5, 11.0 µg/ml.hr). In females these values in week 5 were also higher (1.7, 8.1, 32.1, 69.2 µg/ml.hr) vs day 0 (0.57, 3.5, 2.9, 9.0 µg/ml.hr). Thus the drug accumulates over time. The combination not only increased the lovastatin (by 6-15 fold) and hydroxy-lovastatin exposure (by up to 10 fold) compared to lovastatin alone, but also increased the total drug exposure by up to 4-8 fold. At two high dose combinations, 2/20 deaths per group were drug related (three were attributed to skeletal muscle degeneration and the fourth one due to hepatocellular single cell necrosis). Two highest dose combinations produced clinical signs (alopecia of the hindquaters, urogenital staining, abnormal thinness, coolness to touch, abnormal gait, stool and haircoat, chromorhinorrhea, scabs,

hypoactivity), decreased body weights (by up to 11-15%), increased serum transaminases (ALT were not increased significantly but AST values in these groups were males: 154, 242 vs 100-109 IU/L in both controls, females 117, 490 vs 105-113 IU/L in controls, and AP increases were males 351, 394 vs 189-206 IU/L in both controls, females 295, 287 vs 126-130 IU/L in controls). Also in above two combinations there were minimal decreases in globulin (2.7-3.2 vs 1.6 g/dl in controls), and slight increases in albumin/globulin ratios (1.7-1.8 vs 1.3-1.4 in both controls). Toxicity was observed in the liver, skeletal muscle, tongue and stomach. The liver weights in females increased by up to 20%. In the liver of rats, mitotic figures (11/20, 12/20 vs 1/40 lova and vehicle controls) single cell necrosis (17/20, 17/20 vs 5/40 in both controls), periportal hepatocellular hypertrophy (18/20, 18/20 vs 10/40 in both controls), hepatocellular vacuolation (4, 3, vs 0 in controls), pigment accumulation (1, 1, vs 0 in controls), mid zonal/focal necrosis (4, 5, vs 0 in controls), Kupffer cell hypertrophy (5/20, 10/20, vs 2/40 in lova controls), centrolobular hypertrophy (14, 14 vs 1 in lova controls), and biliary hyperplasia (19/20, 19/20, vs 14/40 in lova/vehicle controls) was observed. In the skeletal muscle, at two high dose combinations toxicity was observed in myofiber degeneration/regeneration, mixed cellular infiltration, interstitial-edema/fibrosis, and were seen with higher incidence and severity in females (all in 1-2 of 10 rats vs 0-1 in controls, but higher severity). In the glandular stomach (with low incidence and severity) these doses produced hyperkeratosis, acanthosis, submucoal edema and/or ulceration (all in 1-2 of 10 rats vs 0-1 in controls). At high-mid (250/50 mg/kg of the drug in M/F + 100 mg/kg lovastatin), and high-high dose combinations (750/250 mg/kg of the drug in M/F + 100 mg/kg lovastatin), there were mortalities, clinical signs, significantly increased transaminases, and histopatholgic changes in the liver (mitotic figures, single cell necrosis, hepatocellular vacuolation, pigment accumulation in Kupffer cell/hypertrophy and biliaty hyperplasia), muscle, and stomach, **the tolerated doses of the combination drug in 3-month toxicity study in rats were not established as liver toxicity (mitotic figures) was noted with all combinations of the drug (SCH-58235 in males/females) + lovastatin.**

Sponsor indicates that mevalonate supplementation ameliorated the increases in ALT and this suggests a pharmacolgical effect of the drug, and these ALT increases have not been observed in 2-week clinical trials in drug combination with lova + simvistatin. **Sponsor states that it is possible that the regional induction of ALT activity within tissues, prolonged half life, and leakage from otherwise normal hepatocytes may also cause increased ALT levels.**

8. Three-Month Oral Gavage Toxicity Study of SCH 5823- in combination with lovastatin in dogs (Study No. 99013):

Sponsor's ID Study #: 99013
Amendment #, Vol. #, and page # : 049, 21.7, page 10.
Conducting laboratory : Schering-Plough Research Institute, Lafayettte, NJ.
Date of study initiation and final report: october 22, 1998 and november 1, 1999
GLP compliance: Yes
QA Report: Yes () No (X), Is the evaluation based on a final, QA report: No.
Methods: This study examined the effects of SCH-5823- (at 0.03, 0.3, 30 and 30 mg/kg/day) in combination with lovastatin (2, 2, 20, and 60 mg/kg respectively) for 3-months in dogs.

Dosing information:
species: Beagle dogs.
#/sex/group or time point: 4/sex/group
age: ≈ Not provided
weight: Not provided
satellite groups used for toxicokinetics: N/A
Dosage groups in administered units: Five groups (4-dogs/sex/group) were given oral SCH-5823* by gavage (once daily) at doses of 0, 0.03, 0.3, 30 and 30 mg/kg/day) in combination with lovastatin (0, 2, 2, 20, and 60 mg/kg respectively) for 3-months. Control animals received the vehicle only. One additional group of dogs received lovastatin alone at 60 mg/kg/day.

Route, form, volume, and infusion rate (if i.v.): Oral (via gavage).
Drug, lot #: Not provided.
Formulation/vehicle: Not provided

Times at which Observations are made: These are approximations, as no details were provided:

Clinical signs/Physical exams: Daily
Body weights: prior to dosing, and weekly thereafter.
Food consumption: Daily.
Hematology/Coagulation: prior to dosing, during weeks 5, 13.
Clinical chemistry: prior to dosing, during weeks 5, 13.
Urine analysis: prior to dosing, during weeks 5, 13.

Gross pathology: At sacrifice in week 14.
Organs weighed: *Marked organs in the appended Table were weighed.
Histopathology: At sacrifice
Toxicokinetics: Blood was collected, no details are provided.

Results:
Mortality: None

Clinical Signs: No mention of it.

Body weight/Food consumption: At low-mid, high-mid and high-high dose combination decreased body weight gain was observed in females (on day 91 body weight in females was 7.0, 6.7 and 6.8 g respectively in the above groups vs 7.2-7.3 g in vehicle and lova controls, in males these were 9.3, 8.9, 8.2 vs 8.4-9.4 g in controls). No drug related effects were observed on food consumption.

Ophthalmic Examination: No drug related effects were observed.

Electrocardiograms: No drug related effects were observed.

Hematology: In the mid-high and high dose combination, hemoglobin, RBC counts and hematocrit were slightly decreased and reticulocyte counts and mean corpuscular hemoglobin conc were slightly increased during week 13. PT was slightly increased at these doses (males 8.7 and 9.3 vs 7.7 sec in vehicle and lova controls, females 8.7 and

10.3 vs 8.0-8.3 sec in vehicle and lova controls).

