1. A 3-month oral Dietary Toxicity Study of SCH-58235 in combination with pravastatin in Rats (Study No. SN 99489)

Key study findings: Co-administration of SCH 58235 (males 15, 250, 250, 750 mg/kg/day, females 15, 50, 50, 150 mg/kg/day) + pravastatin (25, 25, 250, 250 mg/kg/day) in rats increased the exposures to SCH 58235 and also to pravastatin. The two HD combination doses produced clinical signs in rats and/or mortality. All combination doses decreased mean BW (by 5-13%) and weight gains (by 10-23%) in males, and similar decreases in weights (5-8%) and weight gains (12-17%) in females at MD-HD combination. In males two HD combination not only produced increases in plasma AST, ALT, & AP levels (by 2-fold), and SDH (by 4-6 fold) but also produced toxicity in the liver (hyperplasia, biliary hypertrophy, while mitotic figures & vacuollation), skeletal muscle (myofiber degeneration) and stomach (acanthosis). In females, all combination doses increased liver weights by 18-43%, and produced liver histopath changes of higher severity. The NOAEL in this 3-month rat study for SCH 58235/ pravastatin was 250/25 mg/kg/day in males, and <15/25 mg/kg/day in females.

Study no: SN 99489
Volume #, and page #: 1.105, page 1 (reference 53)
Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 2/18//2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-01, pravastatin sodium (SCH57096) 75793-008
Formulation/vehicle: [redacted] (meal)

Methods:
Species/strain: Sprague-Dawley rats/Crl:CD (SD)BR VAF/PLUS
#/sex/group or time point (main study):10/sex/dose
Satellite groups used for toxicokinetics or recovery: Additional 36/sex/dose for TK study.
Age: Approximately 6 weeks of age
Weight: Males 180-202 g, females 127-155 g.
Doses employed: SCH 58235, males 15, 250, 250, 750 mg/kg/day, females 15, 50, 50, 150 mg/kg/day) + Pravastatin sodium (SCH 57096) 25, 25, 250, 250 mg/kg/day, controls received vehicle or 250 pravastatin
Route of administration: SCH 58235 dietary, pravastatin by oral gavage

Parameters and endpoints evaluated:

Three-Month Oral Toxicity and Toxicokinetic Study of SCH 58235 (Diet) Co-Administered with Pravastatin (Gavage) in Rats (SN 99489): **Study Design**

Group	Test/Control Article	No. of Rats/Sex		SCH 58235 Estimated Total Daily Dose (mg/kg)		Pravastatin (SCH 57096)			Duration of Dosing (Days)
		Toxicity Portion	Satellite Portion	M	F	Total Daily Dose[b] (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	
C1	Vehicle Control (Feed/ Methylcellulose)	10	0	0	0	0	5	0	96 to 98
C2	Pravastatin Control	10	36[a]	0	0	250	5	50	96 to 98
T1	Low-Dose Combination	10	36[a]	15	15	25	5	5	96 to 98
T2	Low-Mid-Dose Combination	10	36[a]	250	50	25	5	5	96 to 98
T3	High-Mid-Dose Combination	10	36[a]	250	50	250	5	50	96 to 98
T4	High-Dose Combination	10	36[a]	750	150	250	5	50	96 to 98

a: These animals were evaluated for toxicokinetic parameters only.

b: Doses of pravastatin are expressed as the sodium salt. When expressed as the free acid, daily doses of pravastatin were 238, 23.8, 23.8, 238 and 238 mg/kg for Groups C2, T1, T2, T3 and T4, respectively.

Three-Month Oral Toxicity and Toxicokinetic Study of SCH 58235 (Diet) Co-Administered with Pravastatin (Gavage) in Rats (SN 99489): **Observations and Measurements**

Investigation	Performed	Investigation	Performed
Viability	Daily	Hematology	Weeks 4 and 12
Clinical Observations	Daily	Coagulation	Week 14/15
Body Weight	Weekly beginning Week –1; and days of randomization and terminal sacrifice	Serum Chemistry	Weeks 4 and 12
Food Consumption	Weekly	Urinalysis/Urine Chemistry	Weeks 4 and 12
SCH 58235 Intake (Calculated)	Weekly	Organ Weights	Yes
Ophthalmoscopic Examinations	Once pretest, Weeks 5 and 13	Necropsy (Macroscopic Observations)	Yes
Plasma Analysis for SCH 58235 and Pravastatin	Day 0 and Week 5 (1, 2, 4, 6, 12 and 24 hrs after pravastatin administration)	Histopathology (Microscopic Observations)	Yes[a]

a: The following organs/tissues were examined microscopically: all organs/tissues collected from toxicity portion rats in the vehicle control, pravastatin control and high-dose combination groups, as well as toxicity portion rats that died or were sacrificed prior to scheduled necropsy; all collected gross findings; and liver, skeletal muscle and stomach from toxicity portion rats in all other dose groups.

Organs weighed: Organs weighed are listed in the Table below

Table. Tissues collected for organ weights in the 3-month rat tox study of SCH 58235 + pravastatin

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

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

Histopathology: This was performed at sacrifice in the vehicle & pravastatin controls and high dose combination animals, listed below in the histopathology Table. Liver, skeletal muscle and stomach were identified as target organs of toxicity by the pathologist and were examined in all other dose groups

Table. Tissues collected for histopath evaluation in the 3-month rat tox study of SCH 58235 + pravastatin

Tissues Collected	
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[a]	Seminal Vesicles
Brain	Skeletal Muscle - Biceps Femoris, Diaphragm, Intercostal and Sublumbar[d]
Epididymides	Skin
Esophagus	Small Intestine – Duodenum, Jejunum, Ileum
Eyes (plus Optic Nerve)	Spinal Cord – Thoracolumbar
Harderian Glands	Spleen
Head[b]	Stomach
Heart	Testes
Kidneys	Thymus
Large Intestine – Cecum and Colon	Thyroid Gland
Liver	Tongue
Lungs (plus Bronchi)	Trachea
Lymph Nodes (Mandibular and Mesenteric)	Urinary Bladder
Mammary Gland[c]	Uterus (plus Cervix)
Ovaries	Vagina
Pancreas	Animal Identification[b]
Parathyroid Gland(s)[c]	

a: Bone marrow smears were prepared for all toxicity portion rats except those found dead during the morning viability check and as noted in Appendix 1. Smears were not evaluated because it was not warranted by changes in the peripheral blood.

b: Collected but not processed

c: Examined histopathologically when present in routine section

d: The sublumbar muscles were collected in situ with a segment of the lumbar vertebral column. The left lateral sublumbar muscles were prepared for histopathology.

Toxicokinetics: Days 0 and week 5, at 1, 2, 6, 12 and 24 hrs. Conjugated and unconjugated drug was measured.

Results:

Mortality: One male in the HD combo group (on day 42) was sacrificed in moribund condition. In this male hindquarter weakness correlated with moderate myofiber degeneration in the histopath. In the above HD male, BW/FC decreased during weeks 6-7 by 6-16% and by 16-46% respectively. This animal had clinical signs of chromorhinorrhea, a rough hair coat, dehydration, hypoactivity, loose stool, yellow urogenital staining, bilateral chromcacryorrhea and hindquarter weakness. Hindquarter weakness correlated with moderate myofiber degeneration of skeletal muscle in the histopathology. Sponsor states that myofiber degeneration of skeletal muscle has been previously seen with pravastatin and other HMG-CoA reductase inhibitors. Three other deaths were considered incidental. One F in pravastatin control group, one F in HD combination were found dead on day 26 after blood collection due to hemorrhage following jugular veinpuncture, one male at LD combination was sacrificed due to moribund condition due to ruptured/perforated esophagus.

Clinical signs: At high-mid dose combination, clinical signs were similar to the one described in a HD combination animal that was sacrificed above (no summary Table

was provided). At a HD combination, males had alopecia in hindlimbs (1/10 of rats) or abdomen (1/10 M & 1/10 F) and transient scant stools (1/10 M). Sponsor states that alopecia has been observed before with simva, lovastatin, pravastatin, and combination of SCH 58235 +simvastatin in rats.

Body weights: In males the mean BW were decreased with all combinations by 5-13% and weight gain by 10-23% compared to pravastatin or vehicle controls. In females at mid and high combination doses, BW and weight gains were decreased by 5-8% and 12-17% respectively. In week 14 mean Bws in males were 430, 418, 407, 407, 393, 375 g in vehicle control, pravastatin alone, and at LD, MD, mid-HD, & HD combinations respectively. In females these values were 248, 247, 242, 241, 229, 236 g respectively.

Food consumption: In males at HD combination, food consumption was decreased by 5% during weeks 2-4.

Drug Intake: The mean ezetimibe intake was within 2% of intended doses

Ophthalmoscopy: Sponsor states that no drug related effects were observed, and refers to appendix 5 for ophthalmologist's report. However, in appendix 5, there was only mention of lesions that were identified in two rats i.e. # 57F (which had pale occular fundi) and # 455F (which had focal retinopathy in the left eye), and states that these lesions are not likely to be caused by exposure to an occular oxicant. No other data (including these occurrences in dose groups) are provided in this appendix 5 report.

Hematology/Coagulation: No significant drug related differences were observed

Clinical chemistry: In mid-high and HD combination groups, alanine aminotransferase (minimal), aspartate aminotransferase (min-mild), sorbitol dehydrogenase (mild), AP (min-mild), and albumin/globulin ratios were increased, while globulin conc were lower. In week 12 in males ALT (33, 44, 44, 42, 58, 59 IU/L respectively), AST (131, 147, 127, 134, 245, 354 IU/L respectively), AP (195, 197, 233, 252, 435, 393 IU/L respectively), and SDH (5.4, 8.8, 13.2, 3.7, 20.7, 29.1 IU/L respectively) values were increased at two high combination doses.

Table: Changes in serum chemistry in a 3-month rat toxicity study of SCH 58235 + pravastatin

Test Article-Related Changes in Mean Serum Chemistry Parameters						
		Group				
Finding (Units)	Week	Vehicle Control	Low-Dose Combination	Low-Mid-Dose Combination	High-Mid-Dose Combination	High-Dose Combination
Male Values						
ALT (IU/L)	4	40.8			65.3	88.3
	12	32.7			58.3	59.0
AST (IU/L)	4	135.6			233.3	384.0
	12	130.6			245.1	253.5
SDH (IU/L)	4	4.5			16.7	43.1
	12	5.4			20.7	29.1
AP (IU/L)	4	294.6			666.9	782.3
	12	194.6			435.1	393.3
GLOB (g/dL)	4	2.56			2.16	2.18
	12	3.12			2.59	2.55
A/G	4	1.55			1.92	1.93
	12	1.23			1.52	1.55
CHOL (mg/dL)	12	44.8			24.4	25.1
TRIG (mg/dL)	4	97.0	63.3	44.0	18.3	20.8
	12	111.7	60.9	42.6	16.1	17.1
Female Values						
AST (IU/L)	4	118.1				170.0
	12	128.5				172.1
SDH (IU/L)	4	6.4			12.3	20.2
	12	6.6			14.4	15.1
AP (IU/L)	4	229.6			377.4	399.1
	12	145.8			229.1	245.9
CHOL (mg/dL)	12	44.3				35.1
TRIG (mg/dL)	4	64.1	37.2	22.4	18.8	17.9
	12	70.9	54.3	36.0	21.4	19.0

Urinalysis: No significant drug related differences were observed

Organ weights: Liver weights (absolute and relative) in females increased at all combination doses by 18-43% and relative by 24-54%. Absolute liver weights in females were 6.0, 6.0, 7.9, 7.1, 8.6, 8.3 g respectively. In males these did not change significantly (9.8, 9.6, 9.7, 9.6, 10, 10 g respectively).

