ezetimbe/simvastatin (30/10 mg/kg/day), attenuated the increases in ALT & AP by 53%-57%, & AST by 33%. In the same study histopath findings showed bile duct hyperplasia in ¼ dogs vs 2/4 dogs without mevalonate supplementation, and were considered significant. When ezetimibe + simvastatin (30/10 mg/kg/day), or ezetimibe was given alone (0.03, 0.3, 30, 300 mg/kg/day) in above dog study, all doses of ezetimibe increased the bile cholesterol levels, but did not effect the bile acid levels, as shown in the Table below. In a 3-month dog study with ezetimibe + simvastatin (see the study in a toxicity section), liver enzymes were increased at all combination doses (ezetimibe 0.3-30 mg/kg/day/simvastatin 1-10 mg/kg/day), and histopath findings in liver (hepatocytic cytoplasmic eosinophila was seen at all combinations, and biliary hyperplaisa at high dose combination and was not reversible after 1-month drug free period) were observed, but bile cholesterol levels were not measured in this 3-month dog study . However sponsor states that mechanisms underlying ezetimibe related increases in bile cholesterol concentration in dogs have not been investigated, and animal models are not always predictive of human risk of cholelethiasis.

Table. Summary of bile acids and lipid analysis in dogs (study # SN 00195, 00640, volume 1.9, page 125):

Treatment	Bile Lipid		
	Bile Acids	Phospholipid	Cholesterol
Ezetimibe	NC	NC	↑
Ezetimibe + Simvastatin	↓	↓	↑ (+/-)
Simvastatin	↓	↓	↓
NC = no change; ↑ = increased/higher; ↓ = decreased/lower (+/-) = results not reproducible due to variability in control values between studies			

In rabbits, bile duct hyperplasia (accompanied by peribiliary inflammation and/or fibroplasia) has not been reported after treatment with statins alone, but was observed after ezetimibe + simvastatin (see Tables a and b below). Since biliary epithelium of rabbits is unusually sensitive to irritation, mevalonate supplementation study for 9 days (study # SN 00196, volume 1.165) was conducted in female rabbits (n=3/group). Mevalonate supplementation did not markedly alter the incidence or severity of the hepatic findings with ezetimibe/simvastatin (bile duct hyperplasia accompanied by peribiliary inflammation) in rabbits (1/3, 2/3, 3/3, 3/3, 3/3 at 0, 250/0.5, 250/5, 250/25, 250/25 + mevalonate respectively). Similarly mevalonate did not decrease the severity of ALT increases in rabbits (33, 68, 69, 126, 84 respectively) in above study, suggesting these alterations were not directly linked to inhibition of HMG-CoA reductase. A subsequent study in rabbits (study # SN 01085, vol 1.163) suggested that ezetimibe alone propduces bile duct hyperplasia and peribiliary inflammation at doses as low as 0.3 mg/kg/day for nine days, and with simvastatin these findings were more pronounced (see Tables a, b and c below). However sponsor states that the liver finding is only seen in rabbits and is species specific and is not considered to be clinically relevant. Liver was not the target organ of toxicity in rats, mice or dogs with ezetimibe alone. These studies suggest that although mevalonate supplementation may play some role in increased liver and bone enzymes (ALT, AST, AP) in dogs, this mechanism does not apply to rabbits.

Table a. 9-day oral study design in rabbits

Group Designations				
Group Name	Group Number	SCH 58235 (mg/kg)	Simvastatin (mg/kg)	Mevalonate (mg/kg)
Vehicle Control (0.4% methylcellulose)	C1	0	0	0
Simvastatin Control	C2	0	25	0
Mevalonate Control	C3	0	0	80[a]
Low-Dose Combination	T1	250	0.5	0
Mid-Dose Combination	T2	250	5	0
High-Dose Combination	T3	250	25	0
High-Dose Combination Plus Mevalonate	T4	250	25	80[a]

a: Each rabbit in Groups C3 and T4 received 40 mg/kg (dose volume of 1 ml/kg of a 40 mg/ml solution) of mevalonate twice daily, for a total daily dose of 80 mg/kg.

Table b. Serum ALT values with ezetimibe alone, and with ezetimibe + simvastatin

SCH 58235-Related Differences in Week 2 ALT Values							
Group:	Control	T1	T2	T3	T4	T5	T6
Dose of SCH 58235 (mg/kg):	0	0.003	0.03	0.3	3	30	300
Incidence[a]	0/5	0/5	0/5	0/5	2/5	3/5	5/5
Animal Nos.[b]	-	-	-	-	453 455	551 552 553	NA
Mean (IU/L)[c]	45.0	-	-	-	-	59.8	88.0

NA – Not Applicable

a: Incidence = Number affected/Number examined

b: Animal Nos. = Individual animal identification numbers are indicated only when fewer than all individuals examined are affected

c: Mean is indicated only for groups with values meaningfully different from that of the vehicle control group (except in the case of the vehicle control group itself)

SCH 58235/Simvastatin-Related Differences in Week 2 ALT Values							
Group:	T7	T8	T9	T10	T11	T12	T13
Dose of SCH 58235 (mg/kg):	0	0.003	0.03	0.3	3	30	300
Dose of Simvastatin (mg/kg):	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Incidence[a]	1/5	0/5	0/5	4/5	3/5	3/5	4/5
Animal Nos.[b]	752	-	-	1052 1053 1054 1055	1151 1152 1154	1251 1254 1255	1351 1352 1353 1355
Mean (IU/L)[c]	-	-	-	85.0	50.2	60.0	69.2

a: Incidence = Number affected/Number examined

b: Animal Nos. = Individual animal identification numbers are indicated only when less than all individuals examined are affected

c: Mean is indicated only for groups with values meaningfully different from that of the vehicle control group

Table c. Histopath changes with ezetimibe alone, and with ezetimibe + simvastatin

SCH 58235-Related Histopathologic Findings				
Group:	T3	T4	T5	T6
Dose of SCH 58235 (mg/kg):	0.3	3	30	300
Sex:	Females			
Organ/Finding/Severity	Incidence[a]			
Liver				
-Inflammation, peribiliary minimal	1/5	2/5		3/5
-Hyperplasia, bile duct minimal		1/5	1/5	1/5
a: Incidence = Number affected/Number examined				

SCH 58235/Simvastatin-Related Histopathologic Findings				
Group:	T10	T11	T12	T13
Dose of SCH 58235 (mg/kg)	0.3	3	30	300
Dose of Simvastatin (mg/kg):	0.5	0.5	0.5	0.5
Sex:	Females			
Organ/Finding/Severity	Incidence[a]			
Liver				
-Inflammation, peribiliary minimal	4/5	1/5	4/5	4/5
-Hyperplasia, bile duct minimal	2/5	2/5		2/5
a: Incidence = Number affected/Number examined				

The combination of ezetimibe + statins produced synergistic lowering of serum cholesterol in dogs (especially at high doses of two compounds). In contrast, clinical studies have shown that the cholesterol lowering effect of two drugs is additive. Sponsor states that in dogs, at lower doses the effect of two drugs on cholesterol lowering was additive, this si similar to what is seen in humans. Therefore, dog may be a good representative model of pharmacodynamic additivity of what is seen with the ezetimibe + stain combination in humans. However there are some species differences in the cholesterol profile in dogs and humans, in dogs HDL cholesterol predominates, while in humans LDL cholesterol predominates.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS

A. DETAILED CONCLUSIONS ON MONOTHERAPY

SCH 58235 is a lipid lowering drug, which blocks the intestinal absorption of cholesterol. The mechanism of action of this drug is unknown but it has ACAT inhibitor activity. It is indicated alone or with HMG-CoA reductase inhibitors (statins) for the reduction of elevated LDL cholesterol. The recommended human dose is 10 mg/day.