Biochemistry: In all treated dogs, minimal to marked increases in ALT were observed compared to vehicle controls during weeks 5, and 13. In five groups (vehicle controls, lova control, low-low, low-mid, mid-high and high dose combination groups), the mean ALT values in males were 38, 57, 129, 173, 1574, and 676 IU/L respectively in week 13. Similar ALT increases were also seen in females (43, 74, 95, 200, 1005, 671 IU/L resp in above 5 groups). AST (males week 13: 114-127 vs 34-47 IU/L in both controls) and AP increases (330-454 vs 84-191 IU/L in both controls) were mostly seen with mid-high and high dose combination during weeks 5 and/or 13. Also in above two combinations there were decreases in total protein, and albumin values vs controls. Albumin/globulin ratios were decreased at the above two dose combinations (0.7-0.9 vs 1.1 in both controls). The drug + lovastatin at all doses decreased the serum chol and TG values. No effects on urine analysis were observed.

Organ Weights: No mention of it.

Gross pathology: No mention of it.

Histopathology: In the liver of dogs (at mid-high, and high dose combination), bile duct hyperplasia (6/8, 8/8 dogs vs 2/8 in lova controls), Kupffer cell hypertrophy (8/8, 7/8 dogs vs 2/8 in lova controls), and cholestasis (2/4 females vs none in controls) was observed. Cholestasis consisted of rare bile canaliculi distended with linear to globular deposits of greenish pigment in a few scattered lobules predominantly located adjacent to the gallbladder. Also at the above two dose combinations, mild spermatogenic alteration and luminal cellular debris was observed in the testis of dogs (1/4, 2/4 vs none in controls)

Sponsor stated that these are class statin effects on liver and testis, and therefore predictable.

Toxicokinetics. In the subsequent amendment, this TK study was provided separately (Study No. 99013). Since there was no control SCH 58235 group included in the present TK study, the sponsor has compared it to a previous TK study (SN 96454, a 6-month oral tox study with the drug alone in dogs, data not yet available). Systemic exposure to unconjugated and to total SCH 58235 increased by 1.5 fold on day 31 vs day 1 with SCH 58235 (30 mg/kg/day) + lovastatin (60 mg/kg/day). Sponsor states that the above systemic exposures were not significantly different because there was a large variability in the data. The lovastatin and hydroxylovastatin exposures were 1.6 to 1.9 fold higher vs that with lovastatin alone on days 0 and 31. See the Tables 3 and 4 below.

, Table 3: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 on day 0 and day 31 in dogs:

Dose[b] (mg/kg)	Gender	SCH 58235 AUC(0-24 hr) (ng·hr/mL)					
		Total		Unconjugated		Conjugated	
		Day 0	Day 31	Day 0	Day 31	Day 0	Day 31
0.03/2	M[c]	10.0[e]	28.5[e]	2.49[f]	0.514[e]	10.5[e,h]	63.2[e,h]
	F[c]	14.3[e]	NA[a]	1.20[f]	0.295[e]	13.0[e]	NA
	M & F[d]	12.2[f]	NA	1.84[c]	0.405[f]	11.8[f]	NA
0.3/2	M	43.4	45.1	3.79[g]	NA	58.8[g]	45.0
	F	39.4	55.8	NA	2.63[f]	NA	35.6[f]
	M & F	41.4	50.4	NA	NA	NA	41.9[g]
30/20	M	6480	2750	492	185	6000	2570
	F	3050	2660	160	143	2890	2520
	M & F	4770	2710	326	164	4440	2540
30/60	M	2950	4380	131	95.5	2820	4190
	F	3260	4840	154	89.0	3100	4750
	M & F	3100	4610	142	92.3	2960	4470

a: NA: Data not amenable to pharmacokinetic analysis

b: Dose SCH 58235/ Dose lovastatin

c: n=4; d: n=8; e: n=1; f: n=2; g: n=6;

h: AUC for conjugated SCH 58235 was over estimated due to a non reported value at 2 and 12 hr on Day 0 and 31, respectively.

, Table 4: Systemic exposures (AUC 0-24 hr) to lovastatin and hydroxylovastatin on day 0 and day 31 in dogs:

Dose[a] (mg/kg)	Gender	AUC(0-24 hr) (ng·hr/mL)			
		Lovastatin		Hydroxylovastatin	
		Day 0	Day 31	Day 0	Day 31
0.03/2	M[b]	41.0[d]	38.5[e]	111[g]	150[i]
	F[b]	16.9[e]	26.9	39.8[e]	84.4[e]
	M & F[c]	26.5[f]	30.7[g]	68.4[f]	101[h]
0.3/2	M	31.0[e]	31.3	68.5[d]	90.6
	F	62.3[d]	106[d]	155[e]	207[e]
	M & F	43.5[f]	56.1[g]	120[f]	140[h]
30/20	M	767	1130	1990	1520
	F	501	2290	871	2330
	M & F	634	1710	1430	1920
30/60	M	2080	3600	2810	5210
	F	2570	3280	3610	4310
	M & F	2320	3440	3210	4760
0/60	M	1990	2140	2060	1580
	F	898	1470	1370	1020
	M & F	1440	1800	1720	1300

a: Dose SCH 58235/ Dose lovastatin

b: n=4; c: n=8; d: n=2; e: n=3;

f: n=5 g: n=6; h: n=7; i: n=1

Toxicology summary: In summary, in a 3-month combination toxicity study in dogs, SCH-5823 - doses of 0.03, 0.3, 30 and 30 mg/kg/day were used, in combination with 2, 2, 20, and 60 mg/kg/day of lovastatin. In addition one group of dogs received lovastatin alone at 60 mg/kg/day. The total drug (drug + glucuronide) exposure was generally increased on day 31 ($AUC_{0\text{-}24h}$ values in M+F on day 31 were 29, 50, 2710, 4610 ng.hr/ml) vs day 0. (12, 41, 4770, 3100 ng.h/ml respectively). The presence of lovastatin increased the SCH 58235 exposure on day 31, and also increased exposure to lovastatin and hydroxy-lovastatin by 2-3 fold (vs lovastatin alone), suggesting drug metabolism interaction in dogs. All combinations the serum ALT increased compared to the vehicle controls during weeks 5 and 13. In mid-high and high dose combination groups, mean serum ALT increase was moderate to severe (670-1570 vs 38-74 in vehicle and lovastatin controls, in week 13). In these above two dose combinations, AST (week 13: 114-128 vs 34-47 IU/L in two controls) and AP (330-454 vs 84-191 IU/L in controls) also increased, whereas total protein, albumin and A/G ratios (0.7-0.9 vs 1.1 in both controls) were decreased. In liver, bile duct hyperplasia, Kupffer cell hypertrophy (8/8, 7/8 dogs vs 2/8 in lova controls), and cholestasis (2/4 females vs none in controls) was observed. Also at the above two dose combinations, mild spermatogenic alteration and luminal cellular debris was observed in the testis of dogs (1/4, 2/4 vs none in controls)

Since, at mid-high (30 mg/kg drug+20 mg/kg lovastatin), and high-high dose combinations (30 mg/kg drug+60 mg/kg lovastatin), serum ALT were significantly increased, and histopatholgic changes in the liver (bile duct hyperplasia, Kupffer cell hypertrophy and cholestasis) were observed in all 4/4 dogs, along with mild spermatogenic alterations in the testis of dogs, **the tolerated doses of the combination drug in 3-month toxicity study in dogs may be 0.3 mg/kg of SCH-58235 + 2 mg/kg of lovastatin.**