Table: Liver weights changes in females in a 3-month rat toxicity study of SCH 58235 + pravastatin

Test Article-Related Organ Weight Changes: Percent Difference from Vehicle Control Mean								
Group:	Low-Dose Combination		Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F
Organ	Percent Difference from Vehicle Control Mean (%)							
Liver								
-Absolute weight		+30.87		+17.99		+43.01		+36.66
-Relative weight[a]		+31.02		+24.02		+53.16		+42.25
a: Relative to body weight								

Gross pathology: One female in HD combination had enlarged liver, which was correlated with periportal hepatocellular hypertrophy in that female

Histopathology: In both sexes histopath findings were observed in the liver (biliary hyperplasia, periportal hepatocellular hypertrophy, single cell necrosis vacuolation,

Kupffer cell pigment accumulation and hepatocellular mitotic figures), skeletal muscle (moderate myofiber degeneration), and stomach (mild acanthosis of non glandular stomach). Myocardial degeneration consistent with an early stage of chronic progressive cardiomyopathy was observed in males in all groups, including pravastatin controls (5/10, 6/10, ne, ne, 1/1, 5/10 respectively, ne=not examined), but its incidence was not different with combination, and sponsor states that it is a common age related finding in rats (which were 6-weeks old at study initiation). Sponsor also states that similar liver, skeletal muscle and stomach toxicity is seen with pravastatin or other HMG-CoA reductase inhibitors

Table: Histopathologic findings in a 3-month rat toxicity study of SCH 58235 + pravastatin

Test Article-Related Histopathologic Findings										
Group:	Pravastatin Control		Low-Dose Combination		Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[a]									
Liver										
-Hyperplasia, biliary										
minimal	8/10	9/10	10/10	7/10	10/10	8/10	1/10		2/10	
mild	1/10			3/10		2/10	9/10	10/10	8/10	10/10
-Hypertrophy, periportal, hepatocellular										
minimal	9/10	8/10	3/10	7/10	7/10	10/10		1/10		
mild	1/10			3/10			10/10	9/10	10/10	10/10
-Single cell necrosis, hepatocellular										
minimal	6/10	4/10	3/10	4/10	3/10	3/10	9/10	10/10	9/10	10/10
mild							1/10			
-Pigment accumulation, Kupffer cell										
minimal	4/10	1/10		2/10		1/10	10/10	9/10	8/10	7/10
-Mitotic figures, hepatocellular										
minimal		2/10					1/10			3/10
-Vacuolation, periportal hepatocellular										
minimal	3/10				1/10		9/10	3/10	10/10	4/10
-Foci of cellular alteration, clear cell										
minimal	2/10		1/10		2/10		4/10	1/10	4/10	2/10
-Foci of cellular alteration, basophilic										
minimal							7/10	7/10	9/10	8/10
-Foci of cellular alteration, eosinophilic										
minimal	3/10	1/10					3/10	2/10	3/10	1/10
Skeletal Muscle										
-Degeneration, myofiber										
moderate							1/10			
Stomach										
-Acanthosis, nonglandular										
mild									1/10	

a: Incidence = Number affected/Number examined

Toxicokinetics: The plasma AUC values are shown in the Table. The co-administration of drug with pravastatin resulted in an increased exposure to total and conjugated ezetimibe. There was accumulation of drug at all doses in females and at mid-high doses in males in week 5 vs day 0. Pravastatin expsosures increased with ezetimibe doses in females, but in males these were increased at high doses of ezetimibe (in week 5).

Table: Systemic exposures (AUC 0-24 hr) to total, conjugated and unconjugated SCH 58235 on day 0 and week 5 in a 3-month rat toxicity study of SCH 58235 + pravastatin:

	Male				Female			
	15/25[a,b]	250/25[a,b]	250/250[a,c]	750/250[a,c]	15/25[a,b]	50/25[a,b]	50/250[a,c]	150/250[a,c]
	Total SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	1520	10400	9930	11900	1670	3470	4300	7710
Week 5	1940	9480	39900	133000	2380	7030	15900	41200
	Conjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	1490	10400	9850	11800	1660	3460	4270	7680
Week 5	1940	9430	39800	133000	2380	7020	15900	41200
	Unconjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
Day 0	ND	72.0	80.6	62.0	ND	ND	ND	ND
Week 5	ND	45.9	59.5	181	ND	10.5	9.27[d]	54.4

a: Dose of SCH 58235 (mg/kg)/pravastatin sodium (mg/kg).
b: Pravastatin free acid equivalent dose is 23.8 mg/kg.
c: Pravastatin free acid equivalent dose is 238 mg/kg.
d: AUC(tf) (ng-hr/mL)
ND = Not determinable

Table: Systemic exposures (AUC 0-24 hr) to pravastatin on day 0 and week 5 in a 3-month rat toxicity study of SCH 58235 + pravastatin

	Male					Female				
	15/25[a,b]	250/25[a,b]	0/250[a,c]	250/250[a,c]	750/250[a,c]	15/25[a,b]	50/25[a,b]	0/250[a,c]	50/250[a,c]	150/250[a,c]
	Pravastatin AUC(0-24 hr) (ng-hr/mL)									
Day 0	385	108	1540	1180	1070	155	99.3	1160	1260	1980
Week 5	92.6	81.5	8020	2150	16500	58.3	83.7	772	1580	2030

a: Dose of SCH 58235 (mg/kg)/pravastatin sodium (mg/kg).
b: Pravastatin free acid equivalent dose is 23.8 mg/kg.
c: Pravastatin free acid equivalent dose is 238 mg/kg.

Toxicology summary: In a 3-month toxicity study of SCH 58235 (males 15, 250, 250, 750 mg/kg/day, females 15, 50, 50, 150 mg/kg/day) + pravastatin (25, 25, 250, 250 mg/kg/day) in rats, the increases in AUC values of the total drug (SCH 58235) were not dose proportional and values were generally higher in week 5 (males 1.9, 9.5, 39.9, 133 µg.h/ml, females 2.4, 7.0, 15.9, 41.2 µg.h/ml respectively) vs on day 0 (males 1.5, 10.4, 9.9, 119 µg.h/ml, females 1.7, 3.5, 4.3, 7.7 µg.h/ml respectively), suggesting accumulation of the drug over time. Thus the combination increased the total and conjugated ezetimibe exposures. The HD combination also increased the pravastatin expposures and values were higher in week 5 (combination males 16.5, females 2.0 µg.h/ml vs pravastatin alone males 8.0, females 0.7µg.h/ml) than on day 0 (males 1.0, females 2.0 µg.h/ml vs pravastatin alone males 1.5, females 1.2 µg.h/ml). The two HD combination doses produced clinical signs in rats and/or mortality. All combination doses decreased mean BW (by 5-13%) and weight gains (by 10-23%) in males, and similar decreases in weights (5-8%) and weight gains (12-17%) in females at MD-HD combination. The decreases in BW were not seen with ezetimibe or pravastatin monotherapy. Two HD combination not only produced increases in plasma AST, ALT, & AP levels (by 2-fold), and SDH (by 4-6 fold) but also produced toxicity in the liver (biliary hyperplasia, hepatocellular hypertrophy in both sexes with increased incidences at all doses in females, mitotic figures & vacuolation at two high doses), skeletal muscle (myofiber degeneration in males at approximately 60 times the human exposure) and stomach (acanthosis in males). The NOAEL in this 3-month rat study was SCH 58235 250 mg/kg/day + 25 mg/kg/day pravastatin in males. In females no NOAEL could be identified as all combination doses not only increased liver weights but produced an increase in more severe liver findings (severity: biliary hyperplasia & hepatocellular hypertrophy was mild in 15/25 mg/kg/day 3/10 vs 1/10 in pravastatin controls). Therefore NOAEL in females was <15 mg/kg/day of SCH 58235 + <25 mg/kg/day of pravastatin.

[ ]

2. A 3-month oral gavage Toxicity Study of SCH-58235 in combination with pravastatin in dogs (Study No. SN 99490)

Key study findings: Co-administration of SCH 58235 (3, 3, 30, 30 mg/kg/day) + pravastatin (1, 5, 5, 10 mg/kg/day) in dogs by gavage did not significantly alter the exposures to SCH 58235 or to pravastatin. All combination doses (including the lowest dose) increased plasma ALT levels, and produced thymus toxicity (decreased weights, size and increased the incidences of thymus atrophy in male dogs). The higher doses (from the doses of 3/5 mg/kg/day of SCH 58235/ pravastatin) increased liver AST & AP levels and produced toxicity not only in the thymus, but also in the liver (bile duct hyperplasia, pigment accumulation in kuffer cells). Additionally, the HD combination produced toxicity in the skin (histiocytoma, inflammation, hyperkeratosis) and lungs (vacuolated alveolar macrophages). The NOAEL in this 3-month dog study could not be established, as the lowest dose increased liver enzymes. The NOAEL may be < 3 mg/kg/day of SCH 58235 + 1 mg/kg/day of pravastatin.

Study no: SN 99490
Volume #, and page #: 1.129, page 1 (reference 60)

Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 1/26//2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-01, pravastatin sodium (SCH57096) 75793-008
Formulation/vehicle: 0.4% (w/v) aqueous methylcellulose

Methods:
Species/strain: Beagle dogs
#/sex/group or time point (main study):4/sex/dose

Age: Approximately 6-10 months of age
Weight: Males 7.9-13 kg, females 5.8-10.7 kg.
Doses employed: SCH 58235, 3, 3, 30, 30 mg/kg/day + Pravastatin sodium (SCH 57096) 1, 5, 5, 10 mg/kg/day, controls received vehicle (0.4% w/v aqueous methylcellulose) or 10 mg/kg/day of pravastatin
Route of administration: SCH 58235 dietary, pravastatin by oral gavage

Parameters and endpoints evaluated:

Three-Month Oral (Gavage) Toxicity and Toxicokinetic Study of SCH 58235 Co-Administered with Pravastatin (SCH 57096) in Beagle Dogs (SN 99490): **Study Design**

Group	Test/Control Article	No. of Dogs/Sex	Total Daily Dose (mg/kg)[a]	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Duration of Dosing (Days)
C1	Vehicle Control: 0.4% Methylcellulose	4	0	5	0	93 or 94
C2	Pravastatin Control: SCH 57096	4	10	5	2	93 or 94
T1	Low-Dose Combination: SCH 58235 SCH 57096	4	 3 1	 2.5 2.5	 1.2 0.4	93 or 94
T2	Low-Mid-Dose Combination: SCH 58235 SCH 57096	4	 3 5	 2.5 2.5	 1.2 2.0	93 or 94
T3	High-Mid-Dose Combination: SCH 58235 SCH 57096	4	 30 5	 2.5 2.5	 12.0 2.0	93 or 94
T4	High-Dose Combination: SCH 58235 SCH 57096	4	 30 10	 2.5 2.5	 12.0 4.0	93 or 94

a: Doses of pravastatin are expressed as the sodium salt. Doses of 1, 5 and 10 mg/kg pravastatin sodium are equivalent to 0.95, 4.8 and 9.5 mg/kg pravastatin free acid.

Parameters and endpoints evaluated continued

Three-Month Oral (Gavage) Toxicity and Toxicokinetic Study of SCH 58235 Co-Administered with Pravastatin (SCH 57096) in Beagle Dogs (SN 99490): **Observations and Measurements**			
Investigation	Performed	Investigation	Performed
Viability	At least once daily, beginning Week -4	Hematology	Twice pretest, Weeks 4 and 12
Clinical Observations	Daily beginning Week -1	Coagulation	Twice pretest, Weeks 4 and 12
Body Weight	Weekly beginning Week -3 and days of randomization and terminal sacrifice	Serum Chemistry	Twice pretest, Weeks 4 and 12
Food Consumption (Estimated)	Daily beginning Week -1	Urinalysis/Urine Chemistry	Twice pretest, Weeks 4 and 12
Ophthalmoscopic Examinations	Once pretest, Weeks 4 and 12	Organ Weights	Yes
General Veterinary Examinations	Once pretest, Weeks 4 and 12	Necropsy (Macroscopic Observations)	Yes
Physical Examinations (body temperature, respiratory and heart rates, blood pressure) and Electrocardiograms	Twice pretest, Weeks 5/6 and 13	Histopathology (Microscopic Observations)	Yes[a]
Plasma Analysis for SCH 58235 and Pravastatin	Days 0 and 29 (1, 2, 4, 6, 12 and 24 hrs after dosing)		
a: The following organs/tissues were examined microscopically: all organs/tissues collected from dogs in the vehicle control, pravastatin control and high-dose combination groups; all collected gross findings; and liver and thymus from dogs in all other dose groups.			