A1. In pharmacology studies, the drug was a most potent inhibitor of cholesterol absorption in cholesterol-fed monkeys with an ED_{50} of 0.0005 mg/kg/day (or 0.5 µg/kg/day). In rats and hamsters it inhibited cholesterol with ED_{50} of 30-40 µg/kg, it was more potent in dogs (with the ED_{50} of 7 µg/kg), and less potent in mice (with the ED_{50} of 700 µg/kg). In all above studies the drug worked when animals were fed high cholesterol diet. No effects on plasma cholesterol were observed in animals on normal chow diets. The drug is mainly metabolized to phenolic glucuronide in all species tested, and glucuronide was more potent than the parent compound in inhibiting the absorption of cholesterol (90% vs 70% parent). However, ACAT inhibotry activty of glucuronide is

lower than of parent, suggesting either there is an undescribed mechanism of action or de-glucuronidation resulting in reformation of parent in the intestine is significant. The parent shows ACAT inhibitory activity, this may still explain mechansim of action.

A2. In safety or secondary pharmacology studies, both acute and chronic effects of the drug have been examined. In acute studies the drug did not have an effect on GI morphology or motility. It did not affect intestinal transit time in conscious rats (47.4 vs 45.2% in controls, vs atropine 18.4%*, $p<0.05$), and did not produce gastric lesions. It did not affect urine volume (1.2 vs 1.4 ml/5hrs in controls) or sodium excretion (0.147 vs 0.157 meq/5 hrs in controls) in rats. It also had no effects on blood pressures or heart rates in conscious normotensive rats in acute studies. In chronic studies, the ECG effects of the drug (including effects on heart rates, blood pressures, respiration rates/body temperatures) were examined in the 6-12 month oral (gavage) toxicity studies in dogs at doses up to 300 mg/kg/day or 7-9 fold the human doses, based on AUC exposures) and these were negative. Effects of the drug on behavior, autonomic and neurologic function were examined in a single dose acute study in male rats (at 3, 10 & 30 mg/kg), where modest to marked effects on passivity (body elevation), as well as *neurologic* (change in gait, limb position), and *autonomic effects* ('excretions') were observed at 10 mg/kg and 30 mg/kg (all 6/6 rats at 30 mg/kg had diarrhea). Thus although no clinical signs at doses of 20, 100, 500, and 1500 mg/kg/day were observed in the standard rat toxicity study, a single dose study in male rats showed modest to marked effects on passivity, body elevation, limb position, and changes in gait.

A3. General Toxicology Studies on Monotherapy

In acute tox studies, oral doses of SCH 58235 in mice, rats and dogs (3000-5000 mg/kg/day) were well tolerated, as no mortality was observed. Single intraperitoneal (ip) doses of 2000 mg/kg/day were lethal in both, mice (died within 1-3 days) and rats (females died after 7 days). The drug decreased mean BW gain in mice (2000 mg/kg) and rats (1000-2000 mg/kg) following ip dosing, and induced clinical signs at 2000 mg/kg in mice (scant feces, hypoactivity, tremors, urogenital staining) and rats (distended abdomen, dehydration, scant/loose feces, hypoactivity, tremors, urogenital staining). Macroscopic changes after ip dosing showed irritation and inflammation from the drug.

In a 6-month dietary toxicity study of SCH 58235 (Zeita) in rats, higher doses (0, 250, 750, 1500 mg/kg/day) were used in males than in females (0, 50, 250, 500 mg/kg/day). The AUC exposures of the drug increased in less than proportional manner. The values of the total drug were higher in week 25 (AUC 0-24 hrs males 6.6, 9.4, 12.7 µg.h/ml, females 6.1, 13.7, 12.5 µg.h/ml respectively) vs in week 4 (males 3.5, 6.5, 10.4 µg.h/ml, females 4.5, 7.8, 8.8 µg.h/ml respectively), suggesting accumulation of the drug over time. The drug produced significant increases in the plasma AST levels in females at a high dose (246 vs 143 IU/L in controls). In ophthalmoscopic exams in male rats increases in corneal crystals or chromodacryorrhea was observed (0/15, 2/15, 1/15, 3/15), however no drug related associated histopath findings were observed in eyes. At a high dose in males, the target organs of toxicity may be bone marrow (hyperplasia in 3/15 vs 0/15 in controls), lymph nodes (accumulation in plasma cell in 8/15 vs 4/15 controls), and heart (mononuclear cellular infiltration in 8/15 vs 5/15 rats, myocardial degeneration in 1/15 vs 0/15 controls). In females the target organs at a high dose are adrenals (vacuolation, hypertrophy pigment accumulation in 1-2/15 vs 0/15 controls), kidney (glomerular nephropathy in 8/15 vs 1/15 controls) and heart (myocardial

degeneration in 1/15 vs 0/15 controls). Since histopath changes were not examined at low-mid doses in rats, it is unknown if there was a dose related trend in any of these findings. The NOAEL in the 6-month rat study may be 750 mg/kg/day in males and 250 mg/kg/day in females (or 15-20 fold the human exposure of 0.64, based on recommended 10 mg/day dose in humans).

In a 6-month dietary toxicity study of Zeita in dogs, doses of 0, 30, 100 and 300 mg/kg/day were used. The AUC exposures of the drug increased in less than dose proportional manner. The values of the total drug were generally higher in week 25 (2.1, 2.8, 4.9 µg.h/ml at 30, 100 and 300 mg/kg/day respectively in male + female dogs) vs on day 1 (1.6, 4.0, 3.5 µg.h/ml respectively), suggesting some accumulation of the drug over time. In ophthalmoscopic exams in week 26, mild partial to complete loss of pigmentation in the tapetal region of one or both eyes was observed in dogs (0/8, 2/8, 0/8, 1/8 respectively). The target organs of toxicity at a high dose in males are heart (mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls) and in females spleen (extramedullary hematopoiesis of minimal severity in 2/4 vs 1/4 in controls). The NOAEL dose of the drug in 6-month toxicity study in dogs may be 300 mg/kg/day (7 fold the human exposure), since histopath changes were observed in 1 to 2 animals at a high dose.

In a 1-year dietary toxicity study of Zeita in dogs, doses of 0, 30, 100 and 300 mg/kg/day were used. The AUC exposures of the drug increased in less than proportional manner. The values of the total drug were higher in week 51 (2.8, 5.0, 6.4 µg.h/ml at 30, 100 and 300 mg/kg/day respectively in male + female dogs) vs on day 1 (2.1, 2.7, 3.3 µg.h/ml respectively), suggesting accumulation of the drug over time. At low-mid doses, absolute mean heart weights tended to be lower in males (98.8, 86.7, 81.3, 90.2 g at 0, 30, 100, 300 mg/kg/day respectively) but were not associated with any histopath findings. However at a high dose ezetimibe produced toxicity in the lymph nodes in 8/8 vs 4/8 controls. Lymph nodes toxicity included minimal pigment accumulation in mandibular (in 7/8 vs 3/8 controls), lymphoid mesenteric hyperplasia of minimal to mild severity (in 6/8 vs 2/8 controls), minimal mandibular hemorrhage & erythrophagocytosis in females (in 1/4 vs 0/4 controls), and erythrophagocytosis mesenteric minimal to mild in 6/8 vs 2/8 controls). The NOAEL in a 1-year toxicity study in dogs was 100 mg/kg/day (8 -fold the human exposure).