Appendix: Histopathology Inventory for IND [redacted], 3-month studies of drug + simvastatin or lovastatin in rats & dogs

Species	Rat				Dog			
	3-mont drug + simva	3-mont drug + lova			3-mont drug + simva	3-mont drug + lova		
Adrenals	X*	X			X*	X*		
Alimentary tract								
Aorta	X	X			X	X		
Bone Marrow smear	X	X			X	X		
Bone (femur/sternum)	X	X			X	X		
Brain	X*	X			X*	X*		
Cecum	X*	X			X*	X*		
Cervix								
Colon	X*	X			X*	X*		
Duodenum	X	X			X	X		
Epididymis								
Epididymides	X	X			X	X		
Esophagus	X	X			X	X		
Eyes	X	X			X	X		
Fallopian tube								
Femur								
Gall bladder					X	X		
Harderian gland								
Head								
Heart	X*	X			X*	X*		
Hypophysis								
Intestine(small-duodenum,jejunum,ileum)	X*	X			X*	X*		
Intestine(large-cecum,colon)	X*	X			X*	X*		
Kidneys	X*	X			X*	X*		
Lachrymal gland								
Larynx and pharynx								
Liver	X*	X			X*	X*		
Lungs	X*				X*	X*		
Lymph-nodes submaxillary	X	X			X	X		
Lymph nodes, mesenteric	X	X			X	X		
Mammary Gland	X	X			X	X		
Ovaries	X*	X			X*	X*		
Pancreas	X	X			X			
Parathyroid								
Peripheral nerve								
Pharynx								
Pituitary	X*	X			X*	X		

Prostate	X*	X			x*	x*		
Rectum	X*	X			X*	X*		
Salivary gland	X	X			X	X		
Sciatic nerve	X	X			X	X		
Seminal vesicles	X	X						
Skeletal muscle	X	X			x	x		
Skin	X	X			X	X		
Spinal cord	X	X			X	X		
Spleen	X*	X			X*	X*		
Sternum								
Stomach	X*	X			X*	X*		
Testes	X*	X			X*	X*		
Thymus	X*	X			X*	X*		
Thyroid	X*	X			X*	X*		
Tongue	X	X			X	X		
Trachea	X	X			X	X		
Urinary bladder	X	X			X	X		
Uterus	X*	X			X*	X*		
Vagina	X	X			X	X		
Zymbal gland								

- *organ weight obtained

V. A. GENETIC TOXICOLOGY WITH MONOTHERAPY:

Studies with SCH 58235 without impurities

Following two genotoxicity studies were reviewed under IND [redacted] on 3/27/07

1. MUTAGENICITY STUDIES (vol 1.16)
REVERSION TEST WITH *S. typhimurium*:
Note: Study performed by Schering Plough Research Institute. Study completed: 6/21/96. GLP statement was provided.
Purpose: *In Vitro* assessment of mutagenic potential of SCH 58235 using a [redacted] assay ("Ames Test").
Experimental Design:
Salmonella Strains: TA1535, TA97a; TA98, TA100, TA102
E.coli strain: WP2uvrA
Metabolic Activation System:
Test article: 1.6 to 5000 ug/plate (Batch#: 95-58235-ZZX-05) but ppt. seen
Vehicle control: DMS0
Positive control without S9: 9-aminoacridine
Positive control with S9: 2-aminoanthracene (2.5 to 20 ug/plate)
Criteria for positive result: increase at least 2-fold above solvent controls; a dose-response in at least 2 dose levels; reproducible in independent trials as well as analysis of concurrent and historical control data.
Results: negative
Evaluation: Ppt was observed at doses of 625 ug/plate and prevented evaluation at doses of 2500 ug/plate. There was no cytotoxicity at any doses tested. Assay appears to be valid.

2. CHROMOSOME ABERRATION STUDY IN HUMAN LYMPHOCYTES (vol 1.16)
P 6372. March 1996. [redacted]
Batch#: 95-58235-ZZX-05
Cells: two donors (one male and one female)
Negative control: culture medium and cells
Solvent control: 1% DMSO
Positive control: mitomycin C (nonactivation assay) and cyclophosphamide (activation assay)
Non-activation treatment times: 24 h with 27 hour harvest and 48 hours (and 51 hour harvests) and 3 hours (and 24 hour harvest).
Activation treatment time: 3 hours (with 24 and 48 hour harvests)
Doses: up to 125 ug/ml (female donor) and 30 to 60 ug/ml (male donor).

There were no significant increases in any assay (except for the positive controls).

3) Study Title: Effects of SCH 58235 on in Vivo Micronuclei in Mice.

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

Study no: 96452
Volume #, and page #: Volume 42, page 1 (Reference 33)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 5/6/1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: Lot #: 96-58235-X-01

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, males 25.3-31.1 g, females 21.7-28 g.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a pilot study in mice, where 6 mice (ICR) per sex/dose were given intraperitoneal (IP) doses of 250, 500, 1000, 1500, 2000 mg/kg/day. Mice were observed for clinical signs/BW for 4 days post dosing. Doses of 2000 mg/kg/day (2/6 males and 1/6 females) produced mortalities at 72 hrs after dosing. No additional mortality was observed at 96 hrs after the initiation of dosing. Clinical signs at 1000 mg/kg/day included rough hair coat, while urogenital staining and hypoactivity in both sexes was observed at 1500-2000 mg/kg/day. Bone marrow toxicity (decrease in mean PCE/NCE) was observed in males at doses of ≥250 mg/kg/day (1.15, 0.99, 0.89, 0.74, 0.55, 0.33, or by 86 to 29% at 0, 250, 500, 1000, 1500, 2000 mg/kg/day) and in females at 500 mg/kg/day (i.e. from 98% in control to 81, 54, 44, 30% respectively). Based on mortality and clinical signs, 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: Doses of 250-2000 mg/kg/day were used in the range finding study. Mortality in mice was observed after 72 hrs at a high doses (males 0/6, 0/6, 0/6, 0/6, 0/6, 2/6 at 0, 250, 500, 1000, 1500, 2000 mg/kg/day, in females 1/6 mice died at a high dose vs none in other groups). Bone marrow toxicity was noted from 250 mg/kg/day. Since clinical signs (rough hair coat, urogenital staining, hypoactivity) were observed from 1000 mg/kg/day, high doses of 800 mg/kg/day were chosen for the main micronucleus test.

Test agent stability: The prepared drug at 50-200 mg/ml was stable for up to 7 days at the ambient laboratory conditions of temperature and light.

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. The reason for two consecutive daily doses and two harvest times was to ensure that the peak of micronuclei induction was not missed. 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 —— vs —— 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 suppression

(mean PCE/MCE was 58-87% in males and 67-99% in females at 200-800 mg/kg/day, 40-52% in positive controls, and 104-105% 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 was not cytogenic at doses up to 800 mg/kg/day in an in vivo micronucleus test in mice.