Organs weighed: Organs weighed are listed in the Table below

Table. Tissues collected for organ weights in the 3-month dog tox study of SCH 58235 + pravastatin

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

Histopathology: This was performed at sacrifice in the vehicle & pravastatin controls and high dose combination animals, listed below in the histopathology Table. Liver, and

thymus were identified as target organs of toxicity by the pathologist and were examined in all other dose groups

Table. Tissues collected for histopath evaluation in the 3-month dog tox study of SCH 58235 + pravastatin

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

a: Bone marrow smears were prepared for all dogs but were not evaluated because it was not warranted by changes in the peripheral blood.

b: Collected only from vehicle control group dogs for a non-GLP investigative pharmacology study; any data generated from investigative work on this tissue is not reported.

c: Spleen tissue (remaining after the sections were taken for histological processing) was collected for [redacted] any data generated from investigative work on this tissue is not reported.

d: Collected but not processed

Table. Liver Tissues at HD combination from 2 males and 2 females were subjected to special stains in the 3-month dog tox study of SCH 58235 + pravastatin

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Special Stains

Special Stain	Tissue	Dose Group	Animal No./Sex
Schmorl's Stain for Lipofuscin[a]	Liver	T4	22M, 24M, 124F
		C2	105F
AFIP Method for Lipofuscin	Liver	T4	22M, 24M, 123F, 124F
Hall's Stain for Bilirubin[b]	Liver	T4	22M, 24M, 123F, 124F
Oil Red O Staining Method for Fats/Lipids	Liver	T4	22M, 24M, 123F, 124F
Perl's Method for Iron	Liver	T4	22M, 24M, 123F, 124F

a: Carson FL. Histotechnology: A self-instructional text. Chicago: ASCP Press, 1990:216-18.

b: Luna LG, et al, eds. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York: McGraw-Hill, 1968:174.

Toxicokinetics: Days 0 and week 5, at 1, 2, 6, 12 and 24 hrs. Conjugated and unconjugated drug was measured.

Results:
Mortality: None

Clinical signs: Mild dehydration for several weeks (between weeks 5 and 12) was noted in ¼ M + ¼ F (at low-mid dose combination), and ¼ M +2/4 F (at high-mid dose combination) vs none in other groups. Since it was not seen at a HD combination, sponsor does not consider it significant and not drug related.

Body weights: A slight gradual decrease in mean BW in both sexes was noted in all groups, and this sponsor states was due to low daily ration of food given to dogs (due to SOP specified range). Beginning at week 7, the dogs were given higher scoop of food (at the upper end of specified range). The summary of mean changes in BW weight gains were not provided in dogs.

Note that in rats, BW and BW gains were decreased with all combinations of the drug + pravastatin (by 5-13% and weight gain by 10-23% compared to pravastatin or vehicle controls). Decreases in BW were also seen with the drug + lovastatin and drug + simvastatin in dogs.

Food consumption: No significant drug related differences were observed

Drug Intake: The mean ezetimibe intake was within 2% of intended doses

Ophthalmoscopy: Sponsor states that no drug related effects were observed,

Physical exam/ECG: No significant drug related differences were observed on mean body (rectal) temperatures, respiration rates, heart rates, blood pressures, or ECG findings in weeks 5/6 or 13

Hematology/Coagulation: No significant drug related differences were observed

Clinical chemistry: In all combination groups, alanine aminotransferase (ALT, minimal-moderate) was increased generally in a dose related manner. At mid-high dose combinations, aspartate aminotransferase (AST) and AP levels were also increased. At HD combination decreased globulin (1.7 vs 2.5-2.7 g/dl pretest) and total protein (4.6 vs 5.9-6.2 g/dl pretest) were observed in ¼ male dogs.

Table. Serum liver enzymes (ALT, AST and AP levels), and cholesterol, TG levels in 5 groups of dogs (at 0, pravastatin 10 mg/kg/day, and at 3/1, 3/5, 30/5 30/10 mg/kg/day of SCH 58235/pravastatin respectively)

Week 12	Males	Females
ALT (IU/L)	31, 39, 137, 207, 246, 693	33, 56, 99, 359, 139, 562
AST (IU/L)	33, 36, 47, 50, 51, 83	38, 44, 44, 66, 51, 70
AP (IU/L)	45, 53, 68, 114, 111, 144	87, 83, 85, 161, 134, 178
Cholesterol (mg/dl)	128, 92, 51, 40, 41, 14	143, 97, 69, 32, 35, 25
TG (mg/dl)	18, 17, 11, 12, nc, 12	22, 16, 12, 10, 18, 13

nc= not calculated

Sponsor's Table: Changes in liver enzymes in a 3-month dog toxicity study of SCH 58235 + pravastatin in males

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Incidence, Range of Values (Weeks 4 and 12) and Group Means for Test Article-Related Increased Serum ALT, AST, and AP Activities (Males)						
	ALT (IU/L)		AST (IU/L)		AP (IU/L)	
Group	Week 4	Week 12	Week 4	Week 12	Week 4	Week 12
Males						
Vehicle Control						
Range[a]	—	—	—	—	—	—
Group Mean[a]	30.0	30.5	29.5	33.3	58.3	45.0
Pravastatin Control						
Incidence[b]						
Range						
Group Mean						
Low-Dose Combination						
Incidence	4/4	4/4				
Range	—	—				
Group Mean	142.3	136.8				
Low-Mid-Dose Combination						
Incidence	4/4	4/4			1/4	2/4
Range	—	—			—	—
Group Mean	149.5	206.5			101.0	114.3
High-Mid-Dose Combination						
Incidence	4/4	4/4		1/4	1/4	2/4
Range	—	—		—	—	—
Group Mean	157.5	246.0		51.0	100.3	110.8
High-Dose Combination						
Incidence	4/4	4/4		2/4	2/4	3/4
Range	—	—		—	—	—
Group Mean	202.0	693.8		82.5	87.0	144.3

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

Sponsor's Table: Changes in liver enzymes in a 3-month dog toxicity study of SCH 58235 + pravastatin in females

Incidence, Range of Values (Weeks 4 and 12) and Group Means for Test Article-Related Increased Serum ALT, AST, and AP Activities (Females)

Group	ALT (IU/L)		AST (IU/L)		AP (IU/L)	
	Week 4	Week 12	Week 4	Week 12	Week 4	Week 12
Females						
Vehicle Control						
Range[a]	—	—	—	—	—	—
Group Mean[a]	30.8	32.5	37.5	37.5	89.0	86.5
Pravastatin Control						
Incidence[b]	1/4	1/4				
Range	—	—				
Group Mean	46.0	56.0				
Low-Dose Combination						
Incidence	3/4	3/4				2/4
Range	—	—				—
Group Mean	81.0	98.8				84.5
Low-Mid-Dose Combination						
Incidence	4/4	4/4	2/4	2/4	3/4	3/4
Range	—	—	—	—	—	—
Group Mean	338.3	358.8	56.0	65.8	137.3	160.5
High-Mid-Dose Combination						
Incidence	4/4	4/4			2/4	2/4
Range	—	—			—	—
Group Mean	173.0	138.5			114.5	134.3
High-Dose Combination						
Incidence	4/4	4/4	1/4	2/4	4/4	4/4
Range	—	—	—	—	—	—
Group Mean	226.0	562.3	51.0	69.5	142.0	178.3

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

Sponsor's Table: Changes in serum cholesterol and TG levels in a 3-month dog toxicity study of SCH 58235 + pravastatin in males

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Incidence, Range of Values (Weeks 4 and 12) and Group Means for Test Article-Related Decreased Serum Cholesterol (CHOL) and Triglyceride (TRIG) Concentrations (Males)

Group	CHOL (mg/dL)		TRIG (mg/dL)	
	Week 4	Week 12	Week 4	Week 12
Males				
Vehicle Control				
Range[a]	—	—	—	—
Group Mean[a]	141.0	127.8	22.8	18.0
Pravastatin Control				
Incidence[b]	4/4	4/4	2/4	3/4
Range	—	—	—	—
Group Mean	109.3	92.0	19.8	16.8
Low-Dose Combination				
Incidence	4/4	4/4	3/4	4/4
Range	—	—	BL[c] —	BL —
Group Mean	58.3	51.0	<13.3	<10.5
Low-Mid-Dose Combination				
Incidence	4/4	4/4	3/4	4/4
Range	—	—	BL	BL —
Group Mean	54.5	40.3	<13.0	<12.0
High-Mid-Dose Combination				
Incidence	4/4	4/4	4/4	4/4
Range	—	—	BL —	BL
Group Mean	54.8	41.3	<12.0	---
High-Dose Combination				
Incidence	4/4	4/4	4/4	4/4
Range	—	—	BL	BL —
Group Mean	30.5	14.0	—	<12.0

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

c: Values below analyzer sensitivity (represented as "BL") are not used to calculate group mean.

sponsor's Table: Changes in serum cholesterol and TG levels in a 3-month dog toxicity study of SCH 58235 + pravastatin in females

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Incidence, Range of Values (Weeks 4 and 12) and Group Means for Test Article-Related Decreased Serum Cholesterol (CHOL) and Triglyceride (TRIG) Concentrations (Females)				
	CHOL (mg/dL)		TRIG (mg/dL)	
Group	Week 4	Week 12	Week 4	Week 12
Females				
Vehicle Control				
Range[a]	[illegible]	[illegible]	[illegible]	[illegible]
Group Mean[a]	156.5	142.8	21.3	22.0
Pravastatin Control				
Incidence[b]	4/4	4/4	2/4	1/4
Range	[illegible]	[illegible]	[illegible]	[illegible]
Group Mean	104.5	96.8	17.0	16.3
Low-Dose Combination				
Incidence	4/4	4/4	4/4	4/4
Range	[illegible]	[illegible]	BL[c]- [illegible]	BL [illegible]
Group Mean	74.5	68.5	<12.5	<12.0
Low-Mid-Dose Combination				
Incidence	4/4	4/4	4/4	4/4
Range	[illegible]	[illegible]	BL	BL [illegible]
Group Mean	45.5	31.5	--	<10.0
High-Mid-Dose Combination				
Incidence	4/4	4/4	4/4	4/4
Range	[illegible]	[illegible]	BL [illegible]	BL [illegible]
Group Mean	43.3	35.3	<13.0	<18.0
High-Dose Combination				
Incidence	4/4	4/4	3/4	4/4
Range	[illegible]	[illegible]	BL [illegible]	BL [illegible]
Group Mean	33.8	24.8	<12.7	<12.7

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

c: Values below analyzer sensitivity (represented as "BL") are not used to calculate group mean.

Urinalysis: No significant drug related differences were observed

Organ weights: No significant drug related differences were observed in liver weights. Thymus weights were lower in low-mid, high-mid and high dose combinations (absolute, males 7.2, 5.1, 4.7, 3.5, 4.0, 4.4 g respectively, females 6.0, 7.8, 7.1, 4.3, 3.5, 3.9 g respectively), but sponsor considers this to be due to random biological variation in animals

Gross pathology: A small thymus was noted in ¼ F dogs (at low-mid dose), ¼ F+1/4 M dogs (high-mid dose), ¼ F dogs (high dose) groups and was attributed to physiologic variation in young dogs

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Histopathology: In both sexes histopath findings were observed in the liver (bile duct hyperplasia, of minimal to mild severity, minimal pigment accumulation in Kupffer cells, consistent with lipofuscin). In addition focal or capsular fibrosis in the liver was noted at almost all combination doses in 1/4 dogs vs none in control groups. Note that these findings were not observed in the vehicle or pravastatin controls. Bile duct hyperplasia characterized by hypertrophy of bile duct epithelium with extension of bile ducts into the liver parenchyma and occasionally proliferation or appearance of biliary epithlial cells separate from defined portal sites was observed. Toxicity was also observed in thymus (minimal to moderate atrophy charecterized by a loss of lymphocytes from the cortical region), testes (in ¼ dogs at HD combination vs ¼ dogs in pravastatin control), skin (histiocytoma, inflammation, hyperkeratosis/cysts in 2-3/4 dogs at 3/1 and 30/5 combination of SCH 58235/pravastatin,of minimal and/or mild severity) and lungs (accumulation of vacuolated alveolar macrophages of minimal severity in ¾ male dogs at HD combination vs ¼ male dogs in pravastatin control, mild hemorrhage in ¼ males at LD, and ¼ females at mid-High dose combination vs none in other groups). All the findings were considered incidental by the sponsor except the liver findings.