In a 1-month dietary toxicity study of the drug (Zeita) with impurities in rats, the [redacted] mpurities were qualified. These impurities were present in the drug substance in amounts greater than [redacted] but less than [redacted] . According to ICH Q3A guidance document, these need to be qualified if present in amounts > 0.1%. This study examined the toxicity of SCH 58235 with added impurities in the diet. The impurities used were

[redacted]

The reason why above doses of impurities were selected, because impurities were present in amounts of [redacted] in the drug substance, therefore similar or in some instances slightly higher concentrations of impurities were added then found in the drug substance (see justification in section VIII). The drug dose selection was based on gender related differences in exposure in previous 3-6 month dietary study in rats, where plasma levels had a plateau at 1500 and 500 mg/kg/day in males and females respectively. Therefore these high doses were chosen for this special tox study (males 0, 250, 750, 1500 mg/kg/day, females 0, 50,

250, 500 mg/kg/day). The AUC exposures of the drug increased in less than proportional manner. The values of the total drug in week 4 were (AUC 0-24 hrs males 7.9, 12.0, 11.6 μg.h/ml, females 5.3, 9.7, 11.6 μg.h/ml respectively) not significantly different than in the 6-month toxicity study in rats (in week 25: males 6.6, 9.4, 12.7 μg.h/ml, females 6.1, 13.7, 12.5 μg.h/ml respectively, or in week 4: males 3.5, 6.5, 10.4 μg.h/ml, females 4.5, 7.8, 8.8 μg.h/ml respectively), suggesting that impurities did not effect the drug PK in rats. The target organs of toxicity at a high dose in males may be heart (mononuclear cellular infiltration in 2/10 rats vs 0/10 controls), and lymph nodes (hyperplasia in 1/10 vs 0/10 in controls), and in the female, pituitary gland (diffuse hyperplasia/adenoma in 0/10, 1/1, ne, 2/10 respectively) and spleen (increased extramedullary hematopoiesis in 0/10, 1/1, ne, 1/10 respectively). Note that the pituitay gland toxicity was not observed in a 6-month toxicity study in rats when impurities were not present. The NOAEL in the 1-month rat study with the drug containing impurities may be 750 mg/kg/day in males and 250 mg/kg/day in females (or 15-20 fold human exposure, based on 10 mg/day dose in humans). There was a wide variation in AUC values, but the TK in general appear similar with impurities + ezetimibe than without impurities. The concentration of impurities in the toxicity studies qualify these impurities

AUC (0-24 hrs, μg.h/ml) values of the total drug + impurities in the 1-month rat tox study vs in the 6-month rat tox study (at week 4 and week 25) without impurities are shown below.

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Rat study	Males (250, 750, 1500 mg/kg/day)	Females (50, 250, 500 mg/kg/day)
Drug with impurities	(μg.h/ml)	(μg.h/ml)
I-Month rat study Week 4 values	7.9, 12.0, 11.6	5.3, 9.7, 11.6
Drug without impurities		
6-month rat study, Week 4 values	3.5, 6.5, 10.4	4.5, 7.8, 8.8
Week 25 values	6.6, 9.4, 12.7	6.1, 13.7, 12.5

In a 1-month oral gavage toxicity study of Zeita with impurities in dogs, doses of 0, 30, 100, 300 mg/kg/day were used. Similar doses of impurities were used in dogs as indicated in the above rat study. The AUC values of the total drug increased in less than proportional manner. The $AUC_{0\text{-}24h}$ values of the total drug in week 4 (males+females 3.8, 5.9, 7.4 μg.h/ml respectively) were not significantly different than in the 6-month toxicity study in dogs (in week 25: 2.1, 2.8, 4.9 μg.h/ml respectively, or in week 13: 2.3, 4.6, 6.1 μg.h/ml respectively), suggesting that impurities did not effect the drug PK in dogs. The target organs of toxicity at a high dose were heart (1/4 males had an

enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve, ¼ females had mononuclear cellular infiltration vs 0/4 controls), lymph nodes (brown pigment accumulation in 2/4 M+ 2/4 F vs 0/8 in controls, and lymphoid hyperplasia in ¼ M+ ¼ F vs 0/8 controls), liver (centrilobular vacuolation in ¼ males vs 0/4 controls), lungs (cellular infiltration with neutrophilic focus in ¼ females, metaplasia in ¼ males vs 0/4 controls, histiocytic/lymphohisiocytic multifocal inflammation in ¼ M +1/4 F vs 0/4 controls), ovaries (paraovarian cyst in ¼ females vs 0/4 controls), stomach (mineralization, mucosal focal in 2/4 males vs 0/4 controls). The histopath findings in small intestine (nematodes luminal in **males** 1/4, 2/2, 4/4, 4/4, and **females** 4/4, 2/2, 4/4, 2/2 respectively), and spleen (which was not remarkable in **males** 0/4, 3/3, ne,1/4 and **females** 2/4, 2/2, 3/3, ¼ and findings included siderofibrosis in 1 male at a low dose, and hyperplasia at mid/high doses in females) were observed at all doses. The NOAEL in the 1-month dog tox study with impurities may be <30 mg/kg/day (or 6 fold the human exposure, based on 10 mg/day dose in humans). In conclusion, the TK appear slightly higher with impurities with ezetimibe, than without impurities, sponsor explains that there was a wide variability in mean AUC values (from 18-86%) and these are not significant. The toxicity seen here with impurities in heart, spleen, lungs have been observed previously in 3-6 month studies in dogs without impurities (with ezetimibe). However, toxicity seen in lymph nodes, stomach, liver, ovaries, small intestine with ezetimibe + impurities in dogs here was not previously observed without impurities. Sponsor considers all histopath findings in dogs as common findings, and unrelated to impurities. Sponsor states that no toxicity emerged in rat or dog studies due to impurities, and TK in rats/dog was also not different with drug containing impurities.

AUC (0-24 hrs, µg.h/ml) values of the total drug + impurities in the 1-month dog tox study vs in the 6-month dog tox study (at week 13 and week 25) without impurities are shown below.

Dog study (30, 100, 300 mg/kg/day	Males + females (µg.h/ml)
Drug with impurities	
I-Month dog study Week 4 values	3.8, 5.9, 7.4
Drug without impurities	
6-month dog study, Week 13 values	2.3, 4.6, 6.1
Week 25 values	2.1, 2.8, 4.9

It is important to note that heart was the target organ of toxicity (mostly in males) in the 6-month rat (mononuclear cellular infiltration in 8/15 vs 5/15 rats) and dog (mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls) toxicity studies without impurities. This was also seen in 1-month rat (mononuclear cellular infiltration in 2/10 rats vs 0/10 controls) and dog (1/4 males had an enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve, ¼ females had mononuclear cellular infiltration vs 0/4 controls) toxicity studies with impurities. Sponsor does not offer any explanations as to how ezetimibe

works, or its mechanism of action. As indicated earlier SCH 58235 is a weak ACAT inhibitor, and is ≈ 3-times less potent (with an IC_{50} value of 18 μM) in vitro in the rat liver microsome ACAT assay vs two other standard known ACAT inhibitors (CL2777082 and PD128042 with IC_{50} values of 5-6 μM). The molecular weight of SCH 58235 is 409, and at an IC_{50} of 18 μM (i.e 409 X 18 μM = 7362 μg/1000 ml) or at 7.4 μg/ml it has a significant ACAT inhibitory activity. It can be seen in Table 16 (PK/TK section) that these exposures (6-14 μg.h/ml) are achieved in 1-6 month rat and dog toxicity studies at high doses (of 300-500 mg/kg/day). These exposures are approximately 10 fold the human exposures based on sponsor's AUC exposures of 0.71 μg.h/ml at 10 mg/day dose in humans. SCH 58235 may have some similarities in structure to other ACAT inhibitors (as indicated earlier in the pharmacology section, Roth et. al, J Med. Chem 35: 1609, 1992). The cardiotoxicity with ezetimibe in monotherapy in rats and dogs (generally of low incidences, in a number of studies) may be explained by its weak ACAT activity. This suggests that ezetimibe in fact is an ACAT inhibitor, and may work and act by ACAT mechanism. The sponsor acknowledges that ezetimibe is a week ACAT inhibitor.

A4. Mutagenicity: SCH 58235 was not mutagenic/cytogenic in the following 3 tests: Ames test, in vitro mammalian chromosome aberration test in human lymphocytes, and in vivo micronucleus test in mice. SCH 58235 spiked with impurities was not mutagenic or cytogenic in the following 2 tests: Ames test, and in vivo micronucleus test in mice.