V. B. GENETIC TOXICOLOGY WITH COMBINATION THERAPY (SCH 58235 + STATINS)

1.Studies with SCH 58235 + pravastatin

1A. Effects of SCH 58235 + pravastatin (SCH 57096) on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: SCH 58235 + pravastatin was negative in AMES test

Study no: 99491
Volume #, and page #: Volume 161, page 1 (reference 84)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 3/23/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-01, pravastatin batch # 75793-008

Formulation/vehicle: Dimethylsulfoxide (DMSO)

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA97a, TA98, TA100, TA1535, TA102, and E. coli tester strain WP2 urvA

Dose selection criteria:
Basis of dose selection: SCH 58235 and pravastatin combination was tested at ratio of 1:1 by weight, both drugs were prepared separately in DMSO and then used in the test system. The dose selection was based on the first mutagenicity study (or trial 1) using 50, 158, 500, 1581, 5000 µg/plate in Salmonella and E. coli strains in the plate incorporation method (in the presence and absence of metabolic activation). The dose selection was based on cytotoxicity, the highest dose that could be analyzed without interference from the test article precipitation, or the maximum dose of 5 mg/plate. Based on above dose selection criteria, mutagenicity assays (trials 2, 3 and 4) were conducted at doses ranging from 39-5000 µg/plate. More than one trial was conducted, because there was lack of bacterial growth in plates in some trials.

Range finding studies: The test article was a combination of SCH 58235 and pravastatin at a ratio of 1:1 by weight. Doses of 39-5000 µg/plate were used in all strains in the presence and absence of metabolic activation, and based on cytotoxicity and precipitate formation following doses were selected in trial 4.

Table: The doses and strains used in AMES assay with SCH 58235 + pravastatin (SCH 57096).

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

Test agent stability: Both SCH 58235 (at concentration of 0.15-49.8 mg/ml) and pravastatin (at concentration of 0.1-50 mg/ml) were stable in DMSO for at least 4 hours under the ambient temperature and light

Metabolic activation system: Rat liver microsome S9 fraction.

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

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 approximately 48 hrs at 37^0C 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 compared to the solvent controls in at least one of the six tester strains, and the increase is at least 2 times for strains TA97a, TA98, TA100, TA102, WP2 urvA, and 3 times for strain TA1535, 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: The combination of SCH 58235 + pravastatin at a dose range of 39-5000 µg/plate in at least two independent assays was not mutagenic in any of the tester strains at any doses in the presence or absence of metabolic activation. Precipitate was generally observed from doses of 500 and 1250 µg/plate in the absence and presence of metabolic activation respectively in these assays. A significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). In conclusion, AMES test was negative for the combination of SCH 58235 + pravastatin.

Genetic toxicology summary: SCH 58235+ pravastatin co-administration was negative in AMES test in all tester strains.

1B. Effects of SCH 58235 + pravastatin (SCH 57096) on chromosome aberrations in human peripheral blood lymphocytes

Key findings: SCH 58235 + pravastatin was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

Study no: 99015
Volume #, and page #: Volume 162, page 1 (reference 88)
Conducting laboratory and location: [redacted]

Date of study initiation: 3/14/2000
GLP compliance: Yes
QA reports: yes (X) no ( )

Basis of dose selection: The dose selection was based on cytotoxicity (or mitotic index). Mitotic index was ≥ 50% at the high dose in all assays

SCH 58235 + pravastatin (SCH 57096) Methods:

6.5.7. Chromosome Aberration Study of SCH 58235/Pravastatin Sodium (SCH 57096) in Human Peripheral Blood Lymphocytes (SN 99492)

Methods/Design

Performed by:	[ ]
Study Performed in Compliance with GLP:	Yes
Animals:	In vitro
Target Cells:	Human peripheral blood lymphocytes
Duration of Exposure:	≈4 and ≈19 hours (nonactivation); ≈4 hours (activation)
Sampling Times:	≈22-hour harvest
Test Articles/Formulation:	SCH 58235 and pravastatin sodium (SCH 57096) in a 1:1 ratio by weight, prepared and delivered to the test system separately. All doses are presented as doses of the combination.
Batch Nos.:	SCH 58235: 98-58235-X-01 SCH 57096: 75793-008
Doses Analyzed:	Nonactivation: 7.85, 15.0, 15.7, 30.0, 31.3, 35.0, 40.0 and 62.5 µg/mL Activation: 7.85, 12.5, 15.7, 24.9, 31.3, 49.8, 62.5 and 74.7 µg/mL
No. of Independent Experiments:	Two
No. of Replicate Cultures:	Two/dose
No. of Cells Analyzed:	100/culture, 200/dose
Positive Controls:	Nonactivation: Mitomycin C (Sigma Lot No. 049H2508) Activation: Cyclophosphamide (Sigma Lot No. 073H0846)
Solvent and Final Concentration:	Dimethylsulfoxide (DMSO), 10 µL/mL in tissue culture medium

Genetic toxicology summary: SCH 58235+ pravastatin co-administration was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

1C. Effects of SCH 58235 + pravastatin (SCH 57096) on in Vivo Micronuclei in Mice.

Key findings: SCH 58235 + pravastatin (SCH 57096) was negative in viv o micronucleus test in mice

Study no: 99497
Volume #, and page #: Volume 163, page 1 (Reference 92)
Conducting laboratory and location:

Date of study initiation: 5/30/2000
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug lot #, and % purity: Lot #: SCH 58235 ——— 98-58235-X-01, I, pravastatin batch # 75793-008.

Formulation/vehicle: 0.4% aqueous methylcellulose

Methods:
Test strain and Cells: Mice (Crl:CD-1(CR)BR VAF/PLUS, 8 weeks of age.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a previous in vivo micronucleus study in mice where combined doses of SCH 58235 + pravastatin (ratio 1:1 by weight) were 100, 200, 500, 1000, 1500 mg/kg/day, given ip once a day for two consecutive days. Based on mortality, clinical signs and on the bone marrow toxicity, doses of 150-750 mg/kg/day were chosen for the main micronucleus test. In the main micronucleus test, no mortality was observed in females, but in males at 600 mg/kg/day. 1 male died on day 3. Clinical signs were noted in both sexes, and included rough or hunched posture, rough haircoat, hypoactivity, cold to touch, and were seen in males and females at 300 and 375 mg/kg/day respectively. **At 24 hrs** in male mice, the bone marrow toxicity (decrease in mean PCE/NCE) was observed with increases in doses i.e. 1.04, 0.93, 0.70. 0.75 (or by 89% to 67%) at 0, 150, 300, 600 mg/kg/day, and in females at 750 mg/kg/day (i.e. from 91% in control to 0.85, 0.94, 0.65 (or 95, 103, 71% respectively). **At 48 hrs** in male or female mice, no bone marrow toxicity was observed at any dose (decrease in mean PCE/NCE in males was 1.00, 1.04, 0.88, (i.e decrease was 80, 104, 88%) at 150, 300, 600 mg/kg/day. Similarly in females at 48 hrs bone marrow toxicity was 0.77 in control to 1.05, 0.90, 1.03 (or 136%, 117%, 134% respectively), but sponsor states that no significant increase of micronucleus frequency was observed at any dose in males or females. However above numbers show that bone marrow toxicity was not impressive in this assay.