Special staining was requested by the pathologist in the HD combination dogs (2 males and 2 females). Hall's test for bilirubin was negative in all dogs. Perl's stain for iron was weakly positive in rare kupffer cells in ½ female dogs. One or more stains for lipofuscin (Schmorl's stain, Oil Red O, AFIP acid fast stain) were positive for all 4/4 dogs for scattered kupffer cells. Schmorl's stain also showed slight accumulation of lipofuscin in hepatocytes of 2/2 males and 1/2 female dogs, and sponsor states that it has been shown for other HMG-CoA reductase inhibitors like atorvastatin.

Table: Histopathologic findings in a 3-month dog toxicity study of SCH 58235 + pravastatin (3/1, 3/5, 30/5 30/10 mg/kg/day of SCH 58235/pravastatin)

	Males	Females
Thymic atrophy (min-moderate*)	0/4, 1/4, 2/4, 2/4, ¾, 2/4	¾, 2/4, 2/4, 4/4, 1/4, 3/4
Testicular degeneration of ST (minimal)	0/4, 1/4, 0/4, 0/4, 0/4, 1/4	
Liver, Bile duct hyperplasia (minimal to mild)	0/4, 0/4, 0/4, 0/4, 1/4, 2/4	0/4, 0/4, 0/4, ¼, 2/4, 0/4
Liver, pigment accumultion in Kupffer cells (minimal)	0/4, 0/4, 0/4, 2/4, ¾, 2/4	0/4, 0/4, 0/4, ¼, 2/4, 2/4
Liver, focal/capsular fibrosis (Minimal-mild)	0/4, 0/4, ¼, ¼, ¼, 0/4	0/4, 0/4, 0/4, 0/4, 2/4, 0/4

*-minimal to mild in controls and lo-mid combination treated groups but of moderate severity at HD

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

Test Article-Related Histopathologic Findings						
Group:	Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
SCH 58235 (mg/kg):	3		30		30	
Pravastatin (mg/kg)[a]:	5		5		10	
Sex:	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[b]					
Liver						
-Hyperplasia, bile duct						
Minimal		1/4	1/4	2/4	1/4	
Mild					1/4	
-Pigment accumulation, Kupffer cell						
Minimal	2/4	1/4	3/4	2/4	2/4	2/4
a: Doses expressed as pravastatin sodium						
b: Incidence = Number affected/Number examined						

Toxicokinetics: The plasma AUC values are shown in the Table. The co-administration of drug with pravastatin resulted in a 16-60% decrease in exposure to total, free and conjugated ezetimibe. However, sponsor states that this is due to variability and %CV (which varied between 30-100%) and accounted for the differences. They claim that exposure data from 6 to 12 month tox studies in dogs at 30 mg/kg/day appear to be in agreement with 30/30 mg/kg/day (SCH 58235/pravastatin group) rather that seen at 30/5 mg/kg/day of SCH 58235/pravastatin combination. The total SCH 58235 appears to accumulate at low dose combinations, but not at HD combiantion on day 29 vs day 0. Pravastatin expsosures did not significantly increase with ezetimibe doses, and slight increase (of 30% on day 0 or 29) seen with this combination in both sexes is due to %CV variation of 12-54%.

Table: Systemic exposures (AUC 0-24 hr) to total, conjugated and unconjugated SCH 58235 on day 0 and day 29 in a 3-month dog toxicity study of SCH 58235 + pravastatin:

Group	SCH 58235 (mg/kg)	Pravastatin (mg/kg)	Mean[a] SCH 58235 AUC(0-24 hr) (ng-hr/mL)					
			Total		Conjugated		Unconjugated	
			Day 0	Day 29	Day 0	Day 29	Day 0	Day 29
T1	3	1	625	1468	590	1398	34.4	70.5
T2	3	5	514	806	494	706	20.5	38.0[b]
T3	30	5	5947	4570	4599	4334	209[b]	236
T4	30	10	2210	2122	2091	1980	119	142
a: N=8, male and female data combined								
b: N=7; outliers excluded								

Table: Systemic exposures (AUC 0-24 hr) to pravastatin on day 0 and day 29 in a 3-

month dog toxicity study of SCH 58235 + pravastatin

Group	Pravastatin (mg/kg)	SCH 58235 (mg/kg)	Mean[a] Pravastatin AUC(0-24 hr) (ng-hr/mL)	
			Day 0	Day 29
T1	1	3	212	255
T2	5	3	1192	1581
T3	5	30	1756	1530
C2	10	0	1749	1796
T4	10	30	2271	2314
a: N=8, male and female data combined				

Toxicology summary: In a 3-month toxicity study of SCH 58235 (3, 3, 30, 30 mg/kg/day) + pravastatin (1 , 5, 5, 10 mg/kg/day) in dogs, the increases in AUC values of the total drug (SCH 58235) were not dose proportional and values were generally higher at lower doses on day 29 (1.5, 0.8, 4.6, 2.1 µg.h/ml at 3/1, 3/5, 30/5 30/10 mg/kg/day of SCH 58235/pravastatin respectively) vs on day 0 (0.63, 0.51, 6.0, 2.2 µg.h/ml respectively), suggesting accumulation of the drug over time at two losw dose combinations. The combination did not significantly effect the total (or conjugated and free) ezetimibe exposures. The combination also did not significantly increase the pravastatin expsoures and values were not significantly different on day 29 (0.3, 1.6, 1.5, 2.31 µg.h/ml vs pravastatin alone 1.8 µg.h/ml) than on day 0 (0.2, 1.2, 1.8, 2.27 µg.h/ml vs pravastatin alone 1.8 µg.h/ml). In both sexes, all combination doses produced increases in plasma ALT (by 2-18 fold vs pravastatin control). At MD & HD combinations, AST (by 1.5-2 fold vs pravastatin control) & AP levels (by 1.3-3 fold vs pravastatin control) were also increased in dogs. All combination doses produced significant decreases in cholesterol and TG levels, but produced decreases in absolute thymus weights, small thymus (in gross pathology findings) & increased incidences of thymus atrophy in male dogs. Additionally toxicity was observed in the liver (bile duct hyperplasia, pigment accumulation in kuffer cells consistent with lipofuscin) from the dose of 3/5 mg/kg/day of SCH 58235/pravastatin. Combination produced toxicity in the skin at 3/1 and 30/5mg/kg/day (histiosytoma, inflammation, hyperkeratosis in 0/8, 0/8, 5/5, ne, 4/4, 1/7 dogs respectively, ne=not examined) and in lungs at 30/10 mg/kg/day (accumulation of vacuolated alveolar macrophages with minimal severity in ¾ male dogs vs ¼ in pravastatin control). No NOAEL in this 3-month dog study could be established for the combination and was < 3/1 mg/kg/day of SCH 58235/pravastatin, as all doses increased liver enzyme ALT in dogs, and produced thymus and liver toxicity. Therefore NOAEL in dogs was <3 mg/kg/day of SCH 58235 + <1 mg/kg/day of pravastatin. Sponsor states that liver was the target organ of toxicity in this study and no effect level dose for this combination could be identified. In a 6-month dog study with ezetimibe monotherapy, NOAEL was 300 mg/kg/day. In a 2-year dog study, pravastatin at 25 mg/kg/day produced CNS toxicity (hemorrhage) on day 422.

3. A 3-month oral Dietary Toxicity Study of SCH-58235 in combination with atorvastatin in Rats (Study No. SN 99500)

Key study findings: Co-administration of SCH 58235 (males 15, 15, 250, 250

mg/kg/day, females 15, 50, 50, 150 mg/kg/day) + atorvastatin (10, 30, 30, 100 mg/kg/day) in rats increased the exposures to SCH 58235 and also to atorvastatin (& para-hydroxy atorvastatin) in females. The mid-high dose combinations decreased mean BW (males by 5-13%, females by 5-8%) and weight gains (males by 10-23%, females 12-17%). Two HD combinations produced increases in plasma liver enzymes (AST/AP levels by 2-3 fold), and SDH by 7 fold). All combination doses increased liver weights in females (by 23-28% compared to atorvastatin alone), and produced toxicity in the spleen. In males, mid and/or high dose combinations produced toxicity in the liver (hyperplasia, biliary hypertrophy, single cell necrosis), heart (mononuclear cell infiltration), prostate (cellular infiltration of mononuclear cells and macrophage uretheral), and testes (atrophy in ST, focal, mild to severe). The NOAEL in this 3-month rat study for SCH 58235/ atorvastatin was 15/30 mg/kg/day in males, and could not be identified in females was less than the lowest dose (<15/10 mg/kg/day) in females.

Study no: SN 99500
Volume #, and page #: 1.110, page 1 (reference 54)
Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 3/10//2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-05, atorvastatin 76590-003
Formulation/vehicle: [redacted] (meal)

Methods:
Species/strain: Sprague-Dawley rats/Crl:CD (SD)BR VAF/PLUS
#/sex/group or time point (main study):10/sex/dose
Satellite groups used for toxicokinetics or recovery: Additional 36/sex/dose for TK study.
Age: Approximately 6 weeks of age
Weight: Males 163-183 g, females 123-144 g.
Doses employed: SCH 58235, males 15, 15, 250, 250 mg/kg/day, females 15, 15, 50, 50 mg/kg/day) + atorvastatin calcium 10, 30, 30, 100 mg/kg/day, controls received vehicle or 100 mg/kg/day atorvastatin.
Route of administration: SCH 58235 dietary, atorvastatin by oral gavage

Parameters and endpoints evaluated:

Three-Month Oral Toxicity and Toxicokinetic Study of SCH 58235 (Diet) Co-Administered with Atorvastatin (Gavage) In Rats (SN 99500): **Study Design**

Group	Test/Control Article	No. of Rats/Sex		SCH 58235 Estimated Total Daily Dose (mg/kg)		Atorvastatin (SCH 412387)			Duration of Dosing (Days)
		Toxicity Portion	Satellite Portion	M	F	Total Daily Dose[b] (mg/kg)	Dose Volume (ml/kg)	Dose Conc. (mg/ml)	
C1	Vehicle Control (Feed/ 0.4% Methylcellulose)	10	0	0	0	0	5	0	91-93
C2	Atorvastatin Control	10	36[a]	0	0	100	5	20	91-93
T1	Low-Dose Combination	10	36[a]	15	15	10	5	2	91-93
T2	Low-Mid-Dose Combination	10	36[a]	15	15	30	5	6	91-93
T3	High-Mid-Dose Combination	10	36[a]	250	50	30	5	6	91-93
T4	High-Dose Combination	10	36[a]	250	50	100	5	20	91-93

a: These animals were evaluated for toxicokinetic parameters only.

b: Doses of atorvastatin are expressed as the calcium trihydrate salt. When expressed as the free acid, daily doses of atorvastatin were 92, 9.2, 27.6, 27.6 and 92 mg/kg for Groups C2, T1, T2, T3 and T4, respectively.