A5. Carcinogenicity: In a 2-year carcinogenicty (CAC) study in diet restricted rats at a high dose of 500 mg/kg/day, hepatocellular adenomas were observed in 2/50 females (or 4%) vs none in controls, however these were not statistically significant and were within the historical control range (0-8%) for the same species of rats. Also a decrease in BW (4-7%) and weight gains (7-10%) was observed in male rats at mid-high doses compared to controls. In both rats and mice 2-year CAC studies, no significant neoplastic or other non-neoplastic tumor findings were observed at doses up to 500 mg/kg/day in mice, and up to 1500 and 500 mg/kg/day in male and female rats respectively. The exposure levels at above doses were about 160-220X and 14X the human exposure in mice (males and female respectively) and rats respectively, based on the human dose of 10 mg/day.

A6. Reproductive and Developmental Toxicology Studies on Monotherapy

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

In a segment I fertility study in rats (n=25/group), animals were given oral (by gavage) SCH 58235 at doses of 0, 250, 500, 1000 mg/kg/day. Females were given the drug for

2-weeks prior to mating, throughout mating, and from days 0 to day 7 of gestation, and sacrificed on GD-14. Males were given the drug for 3 weeks prior to mating, during mating, until necropsy. Clinical signs (chromorhinorrhea, reduced fecal pellet, urogenital statining, broken teeth) were increased during gestation in females (observed in total 8 females at mid/high doses) and also during treatment, prior to gestation (observed in total 4 females at mid/high doses). Doses of 250-500 mg/kg/day produced some broken or loose teeth in both sexes, but did not have any affect on the fertility or on the general maternal or paternal reproductive performance, or on the progression of pregnancy in rats. The percentage of resorptions were higher at a high dose (4, 5.4, 6.4, 7.5% respectively), but were not significant and within historical control range. Less animals were pregnant at the highest dose (male/female fertility index was decreased from 96% to 84% respectively), but these were not significantly different. The NOAEL dose in this fertility study was 1000 mg/kg/day (or 1000 times the human dose of 10 mg/day, based on body surface area). However, based on exposures from dietary TK study in rats (at 500-1500 mg/kg/day, $AUC_{0\text{-}24h}$ values of $\geq$ 10.4 µg.h/ml), these values were 15X exposure multiples in humans. **Note that the recommended human dose is 10 mg/day.**

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

In a segment II teratology study in rabbits, pregnant animals (n=20/group) were given oral SCH 58235 by gavage at doses of 0, 250, 500, 1000 mg/k/day from day 7 to day 19 of gestation. Females were sacrificed on day 30 PC. Maternal exposures of the total drug on GD 19 were 71.5, 95.7, 113 µg.h/ml at 250, 500, 1000 mg/kg/day respectively. Maternal NOAEL was 500 mg/kg/day as in females, since mean resorptions were

increased at 1000 mg/kg/day (9.9% vs 4.1% in controls). Developmental NOAEL was 250 mg/kg/day, as all doses (250-1000 mg/kg/day) increased incidence of malformations in fetuses and litters. These included exencephaly (in one fetus and litter at 250 mg/kg/day), agenesis of tail (in one fetus and litter at 250 mg/kg/day), head malformed (in one fetus and litter at 500 mg/kg/day), omphalocele (i.e intestinal and viscera protruding in 1-3 fetuses and litters at all doses vs 1 fetus/litter in controls), and shortened tail in one fetus and litter at 1000 mg/kg/day. Skeletal observations were noted at all doses. These included reduced ossifications in parietals (fetal incidences 0, 3, 5, 0, litter incidences 0, 2, 2, 0) and frontals (fetal/litter incidences 0, 1, 1, 0). Increased focal thickening of ribs at mid/high doses (fetal/litter incidences 0, 0, 2, 1), increased unossified distal humeral epiphysis at a mid dose (fetal 1, 0, 4, 0, litter 1, 0, 2, 0). Extra pair of thoracic ribs were increased at all doses of the drug (fetal incidences 90-107 vs 67 in controls, litter incidences 16-19 vs 14 in controls). Scoliosis was observed in 1 fetus/litter at a high dose (fetal incidences 0.8, litter incidences 5.9 vs none in other groups). Thus maternal NOAEL was 500 mg/kg/day (or 140-fold the human dose of 10 mg/day based on exposures, and ≈ 1000-fold the human dose of 10 mg/day based on body surface area), as increased resorptions (9.9% vs 4.1% in controls) and altered sex distribution (ratios of male/female fetuses was increased at MD & HD 1.2 & 1.4 vs 0.96 in controls). The embryo-fetal NOAEL was 250 mg/kg/day (or 100-fold the human dose of 10 mg/day based on exposures, and ≈ 500-fold the human dose of 10 mg/day based on body surface area), as increased focal thickening of ribs, scoliosis, and malfomations of tail & head were observed at 500 and/or 1000 mg/kg/day.

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

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

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

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

In the rat Segment III study, SCH 58235 was given to pregnant female rats, orally by gavage on gestation days 6 to lactation day 21 (100, 300, 1000 mg/kg/day, n=25/group). The NOAEL for maternal toxicity was 1000 mg/kg/day (or <0.1 times the human dose, based on body surface area). Some clinical signs (dried red material around nose in 0/25, 1/25, 5/25, 1/25 rats respectively, hair loss in 0/25, 0/25, 0/25, 2/25 respectively) and necropsy findings (hair loss in at mid-high doses in 0/23, 0/24, 1/23, 2/24) were observed at 300-1000 mg/kg/day in mothers. Pre/post natal toxicity was observed at all doses. Clinical signs were observed at all doses in F1 pups. These included subcutaneous hemorrhage (number of findings/number of pups with findings 0/0, 1/1, 1/1, 4/3) and uneven hair growth (in 0/0, 0/0, 1/1, 2/2 respectively). Missing tail was observed in one F1 pup at a mid dose. Post weaning clinical signs were also increased in F1 animals (dry material around eyes/ears/nose in 1/1, 5/3, 0/0, 3/3 respectively, increased hair loss in left and/or right forelimbs in male rats (11/2, 28/4, 15/4, 12/3), and females rats (17/4, 46/8, 63/11, 56/8 respectively). One F1 male died at the lowest dose of 100 mg/kg/day on day 155, this death was attributed to the drug administration in mothers. Scheduled necropsy of F1 rats again showed hair loss in 0/24, 2/22, 0/23, 1/25. In F2 pups, tail was missing in 3 pups at mid doses vs none in other groups, and subcutaneous hemorrhage was higher at mid/high doses (0/0, 0/0, 3/3, 2/2 respectively). Thus maternal NOAEL was 1000 mg/kg/day (or 1000-fold the human dose of 10 mg/day, based on body surface area). The embryo-fetal NOAEL was <100 mg/kg/day (or <100 fold the human dose of 10 mg/day, based on body surface area). The latter is based on one fetal death (F1 male died on day 155) at 100 mg/kg/day, and subcutaneous hemorrhages & tail missing at 300-1000 mg/kg/day in both F1 and F2 pups.