Dose groups for the micronucleus test

Dose Group	Dose[a] (mg/kg/day)	Number of Mice Dosed			
		Sacrifice Time[b]			
		24 Hours (Trial 1)		48 Hours (Trial 2)	
		Male	Female	Male	Female
Vehicle Control (0.4% aqueous methylcellulose)	0	6	6	6	6
Low-Dose (SCH 58235 and Pravastatin sodium [SCH 57096])	150	6		6	
	187.5		6		6
Mid-Dose (SCH 58235 and Pravastatin sodium [SCH 57096])	300	6		6	
	375		6		6
High-Dose (SCH 58235 and Pravastatin sodium [SCH 57096])	600	6		6	
	750		6		6
Secondary High-Dose (SCH 58235 and Pravastatin sodium [SCH 57096])	600	2		2	
	750		2		2
Positive Control (Cyclophosphamide)	50	6	6		
	30			6	6

a: The test article was a co-administration of SCH 58235 and pravastatin sodium (SCH 57096) at a 1:1 ratio by weight. SCH 58235 and SCH 57096 were prepared separately and administered by intraperitoneal injection once a day for two consecutive days. All doses were the doses of the combination. The doses of pravastatin in the combined dose, were expressed as the sodium salt. When expressed as the free acid, doses of pravastatin were 71.3, 142.7 and 285.3 mg/kg for males and 89.1, 178.3 and 356.6 mg/kg for females.

b: Time after the second intraperitoneal injection.

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No. of animals used: 6/sex/group/sacrifice time.

Sponsor's summary of mouse bone marrow micronucleus test with SCH 58235 + pravastatin:

The genotoxicity of co-administration of SCH 58235, a cholesterol absorption inhibitor, and pravastatin sodium (SCH 57096), a 3-hydroxy-3-methylglutaryl co-enzyme A reductase inhibitor, at a ratio of 1:1 by weight, was evaluated in a mouse bone marrow erythrocyte micronucleus assay at combined doses of 150, 300, and 600 mg/kg for males and 187.5, 375, and 750 mg/kg for females. The doses of pravastatin in the combined doses are expressed as the sodium salt. When expressed as the free acid, doses of pravastatin were 71.3, 142.7, and 285.3 mg/kg for the males and 89.1, 178.3, and 356.6 mg/kg for the females.

Mortality was observed in one male mouse dosed at 600 mg/kg after the second dose. Rough hair coat was observed in all male and female mice dosed at 600 and 750 mg/kg, respectively. Bone marrow suppression, as determined by decreases of the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) ratio (PCE/NCE ratio), was observed at the 24-hour harvest in males dosed at 300 and 600 mg/kg and females dosed at 750 mg/kg. No decreased PCE/NCE ratio was observed in any other dose groups. No statistically significant increase of micronucleus frequency was observed in male or female mice dosed with the test articles as compared to the vehicle control. The positive control, cyclophosphamide, induced statistically significant increases ($p \leq 0.01$) of micronucleus frequency in both male and female mice.

In conclusion, the co-administration of SCH 58235 and pravastatin sodium (SCH 57096) did not induce micronuclei in bone marrow polychromatic erythrocytes in male or female CD-1® mice under the conditions of this study.

Table. Micronucleus test data with SCH 58235 + pravastatin:

TEST ARTICLE: SCH 58235 and Pravastatin Sodium (SCH 57096)

GROUP (mg/kg/day)		HARVEST TIME	MEAN % MICRONUCLEATED PCEs PER 2000 PCE ± S.E. MALES	FEMALES	MEAN ESTIMATED % MICRONUCLEATED NCEs PER 2000 PCE ± S.E. MALES	FEMALES	PCE:NCE RATIO MEAN ± S.E.
CONTROLS							
VEHICLE	0.4% MC	24 hr	0.12 ± 0.03	0.05 ± 0.04	0.010 ± 0.010	0.000 ± 0.000	1.04 ± 0.06	0.91 ± 0.09
		48 hr	0.11 ± 0.05	0.07 ± 0.02	0.023 ± 0.023	0.014 ± 0.009	1.00 ± 0.05	0.77 ± 0.08
POSITIVE CP	50	24 hr	1.55 ± 0.19*	2.12 ± 0.27*	0.057 ± 0.012	0.180 ± 0.045*	0.54 ± 0.04**	0.57 ± 0.05**
	30	48 hr	0.85 ± 0.07*	1.07 ± 0.14*	0.249 ± 0.078*	0.178 ± 0.007*	0.43 ± 0.03**	0.44 ± 0.05**
TEST ARTICLE	150	24 hr	0.07 ± 0.04		0.000 ± 0.000		0.93 ± 0.06	
	187.5	24 hr		0.03 ± 0.01		0.000 ± 0.000		0.86 ± 0.11
	300	24 hr	0.12 ± 0.04		0.000 ± 0.000		0.70 ± 0.10**	
	375	24 hr		0.09 ± 0.03		0.009 ± 0.009		0.94 ± 0.06
	600	24 hr	0.05 ± 0.03		0.008 ± 0.008		0.75 ± 0.05**	
	750	24 hr		0.05 ± 0.02		0.004 ± 0.004		0.85 ± 0.07
	150	48 hr	0.04 ± 0.02		0.000 ± 0.000		0.80 ± 0.07	
	187.5	48 hr		0.02 ± 0.02		0.014 ± 0.014		1.05 ± 0.13
	300	48 hr	0.07 ± 0.03		0.000 ± 0.000		1.04 ± 0.08	
	375	48 hr		0.04 ± 0.02		0.000 ± 0.000		0.90 ± 0.07
	600	48 hr	0.08 ± 0.06		0.000 ± 0.000		0.88 ± 0.05	
	750	48 hr		0.06 ± 0.01		0.000 ± 0.000		1.03 ± 0.16

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
0.4% MC = 0.4% (w/v) aqueous methylcellulose
Test article = a combination of SCH 58235 and pravastatin sodium (SCH 57096) at a ratio of 1:1 by weight
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
CP = Cyclophosphamide

Genetic toxicology summary: SCH 58235 + pravastatin was not cytogenic up to doses of 600-750 mg/kg/day in an in vivo micronucleus test in mice

2. Studies with SCH 58235 + atorvastatin (SCH 412387)

2A. Effects of SCH 58235 + atorvastatin (SCH 412387) on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: SCH 58235 + atorvastatin was negative in AMES test

Study no: 99502
Volume #, and page #: Volume 161, page 1 (reference 85)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 4/26/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-01, atorvastatin batch # 76590-003

Formulation/vehicle: Dimethylsulfoxide (DMSO)

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA97a, TA98, TA100, TA1535, TA102, and E. coli tester strain WP2 urvA

Dose selection criteria:
Basis of dose selection: SCH 58235 and atorvastatin combination was tested at ratio of 1:1 by weight, both drugs were prepared separately in DMSO and then used in the test system. The dose selection was based on the first mutagenicity study (or trial 1), with 50, 158, 500, 1581, 5000 µg/plate in Salmonella and E. coli strains in the plate incorporation method in the presence and absence of metabolic activation. The dose selection was based on cytotoxicity, the highest dose that could be analyzed without interference from the test article precipitation, or the maximum dose of 5 mg/plate. Based on above dose selection criteria, mutagenicity assay (trials 2) was conducted at doses ranging from 78-5000 µg/plate.