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Parameters and endpoints evaluated continued:

Three-Month Oral Toxicity and Toxicokinetic Study of SCH 58235 (Diet) Co-Administered with Atorvastatin (Gavage) in Rats (SN 99500): Observations and Measurements

Investigation	Performed	Investigation	Performed
Viability	Daily beginning Week -1		
Clinical Observations	Daily beginning Week -1	Coagulation	Week 14
Body Weight	Weekly beginning Week -1; and days of randomization and terminal sacrifice	Serum Chemistry	Weeks 4 and 12
Food Consumption	Weekly beginning Week -1	Urinalysis/Urine Chemistry	Weeks 4 and 12
SCH 58235 Intake (Calculated)	Weekly	Organ Weights	Yes
Ophthalmoscopic Examinations	Once pretest, Weeks 5 and 13	Necropsy (Macroscopic Observations)	Yes
Plasma Analysis for SCH 58235, Atorvastatin and Metabolites	Days 0 and 30 (1, 2, 4, 6, 8 and 24 hrs after atorvastatin administration)	Histopathology (Microscopic Observations)	Yes[a]
Hematology	Weeks 4 and 12		

a: The following organs/tissues from toxicity portion rats were examined microscopically: all organs/tissues collected from rats in the vehicle control, atorvastatin control and high-dose combination groups; all collected gross findings; liver from rats in the low-, low-mid- and high-mid-dose combination groups; and spleen from females in the low-, low-mid- and high-mid-dose combination groups.

Organs weighed: Organs weighed are listed in the Table below

Table. Tissues collected for organ weights in the 3-month rat tox study of SCH 58235 + atorvastatin

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

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

Histopathology: This was performed at sacrifice in the vehicle & atorvastatin controls and high dose combination animals, listed below in the histopathology Table. Liver and spleen were identified as target organs of toxicity by the pathologist and were examined in all other dose groups

Table. Tissues collected for histopath evaluation in the 3-month rat tox study of SCH 58235 + atorvastatin

Tissues Collected	
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[a]	Seminal Vesicles
Brain	Skeletal Muscle - Biceps Femoris, Diaphragm, Intercostal and Sublumbar[d]
Epididymides	Skin
Esophagus	Small Intestine – Duodenum, Jejunum, Ileum
Eyes	Spinal Cord – Thoracolumbar
Harderian Glands	Spleen
Head[b]	Stomach
Heart	Testes
Kidneys	Thymus
Large Intestine – Cecum and Colon	Thyroid Gland
Liver	Tongue
Lungs (plus Bronchi)	Trachea
Lymph Nodes (Mandibular and Mesenteric)	Urinary Bladder
Mammary Gland[c]	Uterus (plus Cervix)
Ovaries	Vagina
Pancreas	Animal Identification[b]
Parathyroid Gland(s)[c]	

a: Bone marrow smears were prepared for all toxicity portion rats but were not evaluated because it was not warranted by changes in the peripheral blood.
b: Collected but not processed
c: Examined histopathologically when present in routine section
d: The subtumbar muscles were collected in situ with a segment of the lumbar vertebral column. The left lateral sublumbar muscles were prepared for histopathology.

Toxicokinetics: Days 0 and 30, at 1, 2, 6, 8, and 24 hrs. Conjugated and free drug was measured.

Results:
Mortality: None

Clinical signs: At a HD combination, one female had persistent hindlimb and/or (1/10 of rats) general alopecia and abdominal distension. Sponsor states that other observations included chromorhinorrhea, chromodacryrorrhea, alopecia, abnormal respiration abnormal stool, abnormal hair coat, and urogenital staining (no summary Table was provided), these were present in all groups or with no dose response relationship.

Body weights: In males, the mean BW were decreased with all combinations by 4-14% and weight gain by 7-24% vs atorvastatin controls (6% and 10% respectively). In

females, BW and weight gains were decreased by 4-7% and 10-15% respectively. In week 13 (day 90) mean Bw gains in males were 247, 223, 230, 211, 208, 188 g in vehicle control, atorvastatin control, and at LD, MD, mid-HD, & HD combinations respectively. In females these values were 109, 105, 98, 99, 99, 93 g respectively.

Table: BW and BW gains in a 3-month rat toxicity study of SCH 58235 + atorvastatin

Table 5 Summary of Body Weight Data - % Difference												
Group:	C1		C2		T1		T2		T3		T4	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Interval	% Difference in Mean Absolute Body Weight Values (Relative to Vehicle Controls)											
Day 90	-	-	-5.86	-2.23	-4.09	-5.04	-8.10	-3.76	-9.96	-4.09	-13.9	-6.64
(N)	10	10	10	10	10	10	10	10	10	10	10	10
	% Difference in Mean Body Weight Gains (Relative to Vehicle Controls)											
Day 0 – Day 90	-	-	-10	-3	-7	-10	-14	-9	-16	-9	-24	-15
(N)	10	10	10	10	10	10	10	10	10	10	10	10
(N) = Number of surviving rats at Day 90												

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

Drug Intake: The mean ezetimibe intake was within 2% of intended doses

Ophthalmoscopy: Sponsor states that no drug related effects were observed, and refers to appendix 5 for ophthalmologist's report. However, in appendix 5, there was only mention of lesions, that were identified in three rats i.e. # 54M (which had iritis in the right eye), # 1M (had focal retinopathy in the left eye), and 309M (focus of hemorrhage nasal to disc in the left eye). It is unknown if these rats were from control or treated groups. Pathologist's report states that these lesions are not likely to be caused by exposure to an occular toxicant. No other data are provided in this appendix 5 report.

Hematology/Coagulation: No significant drug related differences were observed

Clinical chemistry: In HD combination groups, alanine aminotransferase (ALT, minimal), aspartate aminotransferase (AST, min-mild), sorbitol dehydrogenase (SDH, mild), AP (min-mild), and albumin/globulin ratios were increased, while globulin conc were lower. In week 4 in males ALT values were higher at HD combination (44, 61, 55, 65, 56, 100 IU/L respectively), in week 12 in males ALT values were not higher at HD combination (44, 59, 47, 50, 47, 53 IU/L respectively). In week 12, AST (118, 169, 126, 138, 159, 201 IU/L respectively), AP (161, 205, 209, 212, 316, 336 IU/L respectively), and SDH (4.4, 8.8, 5.1, 7.1, 8.9, 9.5 IU/L respectively) values were increased at two high combination doses. Cholesterol in week 12 in males was (49, 47, 44, 42, 52, 29 mg/dl respectively, in females these values in week 12 were not different (52, 64, 59, 57, 57, 52 mg/dl respectively).

Table: Changes in serum chemistry in a 3-month rat toxicity study of SCH 58235 + atorvastatin

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Test Article-Related Changes in Mean Serum Chemistry Parameters							
		Group					
Finding (Units)	Week	Vehicle Control	Atorvastatin Control	Low-Dose Combination	Low-Mid-Dose Combination	High-Mid-Dose Combination	High-Dose Combination
Male Values							
ALT (IU/L)	4	44.2					100.4
AST (IU/L)	4	118.4	152.1		196.2	146.9	342.7
	12	118.1	169.2		137.9	159.3	201.4
SDH (IU/L)	4	5					36
AP (IU/L)	4	322.5			498.3	605.7	739.6
	12	160.6				316.4	335.7
GLOB (g/dL)	4	2.72	2.44	2.30	2.28	2.25	2.00
	12	3.12	2.70	2.67	2.61	2.65	2.57
A/G	4	1.48	1.65	1.75	1.76	1.78	1.98
	12	1.27	1.47	1.47	1.57	1.52	1.57
CHOL (mg/dL)	4	52.5					38.0
	12	49.2					28.9
TRIG (mg/dL)	4	117.3	58.5	43.2	21.6	33.8	22.8
	12	105.5	63.2	42.0	21.9	29.8	19.8
Female Values							
AST (IU/L)	4	108.7					168.3
AP (IU/L)	4	234.1					343.2
	12	121.5					271.4
GLOB (g/dL)	4	2.83					2.42
A/G	4	1.61					1.81
CHOL (mg/dL)	12	51.7					46.1
TRIG (mg/dL)	4	57.0	33.4	29.2	21.9	22.9	20.2
	12	42.2	31.9	26.3	21.7	20.8	17.4

Urinalysis: No significant drug related differences were observed

Organ weights: Liver weights (absolute and relative) in females increased at all combination doses by 23-28% and relative by 29-35%. Absolute liver weights in females were 6.3, 6.1, 7.7, 7.7, 8.1, 7.9 g respectively. In males these did not change significantly (10.1, 8.9, 9.5, 9.6, 10.0, 9.5 g respectively).

Table: Liver weights changes in females in a 3-month rat toxicity study of SCH 58235 + atorvastatin

Test Article-Related Organ Weight Changes: Percent Difference from Vehicle Control Mean								
Group:	Low-Dose Combination		Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F
Organ	Percent Difference from Vehicle Control Mean (%)							
Liver								
- Absolute weight		+23		+23		+28		+25
- Relative weight[a]		+29		+32		+33		+35
a: Relative to body weight								

Gross pathology: No significant drug related differences were observed

Histopathology: In both sexes histopath findings were observed in the liver (biliary hyperplasia, periportal hepatocellular hypertrophy, single cell necrosis and focus of cellular alteration). Sponsor states that similar liver toxicity is also seen with another statin such as lovastatin. A HD combination also showed toxicity in the heart in males (mononuclear cell infiltration of min severity in 2/10 rats vs 1/10 in atorvastatin control), prostate (cellular infiltration of mononuclear cells, or macrophage, 4/10 rats vs 1/10 in the vehicle or atorvastatin control), testes (atrophy in ST, focal, mild to severe at two HD combinations in 1/1 and 1/10 rats respectively vs none in vehicle or atorvastatin controls). In females toxicity in spleen was observed at all combination doses (pigment accumulation, hemosiderin, minimal in 0/10, 0/10, 2/10, 2/10, 2/10, 1/10 rats respectively, hematopoiesis minimal in 0/10, 0/10, 1/10, 0/10, 1/10, 0/10 rats, and congestion in 3/10 rats at HD combination vs none in controls). At two HD combinations, liver findings were of higher severity in rats compared to atorvastatin controls.

Table: Histopathologic findings in a 3-month rat toxicity study of SCH 58235 + atorvastatin

Test Article-Related Histopathologic Findings										
Group:	Atorvastatin Control		Low-Dose Combination		Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[a]									
Liver										
-Hypertrophy, periportal, hepatocellular										
minimal	9/10	4/10	1/10	5/10	9/10	10/10	7/10	10/10	6/10	4/10
mild	1/10						2/10		4/10	5/10
moderate										1/10
-Hyperplasia, biliary										
minimal	9/10	10/10	3/10	10/10	10/10	10/10	9/10	10/10	7/10	5/10
mild	1/10						1/10		3/10	4/10
moderate										1/10
-Single cell necrosis, hepatocellular										
minimal	3/10	1/10					1/10		6/10	6/10
-Focus(i) of cellular alteration										
minimal	3/10		2/10	2/10	1/10	1/10	5/10		5/10	
a: Incidence = Number affected/Number examined										

Toxicokinetics: The plasma AUC values are shown in the Table. The co-administration of drug with atorvastatin at a high dose (30-100 mg/kg/day) resulted in an increased exposure to total and conjugated ezetimibe (by 1.3 fold in males and 1.6 fold in females). There was accumulation of total drug at all doses (in males by 1.4 fold and in females by up to 3 fold) in week 5 vs day 0. There was no consistent trend in atorvastatin

expsosures when co-administered with ezetimibe, which may be due to plasma variability in atorvastatin and atorvastatin metabolite concentrations. Maximal conc of atorvastatin were seen at 1-2 hrs. In week 5, the exposures to atorvastatin, ortho-atorvastatin, and para-hydroxy-atorvastatin increased with increasing atorvastatin doses as seen in the Table below.