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

Repro-tox study	**NOAEL's**	
Segment I study in rats	500 mg/kg/day (≈ 500 times the human dose of 10 mg/day, based on body surface area).	
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day (or ≈ 1000 times the human dose of 10 mg/day, based on body	250 mg/kg/day (or ≈ 250 times the human dose of 10 mg/day, based on body

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

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

Repro-tox study	
Clinical signs	
Segment I study in rats	Chromorhinorrhea, reduced fecal pellet, urogenital statining, broken teeth at 1000 mg/kg/day (≈ 1000 times the human dose of 10 mg/day, based on body surface area)
Segment III study in rats	In F1 rats at 300-1000 mg/kg/day (dried red material around nose, subcutaneous hemorrhage, hair loss). In F2 rats, at all doses of 100-1000 mg/kg/day (pre weaning; subcutaneous hemorrhage, uneven hair growth, post weaning; dry material around eyes/ears/nose, increased hair loss in left and/or right forelimb).
Malformations	
Segment II study in **rats**	Short filamentous tail at 500 mg/kg/day in one fetus, one litter
Segment II study in **rabbits**	Agenesis of tail at 250 mg/kg/day in 1 fetus & 1 litter, shortened tail at 1000 mg/kg/day in 1 fetus & 1 litter, head malformed in 1 fetus & 1 litter at 500 mg/kg/day.
Segment III study in rats	Missing tail in 1 F1 pup at 300 mg/kg/day, & three F2 pups at 300 mg/kg/day
Deaths	

Segment I study in rats	1/25 female rats died at 500 mg/kg/day (on day 7, it was considered accidental, no findings available
Segment II study in rabbits	1/20 rabbits died at 500 mg/kg/day (on GD 9, it was considered incidental, it had its thoracic cavity filled with blood and had dark red focal discoloration and foamy exudate in trachea)
Segment III TK study in rabbits	2/30 rabbits died at 1000 mg/kg/day, one on GD 16 (died shortly after dosing, so necropsy was performed) and the other on GD-17 (necropsy showed white material in the thoracic cavity adhered to all lobes of the right lung and the thoracic wall). Sponsor considers these incidental, since none were seen in the standard segment II in rabbits, although 1/20 rabbit died in that study
Segment III study in rats	1/25 F1 male rat died on day 155 at the lowest dose of 100 mg/kg/day, it had no clinical signs, the animal was internally normal, sponsor acknowledges that this death was attributed to the drug administration in F0 mot hers.

B. DETAILED CONCLUSIONS ON COMBINATION THERAPY (EZETIMIBE + STATINS)

B1. Pharmacology studies with combination of Ezetimibe + statins: When ezetimibe (0.007 mg/kg/day) was given to dogs in combination with lovastatin (5 mg/kg/day), fluvastatin (5 mg/kg/day), or pravastatin (2.5 mg/kg/day) for 14 days, ezetimibe or lovastatin alone had a minimal effect on plasma cholesterol levels in chow fed dogs, but combined caused a ≥ 50% reduction in plasma cholesterol levels, (see Figures 3 & 4 in the pharmacology section). Similarly pravastatin + ezetimibe produced 41% reduction in plasma cholesterol levels, fluvastatin with ezetimibe was more effective (produced 60% reduction in plasma cholesterol levels) than by itself (by 38% alone). Additional studies with simvastatin or atorvastatin (1 mg/kg/day) plus ezetimibe produced significant synergistic 30% reduction in plasma cholesterol levels. The sponsor states that SCH 58235 did not significantly affect the plasma levels of statins in dogs in pharmacology studies and the synergistic effect in lowering plasma cholesterol are due to different mechanisms of action of the two drugs. Ezetimibe effects are due to inhibition of biliary cholesterol absorption, while HMG-CoA reductase inhibitors effects are due to inhibition of a compensatory increase in hepatic cholesterol biosynthesis. In 3-month dog studies, the combination of ezetimibe + statins produced synergistic effects on serum cholesterol levels in dogs. Sponsor explains that the combination effects LDL in humans. These differences may be due to differences in the cholesterol profile in two species, in humans LDL cholesterol predominates while in dogs HDL cholesterol predominates.

B2. General Toxicology and TK Studies on Combination Therapy

In acute toxicity studies, oral doses of SCH 58235 + simvastatin or SCH 58235 + lovastatin in mice and rats (1000/1000 mg/kg/day) were well tolerated, as no clinical signs, changes in BW or mortality was observed. Single intraperitoneal (ip) doses of 100/100 to 500/500 mg/kg/day of SCH 58235 + simvastatin were lethal in both mice and rats (died within 15 minutes to 8 days). Similarly single intraperitoneal (ip) doses of 500/500 mg/kg/day of SCH 58235 + lovastatin were lethal in both mice and rats (died within 15 minutes to 6 days). The combination generally decreased mean BW gain in both mice and rats following ip dosing, and produced clinical signs at almost all doses in mice (hunched posture, moribundity/ and or prostration, scant feces, hypoactivity, urogenital staining) and in rats (all of the above signs plus abdominal swelling, labored breathing, rough hair coat, mild dehydration, nasal discharge). Macroscopic changes after oral or IP dosing were not conducted with the combination in the acute studies. No acute toxicity studies with the combination in dogs were provided.

Acute IP dosing of the combination produced mortality at lower doses (at 100-500 mg/kg) than following monotherapy with ezetimibe in mice/rats (at 1000-2000 mg/kg) by the same ip route. However in acute oral dosing, lower combination doses were tested (1000/1000 mg/kg/day) in rats and mice, compared to the monotherapy with ezetimibe (5000 mg/kg), and no mortalities were seen in both studies. Also, dog appears to be a good model for statin drugs, but dogs were not tested orally with the combination, and were only tested orally with monotherapy. IP dosing was more toxic, this may be because residual compound like material was seen in the abdomen cavity after ip dosing, which sponsor states may have caused severe irritation and inflammation. The acute studies were carried out with lovastatin and simvastatin, these are both inactive lactone forms of statins, and are hydrolyzed in vivo in to the corresponding more active beta hydroxy acids (which are potent active inhibitors of HMG-CoA reductase), it is unknown if pravastatin, atorvastatin etc. (the open acid forms of statins) would have resulted in different acute toxic profiles with the combination. The acute toxicity studies with pravastatin, atorvastatin, etc combination were not performed

Multiple dose combination studies of SCH 58235 (Zeita) + statins in rats and dogs

1. In a rat, 3-month combination (drug + pravastatin) toxicity study, SCH-5823 - doses (of 15, 250, 250, 750 mg/kg/day in males, and 15, 50, 50, 150 mg/kg/day in females by diet) were used, in combination with pravastatin (25, 25, 250, 250 mg/kg/day). In addition one group of rats received vehicle or pravastatin alone at 250 mg/kg/day. The total drug (drug + glucuronide) exposure increased in rats in week 5 vs on day 0 ($AUC_{0\text{-}24h}$ values in males at high dose combination were 133 vs 11.9 µg/ml.hr in week 5 vs on day 0, in females these values were 41.2 vs 7.7 ug/ml.hr). The combination not only increased the total drug exposure (by 2-4 fold) but also the pravastatin exposure by up to 2-8 fold (vs pravastatin alone). This suggests drug metabolism interaction in animals, and the hence the toxicity. All dose combination produced decreases in mean body weight vs pravastatin controls (by 5-13% in males) and at two HD combinations in females (by 5-8%). Two high dose combination increased ALT/AST/AP in males (by 2 fold vs pravastatin controls), SDH (by 4-6 fold). In females liver weights increased with all the combinations by 18-43% compared to pravastatin alone. **Toxicity was observed in the liver in females at all doses with higher severity scores (biliary hyperplasia and hepatocellular hypertrophy at the lowest combination in 10/10 vs 9/10 in pravastatin controls, but 3/10 rats in the LD combination had higher severity scores). At two HD combination in males higher toxicity was noted in the liver, stomach** (acanthosis), and skeletal muscle, and one

HD male was sacrificed moribund on day 42 with evidence of myofiber degeneration of skeletal muscle. Since, at 250/50 and 750/250 mg/kg/day of combination (SCH 58235/pravastatin), there were more decreases in body weights, weight gains, increased transaminases, and histopatholgic changes in the liver, muscle and stomach, **the tolerated doses of the combination drug in 3-month toxicity study in male rats may be 250/25 mg/kg of SCH-58235/pravastatin. In females, all combination doses** increased liver weights, and produced more severe liver findings, therefore NOAEL in females was lower than the lowest dose (<15/25 mg/kg/day of SCH 58235/pravastatin).