Range finding studies: The test article was a combination of SCH 58235 and atorvastatin at a ratio of 1:1 by weight. Based on cytotoxicity and precipitate formation doses of 78-5000 µg/plate were used in the presence and absence of metabolic activation in trial 2.

Test agent stability: Both SCH 58235 (at concentration of 0.15-49.8 mg/ml) and atorvastatin (at concentration of 0.1-50 mg/ml) were stable in DMSO for at least 4 hours under the ambient temperature and light

Metabolic activation system: Rat liver microsome S9 fraction.

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

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 approximately 48 hrs at 37^0C 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: 78-5000 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 compared to the solvent controls in at least one of the six tester strains, and the increase is at least 2 times for strains TA97a, TA98, TA100, TA102, WP2 urvA, and 3 times for strain TA1535, 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: The combination of SCH 58235 + atorvastatin at a dose range of 78-5000 µg/plate in at least two independent assays was not mutagenic in any of the tester strains at any doses in the presence or absence of metabolic activation. Cytotoxicity was generally observed from doses of 625 or 1250 µg/plate in the absence and presence of metabolic activation in these assays. A significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). In conclusion, AMES test was negative for the combination of SCH 58235 + atorvastatin.

Genetic toxicology summary: SCH 58235+ atorvastatin co-administration was negative in AMES test in all tester strains.

2B. Effects of SCH 58235 + atorvastatin (SCH 412387) on chromosome aberrations in human peripheral blood lymphocytes

Key findings: SCH 58235 + atorvastatin was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

Study no: 99503
Volume #, and page #: Volume 162, page 1 (reference 89)
Conducting laboratory and location: [redacted]

Date of study initiation: 5/15/2000
GLP compliance: Yes
QA reports: yes (X) no ( )

Basis of dose selection: The dose selection was based on cytotoxicity (or mitotic index). Mitotic index was approximately 50% at the high dose in all assays

6.5.8. Chromosome Aberration Study of SCH 58235 and Atorvastatin (SCH 412387) in Human Peripheral Blood Lymphocytes (SN 99503)

Methods/Design

Performed by:	[ ]
Study Performed in Compliance with GLP:	Yes
Animals:	In vitro
Target Cells:	Human peripheral blood lymphocytes
Duration of Exposure:	≈4 and ≈19 hours (nonactivation); ≈4 hours (activation)
Sampling Times:	≈22-hour harvest
Test Articles/Formulation:	SCH 58235 and atorvastatin calcium (SCH 412387) in a 1:1 ratio by weight, prepared and delivered to the test system separately. All doses are presented as doses of the combination.
Batch Nos.:	SCH 58235: 98-58235-X-01 SCH 412387: 76590-003
Doses Analyzed:	Nonactivation and Activation: 7.9, 15, 15.8, 30, 31.5, 45, 60 and 63 µg/mL
No. of Independent Experiments:	Two
No. of Replicate Cultures:	Two/dose
No. of Cells Analyzed:	100/culture, 200/dose
Positive Controls:	Nonactivation: Mitomycin C (Sigma Lot No. 049H2508) Activation: Cyclophosphamide (Sigma Lot No. 073H0846)
Solvent and Final Concentration:	Dimethylsulfoxide (DMSO), 10 µL/mL in tissue culture medium

Genetic toxicology summary: SCH 58235+ atorvastatin co-administration was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

2C. Effects of SCH 58235 + atorvastatin (SCH 412387) on in Vivo Micronuclei in Mice.

Key findings: SCH 58235 + atorvastatin was negative in vivo micronucleus test in mice

Study no: 99508
Volume #, and page #: Volume 163, page 1 (Reference 93)
Conducting laboratory and location: [redacted]

Date of study initiation: 6/12/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: Lot #: SCH 58235 98-58235-X-01, I, atorvastatin batch # 76590-003.

Formulation/vehicle: 0.4% aqueous methylcellulose

Methods:
Test strain and Cells: Mice (Crl:CD-1(CR)BR VAF/PLUS, 10 weeks of age.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a previous in vivo micronucleus study in mice where combined doses of SCH 58235 + pravastatin (ratio 1:1 by weight) were 200, 400, 800, 1000, 1200 mg/kg/day, given ip once a day for two consecutive days. Based on mortality, clinical signs and on the bone marrow toxicity, doses of 62.5-300 mg/kg/day were chosen for the main micronucleus test. In the main micronucleus test, no mortality was observed in females, but in males at 300 mg/kg/day 2 males died on day 4 (2 days after the final dose). Clinical signs were noted in both sexes, and included rough haircoat, hypoactivity, squinted eye (in females) at 75 mg/kg/day in females and at 150 mg/kg/day in males.

Dose groups for micronucleus test

Dose Group	Dose[a] (mg/kg/day)	Number of Mice Dosed / Sacrifice Time[b]			
		24 Hours (Trial 1)		48 Hours (Trial 2)	
		Male	Female	Male	Female
Vehicle Control (0.4% aqueous methylcellulose)	0	6	6	6	6
Low-Dose (SCH 58235 and atorvastatin calcium [SCH 412387])	75	6	-	6	-
	62.5	-	6	-	6
Mid-Dose (SCH 58235 and atorvastatin calcium [SCH 412387])	150	6	-	6	-
	125	-	6	-	6
High-Dose (SCH 58235 and atorvastatin calcium [SCH 412387])	300	6	-	6	-
	250	-	6	-	6
Secondary High-Dose (SCH 58235 and atorvastatin calcium [SCH 412387])	300	-	-	3	-
	250	-	-	-	3
Positive Control (Cyclophosphamide)	50	6	6	-	-
	30	-	-	6	6

a: Each test article was administered by intraperitoneal injection once a day for two consecutive days.
b: Time after the final intraperitoneal injection.

No. of animals used: 6/sex/group/sacrifice time.

Table. Results of mouse bone marrow micronucleus test with SCH 58235 + atorvastatin in males:

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Test Article: SCH 58235 and atorvastatin calcium (SCH 412387)

Treatment	Dose (mg/kg/day)	Harvest Time (hr)	Mean % Micronucleated PCE (per 2000 PCE ± S.E.)	Mean Estimated % Micronucleated NCE (per 2000 PCE ± S.E.)	PCE/NCE Ratio Mean ± S.E.
Controls					
Vehicle 0.4% MC	(20 mL/kg)	24	0.11 ± 0.03	0.04 ± 0.01	0.56 ± 0.06
		48	0.05 ± 0.02	0.01 ± 0.01	0.78 ± 0.09
Positive (CP)	50	24	1.78 ± 0.29[a]	0.17 ± 0.05	0.47 ± 0.06
	30	48	0.36 ± 0.11[a]	0.07 ± 0.02[a]	0.40 ± 0.05[b]
SCH 58235 and atorvastatin calcium (SCH 412387)	75	24	0.04 ± 0.02	0.01 ± 0.01	0.83 ± 0.06
		48	0.03 ± 0.02	0.00 ± 0.00	0.79 ± 0.07
	150	24	0.09 ± 0.04	0.02 ± 0.01	0.51 ± 0.06
		48	0.05 ± 0.02	0.01 ± 0.01	0.78 ± 0.04
	300	24	0.03 ± 0.02	0.01 ± 0.01	0.61 ± 0.06
		48	0.03 ± 0.01	0.00 ± 0.00	0.60 ± 0.09

[a] Significantly greater than the corresponding vehicle control, $p \leq 0.01$.