Table: Systemic exposures (AUC 0-24 hr) to total, conjugated and unconjugated SCH 58235 on day 0 and week 5 in a 3-month rat toxicity study of SCH 58235 + atorvastatin:

Gender	Male				Female			
Dose[a]	15/10	15/30	250/30	250/100	15/10	15/30	50/30	50/100
	Total SCH 58235 AUC (0-24 hr) (ng-hr/mL)							
Day 0	1937	1735	10796	14158	1852	2043	4010	6269
Week 5	2038	2140	10983	19613	2874	2272	12752	16594
	Conjugated SCH 58235 AUC (0-24 hr) (ng-hr/mL)							
Day 0	1932	1728	10756	14047	1839	2034	4003	6246
Week 5	2030	2125	10923	19531	2859	2272	12673	16574
	Unconjugated SCH 58235 AUC (tf) (ng-hr/mL)							
Day 0	(-[b])	(-[b])	70.1	111	(-[b])	(-[b])	(-[b])	23.4
Week 5	(-[b])	14.9	61.1	83.0	(-[b])	(-[b])	79.2	10.4

a: mg/kg SCH 58235/mg/kg Atorvastatin Calcium (10, 30 and 100 mg/kg atorvastatin calcium trihydrate correspond to 9.2, 27.6 and 92 mg/kg atorvastatin free acid, respectively)

b: Not determined; data were not amendable for toxicokinetic analysis

Table: Systemic exposures (AUC 0-24 hr) to atorvastatin on day 0 and week 5 in a 3-month rat toxicity study of SCH 58235 + atorvastatin

Gender	Male					Female				
Dose[a]	15/10	15/30	250/30	250/100	0/100	15/10	15/30	50/30	50/100	0/100
	Atorvastatin AUC (0-24 hr) (ng-hr/mL)									
Day 0	68.8	244	189	2915	2991	58.9	234	263	2971	6928
Week 5	62.0	222	222	1399	2363	63.0	153	223	2939	1607
	Ortho-hydroxy Atorvastatin AUC (0-24 hr) (ng-hr/mL)									
Day 0	126	579	525	4726	2821	77.2	357	441	3018	4424
Week 5	57.9	253	296	1219	2951	35.2	129	119	1389	1191
	Para-hydroxy Atorvastatin AUC (tf) (ng-hr/mL)									
Day 0	8.04	32.4	29.6	482	322	4.75	31.8	50.0	557	856
Week 5	3.04	19.8	43.6	286	328	6.60	33.5	22.8	573	234

a: mg/kg SCH 58235/mg/kg Atorvastatin Calcium (10, 30 and 100 mg/kg atorvastatin calcium trihydrate correspond to 9.2, 27.6 and 92 mg/kg atorvastatin free acid, respectively)

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Table: Systemic exposures (AUC 0-24 hr) to atorvastatin and its metabolites in a 3-month rat toxicity study of SCH 58235 + atorvastatin

	Increase in exposure when atorvastatin calcium dose increased from 10 to 30 mg/kg (3.0-fold) in combination with 15 mg/kg SCH 58235		Increase in exposure when atorvastatin calcium dose increase from 30 to 100 mg/kg (3.3-fold) in combination with 250 (males) or 50 (females) mg/kg SCH 58235	
	Males	Females	Males	Females
Atorvastatin [AUC(0-24 hr)]	3.6-fold	2.4-fold	6.3-fold	13-fold
Ortho-hydroxy atorvastatin [AUC(0-24 hr)]	4.4-fold	3.7-fold	4.1-fold	12-fold
Para-hydroxy atorvastatin [AUC(tf)]	6.5-fold	5.1-fold	6.6-fold	25-fold

Toxicology summary: In a 3-month toxicity study of SCH 58235 (males 15, 15, 250, 250 mg/kg/day, females 15, 15, 50, 50 mg/kg/day) + atorvastatin (10, 30, 30, 100 mg/kg/day) in rats, the increases in AUC values of the total drug (SCH 58235) were not dose proportional and values were generally higher in week 5 (males 2.0, 2.1, 11.0, 19.6 µg.h/ml, females 2.9, 2.3, 12.8, 16.6 µg.h/ml respectively) vs on day 0 (males 1.9, 1.7, 10.8, 14.2 µg.h/ml, females 1.9, 2.0, 4.0, 6.3 µg.h/ml respectively), suggesting accumulation of the drug over time. The combination increased the total and conjugated ezetimibe exposures. However, there were no consistent increases in atorvastatin (or metabolite) exposures with the combination, and at HD combination atorvastatin exposures in week 5 were lower in males (males/females 1.4/2.9 µg.h/ml, vs 2.4/1.6 µg.h/ml with atorvastatin alone, on day 0 these values were 2.92/2.97 µg.h/ml vs 3.0/6.9 µg.h/ml with atorvastatin alone), suggesting that the drug may decrease atorvastatin exposures in males, but increase these in females in week 5. The MD-HD combination doses produced decreases in mean BW (by 9-14%) and weight gains (by 14-24%) in males, and similar decreases in weights (4-7%) and weight gains (9-15%) in females. Mid and HD combinations not only produced increases in plasma AST/ AP levels (by up to 2-3 fold), and SDH levels (by up to 7 fold), but also produced toxicity in the liver (liver weights were increased in females at all doses, and produced histopath findings in both sexes of biliary hyperplasia, hepatocellular hypertrophy and single cell necrosis with increased severity). Since all doses produced increases in liver weights in females and toxicity in the spleen, NOAEL could not be identified in females and was <15 mg/kg/day of SCH 58235 + <10 mg/kg/day of atorvastatin . In males, NOAEL was 15 mg/kg/day of SCH 58235 + 30 mg/kg/day of atorvastatin, as higher doses not only produced toxicity in liver but also in the heart, testes and prostate.

4. A 3-month oral Dietary Toxicity Study of SCH-58235 in combination with atorvastatin (SCH 412387) in dogs (Study No. SN 99501)

Key study findings: Co-administration of SCH 58235 (0.3, 3, 3, 30 mg/kg/day) + atorvastatin (1, 1, 10, 10 mg/kg/day) in dogs did not significantly alter the exposures to SCH 58235 or to atorvastatin, suggesting no metabolic interaction between two drugs. However, all combination doses (including the lowest dose) increased plasma ALT

levels. The higher combination doses (from the doses of 3/10 mg/kg/day of SCH 58235/atorvastatin) increased liver AST & AP levels, decreased TP and albumin levels, and decreased liver weights in male dogs by 21-26%. Except the lowest combination dose, all doses produced histopath changes in the liver (bile duct hyperplasia, kuffer cell hypertrophy, increased eosinophilia). Additionally, the HD combination produced toxicity in the heart (hemorrhage in ¼ dogs vs none in other groups) and lungs (1/4 dogs had hemorrhage or fibrosis). The NOAEL in this 3-month dog study could not be established, as the lowest combination dose increased liver enzymes, and higher doses not only produced further synergistic increases in liver ALT levels, but also histopath findings in the liver. The NOAEL may be < 0.3 mg/kg/day of SCH 58235 + 1 mg/kg/day of atorvastatin.

Study no: SN 99501
Volume #, and page #: 1.134-135, page 1 (reference 61)
Conducting laboratory and location: Schering-Plough Research Institute, Lafayette, NJ.
Date of study initiation: 3/10/2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug lot #, and % purity: 98-58235-X-01, atorvastatin calcium trihydrate salt (SCH 412387) 76590-003
Formulation/vehicle: 0.4% (w/v) aqueous methylcellulose

Methods:
Species/strain: Beagle dogs
#/sex/group or time point (main study):4/sex/dose

Age: Approximately 4-7 months of age
Weight: Males 4.9-10.6 kg, females 4.2-9.3 kg.
Doses employed: SCH 58235, 0.3, 3, 3, 30 mg/kg/day + atorvastatin (SCH 412387) 1, 1, 10, 10 mg/kg/day, controls received vehicle (0.4% w/v aqueous methylcellulose) or 10 mg/kg/day of atorvastatin.
Route of administration: SCH 58235 by oral gavage, atorvastatin by oral gavage

Parameters and endpoints evaluated:

Three-Month Oral (Gavage) Toxicity and Toxicokinetic Study of SCH 58235 Co-Administered with Atorvastatin (SCH 412387) in Beagle Dogs (SN 99501): Study Design						
Group	Test/Control Article	No. of Dogs/Sex	Total Daily Dose (mg/kg)[a]	Dose Volume (ml/kg)	Dose Conc. (mg/ml)[a]	Duration of Dosing (Days)
C1	Vehicle Control: Methylcellulose	4	0	5	0	91 to 93
C2	Atorvastatin Control: Methylcellulose Atorvastatin	4	0 10	2.5 2.5	0 4.0	91 to 93
T1	Low-Dose Combination: SCH 58235 Atorvastatin	4	0.3 1	2.5 2.5	0.12 0.4	91 to 93
T2	Low-Mid-Dose Combination: SCH 58235 Atorvastatin	4	3 1	2.5 2.5	1.2 0.4	91 to 93
T3	High-Mid Dose Combination: SCH 58235 Atorvastatin	4	3 10	2.5 2.5	1.2 4.0	91 to 93
T4	High-Dose Combination: SCH 58235 Atorvastatin	4	30 10	2.5 2.5	12.0 4.0	91 to 93
a: Doses of atorvastatin are expressed as the calcium trihydrate salt. When expressed as the free acid, daily doses were 0.92 and 9.2 mg/kg for 1 and 10 mg/kg, respectively, of atorvastatin calcium trihydrate.						

Parameters and endpoints evaluated continued

Three-Month Oral (Gavage) Toxicity and Toxicokinetic Study of SCH 58235 Co-Administered with Atorvastatin (SCH 412387) in Beagle Dogs (SN 99501): Observations and Measurements			
Investigation	Performed	Investigation	Performed
Viability	Daily beginning Week -4	Hematology	Twice pretest, Weeks 3/4 and 13
Clinical Observations	Daily beginning Week -1	Coagulation	Twice pretest, Weeks 3/4 and 13
Body Weight	Weekly (± 1 day) beginning Week -4 and days of randomization and terminal sacrifice	Serum Chemistry	Twice pretest, Weeks 3/4 and 13
Food Consumption (Estimated)	Daily beginning Week -1	Urinalysis/Urine Chemistry	Twice pretest, Weeks 3/4 and 13
Ophthalmoscopic Examinations	Once pretest, Weeks 5, 8 and 12	Organ Weights	Yes
General Veterinary Examinations	Once pretest, Weeks 5 and 13	Necropsy (Macroscopic Observations)	Yes
Physical Examinations (body temperature, respiratory and heart rates, blood pressure) and Electrocardiograms	Twice pretest, Weeks 4 and 12	Histopathology (Microscopic Observations)	Yes[a]
Plasma Analysis for SCH 58235, Atorvastatin and Metabolites	Days 0 and 30 (1, 2, 4, 6, 8 and 24 hrs postdose)	Transmission Electron Microscopy (Ultrastructural Observations)	Yes[b]
a: The following organs/tissues were examined microscopically: all organs/tissues collected from dogs in the vehicle control, atorvastatin control and high-dose combination groups; all collected gross findings; and liver from dogs in all other dose groups. b: Sections of formalin-fixed liver were examined from two males and two females in the vehicle control group, two males and two females in the atorvastatin control group, one female in the high-mid-dose combination group, and one male and two females in the high-dose combination group.			

Organs weighed: Organs weighed are listed in the Table below

Table. Tissues collected for organ weights in the 3-month dog tox study of SCH 58235 + atorvastatin.

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

Histopathology: This was performed at sacrifice in the vehicle & atorvastatin controls and high dose combination animals, listed below in the histopathology Table. Liver was identified as target organs of toxicity by the pathologist and was examined in all other dose groups. Liver sections (formalin-fixed) were evaluated for ultrastructural changes by transmission electron microscopy.

Table. Tissues collected for histopath evaluation in the 3-month dog tox study of SCH 58235 + atorvastatin

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

a: Bone marrow smears were prepared for all dogs sacrificed at the scheduled necropsy but were not evaluated because it was not warranted by changes in the peripheral blood.
b: Collected with the seventh and eighth ribs attached
c: Collected left ventral cervical muscle
d: Collected but not processed

Table. Liver Tissues from atorvastatin group, and at LD, MD & HD combinations were subjected to special stains in the 3-month dog tox study of SCH 58235 + atorvastatin

Special Stains			
Special Stains	Tissue	Dose Group	Animal No./Sex
Hall's Method for Bilirubin,[a] Perl's Method for Iron, Oil Red O Staining Method for Fats/Lipids, Periodic Acid-Schiff Reaction (PAS, diastase resistant), and AFIP Method for Lipofuscin	Liver	Atorvastatin Control	14F
		Low-Dose Combination	23F
		High-Mid-Dose Combination	35M
		High-Dose Combination	47F
a: Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York: McGraw-Hill, Inc., 1968:174.			