2. In a dog, 3-month combination (drug + pravastatin) toxicity study, SCH-5823- doses (of 3, 3, 30, 30 mg/kg/day) were used, in combination with pravastatin (1, 5, 5, 10 mg/kg/day). In addition one group of dogs received vehicle or pravastatin alone at 10 mg/kg/day. The total drug exposure was increased in dogs only at low doses, and $AUC_{0\text{-}24h}$ values on day 29 were 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. These values on day 0 were 0.63, 0.51, 6.0, 2.2 µg.h/ml respectively. The combination did not significantly effect the total ezetimibe exposures, or pravastatin exposures (vs pravastatin alone) in dogs, which suggests no drug metabolism interaction in dogs, but toxicity was still seen in the liver and thymus. All combinations produced increased ALT in both sexes (by 2-18 fold vs pravastatin control, with three HD combinations producing synergistic increases in liver enzymes). At MD-HD combinations (3/5, 30/5, 30/10 mg/kg/day), AST (by 1.5-2 fold vs pravastatin control) & AP levels (by 1.3-3 fold vs pravastatin control) were increased. All combinations significantly decreased cholesterol and TG levels. At 3/5 to 30/10 mg/kg/day (SCH 58235/pravastatin), liver toxicity (bile duct hyperplasia in 0/8, 0/8, 0/8, 1/8, 3/8, 2/8 respectively, pigment accumulation in kupffer cells consistent with lipofuscin in hepatocytes 0/8, 0/8, 0/8, 3/8, 5/8, 4/8 respectively) was observed. HD combination (30/10 mg/kg/day) also produced toxicity in the skin (histiocytoma, inflammation, hyperkeratosis 2-3/4 dogs) and lungs (vacuolated alveolar macrophages ¾ males vs ¼ pravastatin controls), and all combinations produced toxicity in the thymus in males (0/4, ¼, 2/4, 2/4, ¾, 2/4 respectively). No NOAEL in this 3-month dog study could be established for the combination. Therefore, NOAEL may be < 3 mg/kg/day of SCH 58235 + < 1 mg/kg/day pravastatin, as all doses increased liver enzymes (ALT), and slightly higher doses produced liver toxicity in dogs. We concur with the sponsor that no dose effect level could be established in this study due to liver toxicity.

3. In a rat, 3-month combination (drug + atorvastatin) toxicity study, SCH-5823- doses (of 15, 15, 250, 250 mg/kg/day in males, and 15, 15, 50, 50 mg/kg/day in females by diet) were used, in combination with atorvastatin (10, 30, 30, 100 mg/kg/day). In addition one group of rats received vehicle or atorvastatin alone at 100 mg/kg/day. The total drug (drug + glucuronide) exposure was increased in rats in week 5 vs day 0 ($AUC_{0\text{-}24h}$ values in males at high dose combination were 19.6 vs 14.2 µg/ml.hr in week 5 vs on day 0, in females these values were 16.6 vs 6.3 ug/ml.hr). The combination not only increased the total drug expsoure (by 1.5-2 fold) but also increased the atorvastatin (and para-hydroxy atorvastatin) exposure by almost by 2 fold in females in week 5 (vs atorvastatin alone), but not so in males. This suggests drug metabolism interaction in animals, and hence the toxicity. Mid and high dose combinations produced decreases in mean body weight vs atorvastatin controls (by 9-14% in males, 4-7% in females). In females, all combination doses produced toxicity in the liver (increased liver weights in females by 23-28% compared to atorvastatin alone, and hepatocellular hypertrophy 5/10, 10/10, 10/10, 10/10 vs 4/10 in atorvastatin controls), and spleen (pigment accumulation, hemosiderin, hematopoiesis, congestion in 0/10, 0/10, 3/10, 2/10, 3/10,

1/10 respectively). In males, mid and/or high dose combinations produced toxicity in the liver (hyperplasia, biliary hypertrophy, single cell necrosis in 5/10, 5/10 vs 3/10 in atorvastatin controls), heart (mononuclear cell infiltration 2/10, vs 1/10 in atorvastatin controls), prostate (cellular infiltration of mononuclear cells and macrophage uretheral 4/10 vs 1/10 in atorvastatin controls), and testes (atrophy in ST, focal, mild to severe 1/10, 1/10 vs 0/10 in atorvastatin controls). The NOAEL in this 3-month rat study for SCH 58235/atorvastatin was 15/30 mg/kg/day in male. In females, the NOAEL could not be identified due to liver toxicity, and was less than the lowest dose (<15/10 mg/kg/day).

4. **In a dog, 3-month combination (drug + atorvastatin)** toxicity study, SCH-5823 – doses (of 0.3, 3, 3, 30 mg/kg/day) were used, in combination with atorvastatin (1, 1, 10, 10 mg/kg/day). In addition one group of dogs received vehicle or atorvastatin alone at 10 mg/kg/day. The total drug exposure in dogs increased only at two HD combinations, in week 5 vs day 0 ($AUC_{0\text{-}24h}$ values were 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). These values on day 0 were 0.12, 0.66, 0.52, 3.4 µg.h/ml respectively, suggesting accumulation at two HD combinations. The combination did not significantly effect the total ezetimibe exposures, or atorvastatin, ortho-hydroxy atorvastatin, or parahydroxy-atorvastatin exposures (vs atorvastatin alone) in dogs, which suggests no drug metabolism interaction in animals, but toxicity was still seen in the liver at all doses. The atorvastatin expsoures in week 5 with all combinations were 23, 21, 260, 329 ng.h/ml vs atorvastatin alone 473 ng.h/ml. All combinations produced increased ALT in both sexes (by 2-40 fold vs atorvastatin control). At two HD combinations, AST (by 1.5 fold vs atorvastatin control) & AP levels (by 3 fold vs atorvastatin control) were increased, total protein and albumin were decreased, and liver weights were decreased by 21-26%. All combinations significantly decreased cholesterol and TG levels. At 3/10 to 30/10 mg/kg/day (SCH 58235/atorvastatin), liver toxicity (bile duct hyperplasia 0/8, 2/8, 0/8, 0/8, 7/8, 6/8 respectively, kuffer cell hypertrophy with increased pigment accumulation consistent with lipofuscin 0/8, 2/8, 2/8, 2/8, 6/8, 7/8 respectively, increased eosinophilia of hepatocytes 0/8, 2/8, 2/8, 3/8, 8/8, 8/8 respectively) was observed. HD combination produced additional toxicity in the heart in male dogs (hemorrhage acute focal in ¼ vs 0/4 in atorvastatin control) and lungs (fibrosis or hemorrhage at two high doses in 1/8, 1/8 vs 0/8 in atorvastatin controls). No NOAEL in this 3-month dog study could be established for the combination. Therefore, NOAEL may be < 0.3 mg/kg/day of SCH 58235 + < 1 mg/kg/day of atorvastatin, as all doses increased liver enzymes (ALT), and the fact there was a dose related increase in liver enzymes (with two HD combinations producing synergistic increases in liver enzymes) and histopath changes in the liver with increase in doses. We concur with the sponsor that no dose effect level could be established in this study due to liver toxicity.

5. **In a rat, 3-month combination (drug + simvastatin)** toxicity study, SCH-5823 – doses (of 50, 250, and 250 mg/kg/day in males and 12, 50, and 50 mg/kg/day in females by diet) 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 total drug (drug + glucuronide) exposure was increased in rats on day 57 vs day 1 ($AUC_{0\text{-}24h}$ values in males at high dose combination were 48.6 vs 9.5 µg/ml.hr on day 57 vs day 1, in females these values were 14.6 vs 4.0 µg/ml.hr). The accumulation ratios were between 0.9-5.3. The combination not only increased the simvastatin and hydroxysimvastatin exposure by up to 2-6 fold than with simvastatin alone, but also increased the total drug exposure in males by up to 4 fold (at a high dose combinations), not so in females. This

suggests drug metabolism interaction in rats, and the hence the toxicity. All dose combinations produced decreases in mean body weight vs controls (by 6-13% in males at all doses, and by 9% in females at a high dose). High dose combination increased ALT in males (70 vs 33-57 in vehicle or simvastatin controls), AP at all doses (316-382 vs 168), GGT at all doses (3-5 vs 12 in controls). In females liver weights increased with the combination by 60-73% vs 27-28% with simvastatin alone (7.8-9.0 vs 7.2 g with simvastatin alone). Toxicity was observed in the liver (in females at high dose combo hepatocellular hypertrophy 5/10 vs none in any controls), and stomach (acanthosis at high dose in 8/20 vs 2/20 simvastatin controls, hyperkeratosis 8/20 vs 3/20 simvastatin controls, submucosal edema 9/20 vs 1/20 in simvastatin controls). Since, at high-mid, and high-high dose combinations, there were more decreases in body weights, weight gains, increased transaminases, and histopatholgic changes in the liver and stomach, the tolerated doses of the combination drug in 3-month toxicity study in male rats may be 50 mg/kg/day of SCH-58235 + 10 mg/kg of simvastatin, In females the NOAEL was 12 mg/kg/day of SCH-58235 + 10 mg/kg of simvastatin.