[b] Significantly less than the corresponding vehicle control, $p \leq 0.05$.

PCE: Polychromatic erythrocyte

NCE: Normochromatic erythrocyte

CP: Cyclophosphamide

0.4% MC: 0.4% (w/v) aqueous methylcellulose

Test Article: A coadministration of SCH 58235 and atorvastatin calcium (SCH 412387) in a 1:1 ratio by weight; doses were expressed as total dose of combination.

Table. Results of mouse bone marrow micronucleus test with SCH 58235 + atorvastatin in females:

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Test Article: SCH 58235 and atorvastatin calcium (SCH 412387)

Treatment	Dose (mg/kg/day)	Harvest Time (hr)	Mean % Micronucleated PCE (per 2000 PCE ± S.E.)	Mean Estimated % Micronucleated NCE (per 2000 PCE ± S.E.)	PCE/NCE Ratio Mean ± S.E.
Controls					
Vehicle	(20 mL/kg)	24	0.07 ± 0.02	0.01 ± 0.01	0.57 ± 0.05
0.4% MC		48	0.09 ± 0.03	0.02 ± 0.02	0.92 ± 0.05
Positive (CP)	50	24	1.89 ± 0.26[a]	0.13 ± 0.03[a]	0.52 ± 0.05
	30	48	0.25 ± 0.09	0.07 ± 0.03	0.77 ± 0.05
SCH 58235 and atorvastatin calcium (SCH 412387)	62.5	24	0.04 ± 0.02	0.00 ± 0.00	0.70 ± 0.09
		48	0.04 ± 0.02	0.00 ± 0.00	0.87 ± 0.10
	125	24	0.03 ± 0.02	0.00 ± 0.00	0.67 ± 0.08
		48	0.07 ± 0.01	0.01 ± 0.01	0.79 ± 0.09
	250	24	0.04 ± 0.02	0.01 ± 0.01	0.66 ± 0.06
		48	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.10

[a] Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
PCE: Polychromatic erythrocyte
NCE: Normochromatic erythrocyte
CP: Cyclophosphamide
0.4% MC: 0.4% (w/v) aqueous methylcellulose
Test Article: A coadministration of SCH 58235 and atorvastatin calcium (SCH 412387) in a 1:1 ratio by weight; doses were expressed as total dose of combination.

Genetic toxicology summary: SCH 58235 + atorvastatin was not cytogenic up to doses of 250-300 mg/kg/day in an in vivo micronucleus test in mice

3. Studies with SCH 58235 + simvastatin (SCH 57098)

3A. Effects of SCH 58235 + simvastatin (SCH 57098) on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: SCH 58235 +simvastatin was negative in AMES test

Study no: 97119
Volume #, and page #: Volume 161, page 1 (reference 82)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 8/7/1998
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug lot #, and % purity: SZ-58235-96-X-3, simvastatin batch # 38425-111

Formulation/vehicle: Dimethylsulfoxide (DMSO)

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA97a, TA98, TA100, TA1535, TA102, and E. coli tester strain WP2 urvA

Dose selection criteria:
Basis of dose selection: The dose selection was based on the first mutagenicity study (or trial 1) in which doses of 50, 158, 500, 1581 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. The test article was a combination of SCH 58235 and simvastatin at ratio of 1:1 by weight. In the above assay, cytotoxicity was estimated based on approximate 30% decreases in revertant colony counts below concurrent solvent controls and inhibition of bacterial lawn growth. In the first assay the drug did not induce an increase in revertant colony counts in any bacterial starins tested. In the second assay (or trial 2), doses ranged from 39-5000 µg/plate, based on cytotoxic dose levels, the highest capable dose that could be analyzed without interference from the test article precipitate, or the maximum dose of 5 mg/plate. Doses chosen in the second assay are sown in the Table below.

Range finding studies: The test article was a combination of SCH 58235 and simvastatin at a ratio of 1:1 by weight. Based on cytotoxicity and precipitate formation, following doses were selected in trial 2.

Table: The doses and strains used in AMES assay with SCH 58235 + simvastatin (SCH 57098).

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

Test agent stability: Both SCH 58235 (at concentration of 0.15-49.8 mg/ml) and simvastatin (at concentration of 0.1-50 mg/ml) were stable in DMSO for at least 4 hours under the ambient temperature and light

Metabolic activation system: Rat liver microsome S9 fraction.

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

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 (100)	2-Aminoanthracene (5)
Escherichia coli		
WP2uvrA	N-Ethyl-N'-nitro-N-nitrosoguanidine (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 approximately 48 hrs at 37^0C 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 compared to the solvent controls, in at least one of the six tester strains, and the increase is at least 2 times for strains TA97a, TA98, TA100, TA102, WP2 urvA, and 3 times for strain TA1535, 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 assay (at dose range of 50-5000 µg/plate), and in the confirmatory mutagenicity assay (39-5000 µg/plate), the combination of SCH 58235 + simvastatin was not mutagenic in any of the tester strains at any doses in the presence or absence of metabolic activation. Precipitate was observed from doses of 1581 and 1250 µg/plate in the presence or absence of metabolic activation respectively. A significant increase in the number of revertant colonies was observed with the positive controls (with or without S9 mix). In conclusion, AMES test was negative. The revertant colonies in the solvent controls were within the historical control range, see Table below.

Table 5 Historical Solvent Control Values for Dimethylsulfoxide (Revertants/Plate) 1/1990 - 12/1997						
	Bacterial Strain					
	Salmonella typhimurium					*Escherichia coli*
	TA1535	TA97a	TA98	TA100	TA102	WP2uvrA
Nonactivation (-S9)	7-27 (36)	15-131 (38)	14-34 (36)	84-171 (34)	96-368 (39)	18-42 (36)
Activation (+S9)	7-33 (35)	74-179 (37)	20-45 (36)	96-159 (35)	132-460 (34)	17-53 (35)
( ) = Number of studies						

Genetic toxicology summary: SCH 58235+ simvastatin co-administration was negative in the AMES test in all tester strains.