Toxicokinetics: Days 0 and 30, conjugated and unconjugated drug was measured.

Results:
Mortality: None

Clinical signs: None

Body weights: In males, a decrease in BW gain was noted in all combination groups (0/4, 0/4, ¼, ¼, 0/4. 2/4 at 0, atorvastatin control, and at 0.3/1, 3/1, 3/10, 30/10 mg/kg/day of SCH 58235 +atorvastatin respectively). Sponsor states these dogs had a decrease in BW gain during pre-study period which continued during the study period and may have been due to low daily ration of food given to dogs (due to SOP specified range) at this young age (4-7 months of age). The decrease in absolute BW gain in above groups was 0.6, 0.1, 1.0 & 0.3 kg respectively on day 91, compared to day 0. The mean body weight gains in male dogs on day 91 were 9.7, 9.4, 9.3, 9.3, 10.0, 8.6 kg respectively, in females these values were 7.2, 6.6, 6.7, 6.0, 6.7, 6.9 kg respectively.

Note that in studies with rats, BW and BW gins were decreased with all combinations of the drug + pravastatin (by 5-13% and weight gain by 10-23% compared to pravastatin or vehicle controls). Decreases in BW were also seen with the drug + lovastatin and drug + simvastatin in dogs.

Food consumption: No significant drug related differences were observed

Ophthalmoscopy: At two HD combinations 3 dogs (1M+1F/8 dogs, 1M/4 dogs respectively) had multiple grey foci fo the fundus with indistinct margins and bilateral peripheral zones of dullness in the tapetum accompanied by a wide band of hyperreflectivity (that is these dogs had subtle degrees of nyctalopia or night blindness). These findings were then checked in atorvastatin control dogs and 1M and 1F had similar findings in this group. Pathologist report states that these are not likely to be caused by ocular toxicant. Sponsor states that no drug related effects were observed, these may be hereditary, and are attributed to background genetics in the closed beagle research colony.

Physical exam/ECG: One male dog at low-mid dose had thin body condition during week 13 due to 13% decrease in BW compared to day 0. This finding is considered incidental. No significant drug related differences were observed on mean body temperatures, respiration rates, heart rates, & blood pressures. ECG findings showed that all dogs sustained sinus rhythms, and these and all ECG findings were considered incidental and normal variants in beagle dogs.

Hematology/Coagulation: No significant drug related differences were observed

Clinical chemistry: In all combination groups, alanine aminotransferase (ALT, minimal-moderate) was increased and at two HD combinations these were increased in a synergistic manner. At mid-high dose combinations, aspartate aminotransferase (AST) and AP levels were also increased. At two HD combinations decreased total protein and albumin were observed. Sponsor states that total calcium is a measurement of free ionized and protein bound will therefore decline seconday to decreases in serum albumin levels. The mean levels of total calcium in animals were not provided and appeared lower at HD combinations, and sponsor states that these are lower due to decreases in serum protein and albumin concentrations, but free is tightly regulated by hormonal controls, and its concentation is not altered by albumin, but they have not measured the free calcium here.

Table. Serum liver enzymes (ALT, AST and AP levels), and cholesterol, TG, TP and albumin levels in 5 groups of dogs (at 0, atorvastatin 10 mg/kg/day, and at 0.3/1, 3/1, 3/10, 30/10 mg/kg/day of SCH 58235/atorvastatin respectively)

Week 12	Males	Females
ALT (IU/L)	36, 60, 84, 108, 1048, 2157	40, 43, 86, 173, 1934, 1920
AST (IU/L)	38, 35, 48, 46, 104, 158	44, 43, 41, 45, 145, 134
AP (IU/L)	68, 93, 86, 81, 322, 345	95, 103, 112, 118, 389, 355
Cholesterol (mg/dl)	141, 90, 71, 69, 13, 11	156,82, 58, 55, 12, 13
TG (mg/dl)	17, 12, 10, 12, nc, nc	ndp
TP (g/dl)	5.7, 5.6, 5.2, 5.5, 4.7, 5.0	5.6, 5.4, 5.5, 5.4, 5.0, 4.8
Albumin (g/dl)	2.8, 2.9, 2.7, 2.9, 2.4, 2.4	3.0, 3.0, 2.9, 2.9, 2.6, 2.6

nc= not calculated
ndp=no mean data provided

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Sponsor's Table: Changes in liver enzymes in a 3-month dog toxicity study of SCH 58235 + atorvastatin in males

Incidence, Range of Values (Weeks 3 and 13) and Group Means for Increased Serum ALT, AST and AP Activities: Males

Group	↑ ALT (IU/L)		↑ AST (IU/L)		↑ AP (IU/L)	
	Week 3	Week 13	Week 3	Week 13	Week 3	Week 13
Males						
Vehicle Control						
Range[a]						
Group Mean[a]	32.5	36.3	33.8	37.5	95.0	68.3
Atorvastatin Control						
Incidence[b]	1/4	2/4				
Range						
Group Mean	41.3	60.3				
Low-Dose Combination						
Incidence	3/4	2/4				
Range						
Group Mean	98.8	83.8				
Low-Mid-Dose Combination						
Incidence	4/4	4/4				
Range						
Group Mean	106.3	108.0				
High-Mid-Dose Combination						
Incidence	4/4	4/4		4/4	2/4	3/4
Range						
Group Mean	391.5	1047.5		104.0	216.0	321.8
High-Dose Combination						
Incidence	4/4	4/4	1/4	4/4	3/4	4/4
Range						
Group Mean	1133.5	2157.3	67.0	158.0	198.8	344.8

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

Sponsor's Table: Changes in liver enzymes in a 3-month dog toxicity study of SCH 58235 + atorvastatin in females

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Incidence, Range of Values (Weeks 3 and 13) and Group Means for Increased Serum ALT, AST and AP Activities: Females

Group	↑ ALT (IU/L)		↑ AST (IU/L)		↑ AP (IU/L)	
	Week 3	Week 13	Week 3	Week 13	Week 3	Week 13
Females						
Vehicle Control						
Range[a]						
Group Mean[a]	29.5	39.8	34.3	44.0	149.3	94.5
Low-Dose Combination						
Incidence	2/4	2/4				
Range						
Group Mean	112.8	85.8				
Low-Mid-Dose Combination						
Incidence	4/4	4/4				
Range						
Group Mean	156.3	172.5				
High-Mid-Dose Combination						
Incidence	4/4	4/4	2/4	4/4	2/4	4/4
Range						
Group Mean	990.3	1934.0	81.8	144.5	224.0	389.3
High-Dose Combination						
Incidence	4/4	4/4	1/4	4/4	3/4	4/4
Range						
Group Mean	657.0	1919.5	52.8	134.0	220.5	355.0

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

Sponsor's Table: Changes in TP and albumin levels in a 3-month dog toxicity study of SCH 58235 + atorvastatin in males + females

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Incidence, Range of Values (Weeks 3 and 13) and Group Means for Decreased Serum Total Protein (TP) and Albumin (ALB) Concentrations				
	↓ TP (g/dL)		↓ ALB (g/dL)	
Group	Week 3	Week 13	Week 3	Week 13
Males				
Vehicle Control				
Range[a]				
Group Mean[a]	5.45	5.70	2.90	2.83
Low-Mid-Dose Combination				
Incidence[b]				1/4
Range				-
Group Mean				2.88
High-Mid-Dose Combination				
Incidence	2/4	3/4	2/4	3/4
Range				
Group Mean	4.73	4.73	2.70	2.40
High-Dose Combination				
Incidence	2/4	2/4	1/4	3/4
Range				
Group Mean	4.95	5.08	2.78	2.40
Females				
Vehicle Control				
Range				
Group Mean	5.20	5.60	3.03	3.00
High-Mid-Dose Combination				
Incidence	2/4	3/4	2/4	4/4
Range				
Group Mean	5.00	4.98	2.90	2.55
High-Dose Combination				
Incidence	4/4	4/4	2/4	4/4
Range				
Group Mean	4.90	4.78	2.90	2.58

a: Group mean is calculated from all individuals within a group. Range represents only those individual animals with abnormal values (except for the vehicle control group where the range comprises all vehicle control values).

b: Incidence = Number affected/Number examined

Urinalysis: No significant drug related differences were observed

Organ weights: Mean absolute and relative liver weights were decreased in male dogs at two high dose combinations. The mean data on liver weights in males were missing from sponsor's Table 17, and were only shown for treatment groups in females (the controls were missing in females).

Sponsor's Table: Changes in liver tissue weights in a 3-month dog toxicity study of SCH 58235 + atorvastatin

Test Article-Related Organ Weight Changes				
Group:	High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F
Organ	Percent Difference from Vehicle Control Mean (%)			
Liver				
-Absolute weight	-21.4		-26.1	
-Relative weight[a]	-23.5		-16.2	

a: Relative to body weight

Gross pathology: Liver in ¼ male dogs at HD combination, and two female dogs (¼ each at 0.3/1 & 3/1 mg/kg/day of SCH 58235/atorvastatin) had altered surface (irregular liver), or accentuated lobular pattern in liver, or mottled red tan discoloration of liver.

Histopathology: In both sexes histopath findings were observed in the liver. These included minimal increase in cytoplasmic eosinophilia of hepatocytes, minimal to mild bile duct hyperplasia, minimal to mild kupffer cell hypertrophy with increased pigment accumulation consistent with lipofuscin, & minimal hypertrophy of periportal hepatocytes. Limited ultrastructural evaluation indicated that periportal hypertrophy correlated with mild to moderate proliferation of the smooth endoplasmic reticulum. Increase in cytoplasmic eosinophilia was associated with mildly decreased cytoplasmic glycogen. Mild hepatocellular lipofuscin accumulation was identified in the absence of microscopic correlate in the HD combination group. Sponsor states that no NOAEL could be identified due to liver toxicity, but findings seen are not unique with this combination and are seen in dogs with other HMG-CoA reductase inhibitors.

Special stains requested by the pathologist were performed in the atorvastatin controls and in LD, MD & HD combination dogs (n=1/group). These special stains indicated that hypertrophy and cytoplasmic accumulation of pigment in kupffer cells was due to lipofuscinosis. Positive staining for lipofuscin was identified in all dogs in above groups using diastase-resisitent PAS, Oil Red O, and AFIP lipofuscin stains. Kupffer cells in all above 4 dogs (in four groups) did not react with Hall's stain (i.e. test for bilirubin was negative), or Perl's stain (i.e. test for ferric iron was negative). Sponsor states that kupffer cell hypertrophy has been shown for other HMG-CoA reductase inhibitors like lovastatin alone, or lovastatin + SCH 58235.