6. In a dog, 3-month combination (drug + simvastatin) toxicity study, with 1-month recovery period, SCH-5823 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. One group of dogs also received simvastatin alone at 10 mg/kg/day. The total drug (drug + glucuronide) exposure was not altered on day 56 vs day 1 ($AUC_{0\text{-}24h}$ values in M+F at high dose combination on day 56 were 4010 vs 5350 ng.hr/ml on day 1). The combination did not increase the SCH 58235 exposure, but exposure to simvastatin and hydroxysimvastatin increased by 2.5 and 1.7 fold (vs simvastatin alone), suggesting drug metabolism interaction in dogs. In all treated dogs, minimal to marked increases in serum ALT was observed compared to vehicle controls (6/8, 8/8, 8/8, 12/12 vs 5/8 with simvastatin alone). 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). 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 with mid-high dose combinations, but these were reversible after discontinuation of the drug. In liver, minimal hepatocytic cytoplasmic eosinophilia was observed in 1-2/ 4 dogs at low-mid dose combination, but in 5-6/6 dogs at high dose combinations, along with biliary hyperplasia (4-5/6 dogs vs none in simvastatin controls). Since liver histopathology was observed at all combinations and was not reversible, the tolerated doses of the combination drug in 3-month toxicity study in dogs with (SCH-58235 + simvastatin) could not be established, and was < 0.3/1 mg/kg/day of SCH 58235/simvastatin.

7. In a rat, 3-month combination (drug + lovastatin) toxicity study, 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 vs day 0 ($AUC_{0\text{-}24h}$ values in females at high dose combination in week 5 were 69.2 vs 9.0 µg.hr/ml on day 0, in males these values were 81.2 vs 11.0 µg.hr/ml on day 0). The AUC values of total ezetimibe in week 5 in males were 4.5, 7.5, 116, 81 µg.hr/ml respectively and in females were 1.7, 8.1, 32.1, 69.2 µg.hr/ml respectively. 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. This suggests drug metabolism interaction in animals. At two high dose combinations 2/20 rats died/group and these deaths were attributed to the drug related skeletal muscle degeneration and/or hepatocellular single cell necrosis. Two highest dose combinations produced clinical signs (alopecia of the hindquaters, urogenital staining, abnormal thinness, chromorhinorrhea, hypoactivity), decreased body weights (by up to 11-15%), increased serum transaminases (ALT were not increased significantly but AST/AP values increased by up to 2-5 fold), decreased globulin (2.7-3.2 vs 1.6 g/dl in controls), and slightly increased albumin/globulin ratios (1.7-1.8 vs 1.3-1.4 in both controls). The liver weights in females were increased by 14-20%, and toxicity was observed in the liver including mitotic figures in 6/20, 2/20, 11/20, 12/20 at all combinations vs 0/20, 4/20 in vehicle & lovastatin controls, single cell necrosis at two HD combinations (in 17/20, 17/20 rats vs 5/40 in the vehicle/lovastatin controls), periportal hepatocellular hypertrophy (in 18/20, 18/20 vs 10/40 in both controls), hepatocellular vacuolation (in 4/20, 3/20, vs 0/20 in controls), pigment accumulation (1, 1, vs 0 in controls), mid zonal/focal necrosis (4, 5, vs 0 in controls), Kupffer cell hypertrophy (2, 2, vs 1 in lova controls), centrolobular hypertrophy (14, 14 vs 1 in lova controls), and biliary hyperplasia (19, 19, vs 14 in lova control). Target organs of toxicity at two HD combinations were also skeletal muscle (myofiber degeneration/regeneration, mixed cellular infiltration, interstitial-edema/fibrosis), and glandular stomach (single cell necrosis). Since, all dose combinations increased mitotic figures in the liver, the tolerated doses of the combination drug (SCH-58235/lovastatin) in 3-month toxicity study in rats could not be established. NOAEL doses were <50/10 mg/kg/day of SCH 58235/lovastatin in males (and <12/10 mg/kg/day in females)

8. **In a dog, 3-month combination (drug + lovastatin)** toxicity study, 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 increased on day 31 vs day 0 ($AUC_{0\text{-}24h}$ values in M+F at high dose combination on day 31 were 4610 vs 3100 ng.hr/ml on day 1). The combination 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. The expsoures of lovastatin and hydroxylovastatin increased more in females than males, this may explain the statin like cholestasis in livers of female dogs. The combination (at all doses) increased the serum ALT in all dogs (by 2-28 fold). In mid-high and high-dose combination groups, mean serum ALT increase was moderate to severe (10-20 fold above lovastatin control), AST and AP also increased by 2-3 fold above lovastatin controls, whereas total protein, albumin and A/G ratios (0.7-0.9 vs 1.1 in both controls) were decreased. The two HD combinations increased liver toxicity (bile duct hyperplasia, Kupffer cell hypertrophy in 8/8, 7/8 dogs vs 2/8 in lovastatin controls, and cholestasis 2/4 females vs none in controls), and testicular toxicity (mild spermatogenic alteration and luminal cellular debris in 1/4, 2/4 vs none in control dogs). 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 was significantly increased, and histopatholgic changes in the liver (bile duct hyperplasia, Kupffer cell hypertrophy and cholestasis), and testis were noted, 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.

Note that the measurement of lipid soluble vitamins (A & D) with the combination or with ezetimibe alone were not examined in the chronic animal tox studies. Sponsor states that in general no NOAELs could be identified in ezetimibe/statin dog studies mainly

because of increased serum ALT levels. Bile duct hyperplasia was also a consistent hepatic finding with the combination of ezetimibe + statins. Dog to human exposure multiples at NOEL and LOEL for bile duct hyperplasia in the 3-month studies are shown below:

Table 11 Dog-to-Human Exposure Multiples for Bile Duct Hyperplasia

	NOEL		LOEL	
	Dose[a] (mg/kg)	Exposure Multiple	Dose[a] (mg/kg)	Exposure Multiple
Ezetimibe/Simvastatin (SN 96417)[58]	30/1		30/10	
Total Ezetimibe		9.3		4.8
Simvastatin[b]		1.5		34.4
β-Hydroxy Simvastatin[b]		8.3		86.8
Ezetimibe/Lovastatin (SN 99013)[59]	0.3/2		30/20	
Total Ezetimibe		0.1		3.8
Lovastatin		2.4		80.2
β-Hydroxy Lovastatin		6.3		82.6
Ezetimibe/Pravastatin (SN 99490)[60]	3/1		3/5	
Total Ezetimibe		2.1		1.1
Pravastatin		3.7		22.6
Ezetimibe/Atorvastatin (SN 99501)[61]	3/1		3/10	
Total Ezetimibe		1.1		1.2
Atorvastatin		1.0		12.7
Ortho-Hydroxy Atorvastatin		0.8		19.6
Atorvastatin (SN 99501)[61]	NA[c]		0/10	
Atorvastatin		-		22
Ortho-Hydroxy Atorvastatin		-		16.4

Note:Exposure multiples are based on ratio of AUC values at clinical doses of 10 mg (ezetimibe, simvastatin and atorvastatin) or 20 mg (lovastatin and pravastatin).

a: Doses are presented as dose of ezetimibe/dose of statin

b: Exposure multiples, based on a LOEL of 30 mg/kg of ezetimibe and 10 mg/kg of simvastatin, exclude the minimal bile duct hyperplasia observed in one dog given 3 mg/kg of ezetimibe and 1 mg/kg of simvastatin. In retrospect, the relationship of this finding to test article administration is considered equivocal based on occurrence in a single animal consistent with background incidence, lack of dose response, and results of subsequent coadministration studies. If this dog is not excluded, exposure multiples at the NOEL are <1x for total ezetimibe, 1.2x for simvastatin and 8.0x for β-hydroxy simvastatin.

c: Not Applicable; 10 mg/kg was the only dose of atorvastatin tested in SN 99501.