3B. Effects of SCH 58235 + simvastatin (SCH 57098) on chromosome aberrations in human peripheral blood lymphocytes

Key findings: SCH 58235 +simvastatin was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

Study no: 97120
Volume #, and page #: Volume 161, page 1 (reference 86)
Conducting laboratory and location: [redacted]

Date of study initiation: 7/28/1998
GLP compliance: Yes
QA reports: yes (X) no ( )

Basis of dose selection: The dose selection was based on the precipitation of the test agents and cytotoxicity (or mitotic index). Mitotic index was ≥ 50% at the high dose in all assays. Sponsor's summary on this study is as follows:

SCH 58235 + simvastatin (SCH 57098) study design

6.5.5. Chromosome Aberration Study of SCH 58235/SCH 57098 in Human Peripheral Blood Lymphocytes (SN 97120)

Methods/Design

Performed by:	[ ]
Study Performed in Compliance with GLP:	Yes
Animals:	In vitro
Target Cells:	Human peripheral blood lymphocytes
Duration of Exposure:	≈4 and ≈19 hours (nonactivation); ≈4 hours (activation)
Sampling Times:	≈22-hour harvest
Test Articles/Formulation:	SCH 58235 and SCH 57098 in a 1:1 ratio by weight, prepared and delivered to the test system separately. All doses are presented as doses of the combination.
Batch Nos.:	SCH 58235: SZ-58235-96X-3 SCH 57098: 38425-111
Doses Analyzed:	Nonactivation: 10, 15, 20, 25, 30 and 40 µg/mL Activation: 15, 20, 25 and 30 µg/mL
No. of Independent Experiments:	Two
No. of Replicate Cultures:	Two/dose
No. of Cells Analyzed:	100/culture, 200/dose
Positive Controls:	Nonactivation: Mitomycin C (Sigma Lot No. 116H2511) Activation: Cyclophosphamide (Sigma Lot No. 43H0269)
Solvent and Final Concentration:	Dimethylsulfoxide (DMSO), 10 µL/mL in tissue culture medium

SCH 58235 + simvastatin (SCH 57098) Results:

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Results

Genotoxic Effects:	No genotoxic effects were observed
Effect of the Positive Control:	All positive controls induced statistically significant increases above their respective vehicle controls ($p \leq 0.01$).

Conclusions

Coadministration of SCH 58235 and SCH 57098 (ratio of 1:1, by weight), was negative in inducing chromosome aberrations in cultured whole blood human lymphocytes in the presence or absence of an exogenous metabolic activation system under the conditions of this study.

Genetic toxicology summary: SCH 58235+ simvastatin co-administration was negative in the chromosome aberration assay in cultured whole blood human lymphocytes

3C. Effects of SCH 58235 + simvastatin (SCH 57098) on in Vivo Micronuclei in Mice.

Key findings: SCH 58235 + simvastatin (SCH 57098) was negative in vivo micronucleus test in mice

Study no: 97135
Volume #, and page #: Volume 162, page 1 (Reference 90)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 5/23/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: Lot #: SCH 58235 98-58235-X-02, simvastatin batch # 99-57098-X-02

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.

Dose selection criteria:
Basis of dose selection: The dose selection was based on a previous in vivo micronucleus study in mice where combined doses of SCH 58235 +simvastatin (ratio 1:1 by weight) were 200, 400, 600, 800,1200 mg/kg/day, given ip once a day for two consecutive days. Based on mortality, clinical signs and on the bone marrow toxicity, doses of 0, 150, 300, 600 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 600 mg/kg/day.

No. of animals used: 5/sex/group/sacrifice time

Sponsor's summary of mouse bone marrow micronucleus test with SCH 58235 + simvastatin (SCH 57098):

> The genotoxicity of SCH 58235, a cholesterol absorption inhibitor, co-administered with simvastatin (SCH 57098) was evaluated in a mouse bone marrow erythrocyte micronucleus assay.
>
> SCH 58235 and simvastatin at a ratio of 1:1 by weight were administered by separate intraperitoneal injections at combined doses of 0, 150, 300 and 600 mg/kg in male and female CD-1® mice. Clinical signs of rough hair coat and urogenital staining were observed at 600 mg/kg. Bone marrow suppression, as determined by decreases of the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) ratio (PCE/NCE ratio), was observed in both male and female mice at the mid- and high-doses. No statistically significant increase of micronucleus frequency was observed in male or female mice dosed with the test article as compared to the vehicle control. The positive control, cyclophosphamide, induced statistically significant increases ($p \leq 0.001$) of micronucleus frequency in both male and female mice.
>
> In conclusion, SCH 58235 when co-administered with simvastatin at a ratio of 1:1 by weight did not induce micronuclei in bone marrow polychromatic erythrocytes in male or female CD-1® mice under the conditions of this study.

Genetic toxicology summary: SCH 58235 + simvastatin (SCH 57098) was negative up to doses of 600 mg/kg/day in an in vivo micronucleus test in mice

4. Studies with SCH 58235 + lovastatin (SCH 48176)

4A. Effects of SCH 58235 + lovastatin (SCH 48176) on Salmonella/Escherichia Coli Reverse Mutation Test: (AMES TEST)

Key findings: SCH 58235 + lovastatin was negative in AMES test

Study no: 99014
Volume #, and page #: Volume 161, page 1 (reference 83)
Conducting laboratory and location: Schering Plough Research Institute, NJ.

Date of study initiation: 3/11/1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-5, lovastatin batch # 99-48176-X-01

Formulation/vehicle: Dimethylsulfoxide (DMSO)

Methods:
Strains/species/cell line: Salmonella typhimurium tester strains TA97a, TA98, TA100, TA1535, TA102, and E. coli tester strain WP2 urvA

Dose selection criteria:
Basis of dose selection: SCH 58235 and lovastatin were tested at ratio of 1:1 by weight. Both the test articles were prepared separately in DMSO and then used in the test system. The dose selection was based on the first mutagenicity study (or trial 1) in which dose range of 78 to 5000 µg/plate were used in Salmonella and E. coli strains in the plate incorporation method in the presence and absence of metabolic activation. The dose selection was based on, cytotoxicity (which was estimated based on approximate 30% decreases in revertant colony counts below concurrent solvent controls), the highest capable dose that could be analyzed without interference from the test article precipitate, or the maximum dose of 5 mg/plate. Based on above criteria, mutagenicity assays (trial 2 and 3) were conducted at doses ranging from 39-5000 µg/plate. More than one trial was conducted because solvent controls were not within the historical control range.

Range finding studies: The test article was a combination of SCH 58235 and lovastatin at a ratio of 1:1 by weight. Based on cytotoxicity and precipitate formation, following doses were selected in trial 2.

Table: The doses and strains used in AMES assay with SCH 58235 + lovastatin
Trial 2 was conducted with SCH 58235/SCH 48176 at the following doses:

Bacterial Strains	Doses (µg/plate)	
	Nonactivation Phase	Activation Phase
TA1535	78.13, 156.25, 312.5, 625, 1250	78.13, 156.25, 312.5, 625, 1250
TA97a	39.07, 78.13, 156.25, 312.5, 625	39.07, 78.13, 156.25, 312.5, 625
TA98	312.5, 625, 1250, 2500, 5000	156.25, 312.5, 625, 1250, 2500
TA100	78.13, 156.25, 312.5, 625, 1250	78.13, 156.25, 312.5, 625, 1250
TA102	78.13, 156.25, 312.5, 625, 1250	78.13, 156.25, 312.5, 625, 1250
WP2uvrA	156.25, 312.5, 625, 1250, 2500	156.25, 312.5, 625, 1250, 2500

Test agent stability: Both SCH 58235 (at concentration of 0.15-49.8 mg/ml) and lovastatin (at concentration of 0.1-50 mg/ml) were stable in DMSO for at least 4 hours under the ambient temperature and light

Metabolic activation system: Rat liver microsome S9 fraction.

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