Table: Histopathologic findings in a 3-month dog toxicity study of SCH 58235 + atorvastatin (0.3/1, 3/1, 3/10 30/10 mg/kg/day of SCH 58235/atorvastatin)

	Males	Females
Heart, hemorrhage, acute, focal (minimal)	0/4, 0/4, 0/4, 0/4, 0/4, 1/4	
Lungs, fibrosis, interstitial, focal and/or hemorrhage acute, focal (minimal)	0/4, 0/4, 0/4, 0/4, 2/4, 0/4	0/4, 0/4, 0/4, 0/4, 0/4, 1/4
Liver, Bile duct hyperplasia (minimal to mild)	0/4, 0/4, 0/4, 0/4, 3/4, 4/4	0/4, 2/4, 0/4, 0/4, 4/4, 2/4
Liver, Kupffer cell hypertrophy (minimal-mild)	0/4, 1/4, 1/4, 1/4, 2/4, 4/4	0/4, 1/4, 1/4, ¼, 4/4, 3/4
Liver, increased eosinophilia, cytoplasmic (Minimal)	0/4,1/4, 1/4, 1/4, 4/4, 4/4	0/4, 1/4, 1/4, 2/4, 4/4, 4/4

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

Test Article-Related Histopathologic Findings										
Group:	Atorvastatin Control		Low-Dose Combination		Low-Mid-Dose Combination		High-Mid-Dose Combination		High-Dose Combination	
Sex:	M	F	M	F	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[a]									
Liver										
-Eosinophilia, cytoplasmic, increased minimal	1/4	1/4	1/4	1/4	1/4	2/4	4/4	4/4	4/4	4/4
-Hyperplasia, bile duct minimal mild		2/4					3/4	3/4 1/4	3/4 1/4	2/4
-Hypertrophy, Kupffer cell minimal mild							1/4 1/4	4/4	4/4	3/4
-Hypertrophy, hepatocellular, periportal minimal								1/4	1/4	2/4
a: Incidence = Number affected/Number examined										

Table: Electron microscopy findings in the liver, in a 3-month dog toxicity study of SCH 58235 + atorvastatin

Test Article-Related Transmission Electron Microscopic Findings						
Group:	Atorvastatin Control		High-Mid-Dose Combination		High- Dose Combination	
Sex:	M	F	M	F	M	F
Organ/Finding/Severity	Incidence[a]					
Liver						
-Proliferation, smooth endoplasmic reticulum, hepatocellular mild moderate				1/1	1/1	2/2
-Decreased glycogen, hepatocellular mild	2/2			1/1	1/1	2/2
-Lipofuscin accumulation, hepatocellular mild					1/1	2/2
a: Incidence = Number affected/Number examined						

Toxicokinetics: The plasma AUC values are shown in the Table. The co-administration of drug with atorvastatin did not result in significant changes in exposure to total, free and conjugated ezetimibe, the increases in exposures were dose related, and gender independent. Slightly lower values seen may be due to variability and %CV (which were >50% during week 5) and accounted for the differences. The sponsor states that exposure data from 6 month tox study in dogs at 30 mg/kg/day appear to be in agreement with 30/10 mg/kg/day of SCH 58235/atorvastatin here. The total SCH 58235 appears to accumulate at high dose combinations, but not at LD combination in week 5 vs day 0. Atorvastatin (or ortho-hydroxy atorvastatin/parahydroxy-atorvastatin)

exposures did not significantly change with ezetimibe doses, and slight decrease seen with this combination in both sexes is due to %CV (<50% during week 5). The increase in AUC exposures to atorvastatin, ortho-hydroxy atorvastatin, and parahydroxy-atorvastatin were dose related, and gender independent. Atorvastatin and its metabolite did not accumulate over time

Table: Systemic exposures (AUC 0-24 hr) to total, conjugated and unconjugated SCH 58235 on day 0 and week 5 in a 3-month dog toxicity study of SCH 58235 + atorvastatin:

	Male + Female				
	0.3/1[a]	3/1[a]	0/10[a]	3/10[a]	30/10[a]
	Total SCH 58235 AUC(tf) (ng-hr/mL)				
Day 0	118	658	NA	521	3378
Week 5	124	795	NA	888	3879
	Unconjugated SCH 58235 AUC(tf) (ng-hr/mL)				
Day 0	6.73	41.5	NA	23.8	248
Week 5	3.97	45.1	NA	39.9	339
	Conjugated SCH 58235 AUC(tf) (ng-hr/mL)				
Day 0	112	616	NA	497	3130
Week 5	127	750	NA	848	3541

a: Dose of SCH 58235 (mg/kg)/atorvastatin calcium (mg/kg).
NA = Not Applicable

Table: Systemic exposures (AUC 0-24 hr) to atorvastatin, ortho-hydroxy atorvastatin, and para hydroxy atorvastatin on day 0 and week 5 in a 3-month dog toxicity study of SCH 58235 + atorvastatin

	Male + Female				
	0.3/1[a]	3/1[a]	0/10[a]	3/10[a]	30/10[a]
	Atorvastatin AUC(tf) (ng-hr/mL)				
Day 0	26.9	33.7	302	215	293
Week 5	22.5	21.2	473	260	329
	Ortho-hydroxy Atorvastatin AUC(tf) (ng-hr/mL)				
Day 0	16.9	16.3	166	191	207
Week 5	18.2	15.3	309	368	514
	Para-hydroxy Atorvastatin AUC(tf) (ng-hr/mL)				
Day 0	ND	ND	62.8	39.8	54.7
Week 5	ND	ND	115	88.3	109

a: Dose of SCH 58235 (mg/kg)/atorvastatin calcium (mg/kg).
ND = Not determinable

Toxicology summary: In a 3-month toxicity study of SCH 58235 (0.3, 3, 3, 30 mg/kg/day) + atorvastatin (1, 1, 10, 10 mg/kg/day) in dogs, AUC exposures were slightly higher at two HD combinations, suggesting some accumulation of the total drug (SCH 58235) in week 5 (0.12, 0.8, 0.89, 3.9 µg.h/ml at 0.3/1, 3/1, 3/10, 30/10 mg/kg/day of

SCH 58235/atorvastatin respectively) vs on day 0 (0.12, 0.66, 0.52, 3.4 μg.h/ml respectively). However, presence of atorvastatin in the combination did not significantly effect the total (or conjugated and free) ezetimibe exposures. The combination also did not significantly increase the atorvastatin (or metabolites such as ortho-hydroxy atorvastatin/parahydroxy-atorvastatin) expsoures and values were not significantly different in week 5 (23, 21, 260, 329 ng.h/ml vs atorvastatin 473 ng.h/ml) than on day 0 (27, 34, 215, 293 ng.h/ml vs atorvastatin alone 302 ng.h/ml). In both sexes, all combination doses produced increases in plasma ALT (by 2-40 fold vs atorvastatin control). At two HD combinations, AST (by 1.5-2 fold vs atorvastatin control) & AP levels (by 3 fold vs atorvastatin control) were increased, while total protein and albumin levels were decreased in dogs (see Table). All combination doses produced significant decreases in cholesterol and TG levels. Two HD combinations decreased absolute liver weights in males by 21-26%. At mid-high doses (3/1, 3/10, 30/10 mg/kg/day of SCH 58235/atorvastatin), toxicity was observed in the liver (bile duct hyperplasia, kuffer cell hypertrophy, increased eosinophilia). HD combination produced toxicity in the heart (hemorrhage acute focal) and lungs (fibrosis or hemorrhage). No NOAEL in this 3-month dog study could be established for the combination and was < 0.3/1 mg/kg/day of SCH 58235/atorvastatin, as all doses increased liver enzyme ALT in dogs, and produced liver toxicity. We concur with the sponsor that NOAEL in dogs was < 0.3 mg/kg/day of SCH 58235 + <1 mg/kg/day of atorvastatin.

Following 3-month toxicity studies in rats and dogs with SCH 58235 + simvastatin and SCH 58235 + lovastatin were reviewed IND ——— on 12/14/00 and 4/26/01

5. Three-Month Oral dietary Toxicity Study of SCH 5823– in combination with simvastatin (gavage) in rats (Study No. 97124):

Sponsor's ID Study #: 97124
Amendment #, Vol. #, and page # : 049, 21.4, page 1.
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 (X) No ( ), Is the evaluation based on a final, QA report: Yes.
Methods: This study examined the effects of SCH-5823 - (males at 50, 250, and 250 mg/kg/day, females at 12, 50, 50 mg/kg/day) in combination with simvastatin (10, 10, and 50 mg/kg respectively) for 3-months in rats.

Dosing information:
species: Rats Crl:CD (SD) IGS BR VAF/Plus.
#/sex/group or time point: 10/sex/group
age: ≈ 6-weeks of age
weight: males 159-182 g, females 120-141 g.
satellite groups used for toxicokinetics: n=36 rats/sex/group
Dosage groups in administered units: Three groups (10-rats/sex/group) were given oral SCH-5823 - by diet (once daily) at doses of 50, 250 and 250 mg/kg/day for males, and doses of 12, 50, 50 mg/kg/day for females, in combination with simvastatin (10, 10, and 50 mg/kg respectively by gavage) for 3-months. Fourth (control) group of animals received the vehicle only (0.4% w/v aqueous

methylcellulose). One additional group of rats received simvastatin alone at 50 mg/kg/day. Also 36 rats/sex were used for TK studies.

Route, form, volume, and infusion rate (if i.v.): Oral (via diet).
Drug, Batch #: 96-58235-X-02. Simvastatin: 38425-111
Formulation/vehicle: 0.4% (w/v) aqueous methylcellulose.

Times at which Observations are made:

Clinical signs/Physical exams: Daily
Body weights: Prior to dosing, and weekly thereafter.
Food consumption: Food consumption and drug intake were monitored weekly.
Hematology/Coagulation: prior to dosing, during weeks 4/5, and 14 (coagulation, week 14).
Clinical chemistry: weeks 4/5, and 13.
Urine analysis: weeks 4/5, and 13.
Ophthalmic Examinations: Pretest and weeks 4 and 12.
Gross pathology: At sacrifice in week 13.
Organs weighed: *Marked organs in the appended Table were weighed.
Histopathology: At sacrifice controls and high doses animals, and animals who had gross findings, and liver and stomach toxicity in all rats were examined.

Toxicokinetics: Blood was collected on day 0 and 57 at 1, 2, 4, 6, 12 and at 24 hrs after simvastatin administration.

Results:
Mortality: None

Clinical Signs: No treatment related clinical signs were observed.

Body weight/Food consumption: No effects of simvastatin alone on BW were observed, but all combinations decreased BW (males 397, 396, 367, 374, 343, and females 237, 237, 230, 233, 219 g in vehicle control, simva control, and low, mid, high dose combos respectively) weight gains (males 222, 220, 192, 200, 170, females 107, 106, 99, 100, 88 g in vehicle control, simva control, and low, mid, high dose combos respectively) in both sexes and were seen from week 2 onwards. The BW was decreased by 6-13% at all combos in males, and in females at high dose by 13%. Weight gains were decreased in both sexes at all doses (males by 10-22%, females by 11-21%). No drug related effects were observed on food consumption.

Hematology: In males the RBC counts were slightly lower at all combos (8.7-8.9 vs 9.2 M/ul in controls). The reticulocyte counts were slightly increased in both sexes at all combos (2.3-2.8 vs 1.5-2.1% in controls). No effects on coagulation parameters were seen.

Biochemistry: In the high dose combo male rats, ALT (70 vs 57 IU/L with statin alone), AST (176 vs 108 IU/L with statin alone) was increased. All dose combos increased AP (316-382 vs 168 IU/L with vehicle/statin controls), GGT (3-4 vs 1-3 IU/L with vehicle/statin controls). In females AP (223 vs 124 IU/L in controls) increased at high dose combo, and GGT at all doses (4-5 vs 2 with vehicle controls). Chol decreased in both sexes at high dose combo and TG with all dose combos in both males/females.

Sponsor's Table: Changes in serum cholesterol, TG, ALT, AST, AP & CGT levels in a 3-month rat toxicity study of SCH 58235 + simvastatin.

Toxicologically or Clinically Significant Differences in Mean Cholesterol, Triglycerides, ALT, AST, AP and GGT Values						
	Cholesterol (mg/dL) Week 13	Triglycerides (mg/dL) Week 13	ALT (IU/L) Week 4	AST (IU/L) Week 4	AP (IU/L) Week 13	GGT (IU/L) Week 13
Males						
Vehicle Control	45	64	33	108	168	1
SCH 57098 Control			57			3
Low-Dose Combination		36			316	4
Mid-Dose Combination		30			356	4
High-Dose Combination	28	26	70	176	382	3
Females						
Vehicle Control		46			124	2
SCH 57098 Control		32				
Low-Dose Combination		20				4
Mid-Dose Combination		22				5
High-Dose Combination		20			223	4
SCH 57098 = Simvastatin						

Organ Weights: In females the absolute and relative liver weights were increased above simva controls in all combo groups (absolute 7.2, 7.8, 8, 9 g, and relative 3.2, 3.5, 3.5, 4.3% respectively in above 4 groups).

Gross pathology: Liver was enlarged at high dose combo in 3/10 females.

Histopathology: Toxicity was observed in the liver (single cell necrosis, hepatocellular hypertrophy, vacuolation, and bile duct hyperplasia), and stomach (hyperkeratosis, acanthosis, submucosal edema, and cellular infiltration). Sponsor claims that these stomach toxicities are similar to those found with simvastatin previously.

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