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B3. Mutagenicity: combination of SCH 58235 + statins (pravastatin, atorvastatin, simvastatin and lovastatin) was not mutagenic/cytogenic in the following 3 tests: Ames test, in vitro mammalian chromosome aberration test in human lymphocytes, and in vivo micronucleus test in mice.

B4. Reproductive and Developmental Toxicology Studies on Combination Therapy (SCH 58235 + statins)

1. In a segment II teratology study with **drug+pravastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + pravastatin (125, 250, 500 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or pravastatin alone (500 mg/kg/day). Maternal exposures of the total drug on GD 17 were 9.8, 11.2, 30 µg.h/ml at 1000/20, 1000/250, 1000/500 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, exposures to total drug were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by 2-6 fold when SCH 58235 was co-administered with pravastatin. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Exposure to pravastatin free acid when co-administered with SCH 58235 increased > dose proportional as the pravastatin dose increased from 125 to 500 mg/kg resulting in exposure of 1.7X (18.2 with SCH 58235 + pravastatin vs 10.9 µg.h/ml with pravastatin alone). The sponsor attributes increased salivation to pravastatin at doses ≥250 mg/kg/day. The HD combo (SCH 58235 with pravastatin 500 mg/kg/day) significantly

decreased the body weight gain vs the controls (61* g vs 64-72 g in the vehicle/pravastatin controls) and produced a small increase in fetal visceral variation of dilated ureters (which the sponsor attributes to pravastatin). The HD combo generally increased the incidences (fetal/litter) of skeletal variations consisting of reduced ossification of sternebrae, parietal bone, proximal phalanges (hind paws) compared to pravastatin alone (500 mg/kg/day). These increases are within historical control range, but exceed the mean. **Maternal and developmental NOAELs were both 1000 mg/kg SCH 52835 + 250 mg/kg pravastatin** based on statistically significant decreases in body weight gain at HD (1000 SCH + 500 pravastatin mg/kg/day) in mothers, and general increases in incidences in reduced skeletal ossifications in fetuses at HD combination compared to pravastatin alone (500 mg/kg/day).

2. In a segment II teratology study with **drug+pravastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + pravastatin (5, 25, 50 mg/k/day) from day 6-19 of gestation. Control animals received the vehicle or pravastatin alone (50 mg/kg/day). Maternal exposures of the total drug on GD 19 were 139, 135, 145 µg.h/ml at 1000/5, 1000/25, 1000/50 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with pravastatin. Systemic exposure to unconjugated SCH 58235 was <0.2% of exposure to total SCH 58235. Coadministration of SCH 58235 with pravastatin 50 mg/kg (AUC= 10 µg.h/ml) had no effect on the mean systemic exposure to pravastatin 50 mg/kg/day (9.7 µg.h/ml). All combo doses produced higher fecal findings (fecal stained fur, discolored or soft stool, reduced fecal pellets) than the vehicle or pravastatin control. High dose combo group had significantly decreased BW gain during GD 7-30 (0.23* vs 0.34 kg, *p<0.01). Food consumption was transiently decreased in all combo groups during GD 8-9. 25 and 50 mg/kg pravastatin + 1000 mg/kg SCH 58235 produced malformations of shortened or kinked tail (1/16 & 2/19 litters with 1-2 fetuses effected) along with fused caudal vertebra (5.3-6.9% vs 0% in controls or pravastatin group). A HD combo increased the incidences of skeletal variations such as sternbrae bipartite (31.6% vs 22% with prava alone) and extra pair of thoracic ribs (100% vs 83% with prava alone). **NOAEL for maternal toxicity was 25 mg/kg pravastatin + 1000 mg/kg SCH 58235**, as the higher doses (50 mg/kg pravastatin + 1000 mg/kg SCH 58235) produced significant decreases in BW gain in rabbits. **Developmental NOAEL was 5 mg/kg pravastatin + 1000 mg/kg SCH 58235**, as mid and higher combination doses produced external malformations (in tail) as well as fused caudal vertebrae compared to pravastatin or control group. Sponsor's no effect level doses for both maternal and embryo-fetal toxicity were higher (i.e. 1000 mg/kg/day SCH 58235 and ≤ 50 mg/kg/day pravastatin), despite the fact they acknowledge that HD combo produced decreases in BW gain in rabbits, and Mid-high dose combination of SCH 58235 +pravastatin produced malformations in rabbit fetuses.

3. In a segment II teratology study with **drug+simvastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + simvastatin (5, 10, 25 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or simvastatin alone (25 mg/kg/day). Maternal exposures of the total drug on GD 17 were 3.6, 4.7, 8.1 µg.h/ml at 1000/5, 1000/10, 1000/25 mg/kg/day respectively. In a previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, total drug exposures were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by ≈2 fold when SCH 58235 was co-administered with 25 mg/kg/day of simvastatin. Systemic exposure to

unconjugated SCH 58235 was <2% of exposure to total SCH 58235. Exposures to simvastatin and hydroxy-simvastatin increased by 1.4 to 2 fold at the HD combination (with SCH 58235 + 25 mg/kg/day simvastatin). Fetal body weights were decreased with simva alone (5.3* vs 5.6 g in controls). All combination groups had an increased incidence of right carotid and subclavian arteries arising from the aortic arch in the visceral exam, and in the HD combination group the right subclavian artery was absent. HD combination had increased incidences of bipartite sternebrae which exceeded historical mean and range and increased incidences of a skeletal malformation (hemivertebrae). The maternal NOAEL was 1000 mg/kg SCH 58235 + 25 mg/kg simvastatin. Developmental NOAEL was 1000 mg/kg SCH58235 + 10 mg/kg simvastatin based on visceral findings (involving blood vessels and skeletal malformations and variations in combination with 25 mg/kg simvastatin).

4. In a segment II teratology study with **drug+simvastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + simvastatin (1, 5, 10 mg/k/day) from day 6-19 of gestation. Control animals received the vehicle or simvastatin alone (10 mg/kg/day). Maternal exposures of the total drug on GD 19 were 99, 104, 114 µg.h/ml at 1000/1, 1000/5, 1000/10 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with simvastatin. Systemic exposure to unconjugated SCH 58235 was <2% of exposure to total SCH 58235. Coadministration of SCH 58235 with simvastatin 10 mg/kg (AUC= 20 ng.h/ml) had no effect on the mean systemic exposure to simvastatin 10 mg/kg/day (21 ng.h/ml) or to hydroxysimvastatin. Two of 20 rabbits at HD combination died or were sacrificed, one death was attributed to mechanical injury. ALT was mildly increased for all groups including simvastatin alone on GD 20 (92-172 IU/L vs. baseline 28-35 IU/L). Some animals reached moderate levels (max. 489 IU/L) approximately 10X increase from baseline. Similarly AST was minimally increased for all groups (47-56 IU/L) including the simvastatin alone group compared to baselines of 26.3-26.6 IU/L. MD & HD combination (5 and 10 mg/kg simvastatin + 1000 mg/kg SCH 58235) not only produced short, filamentous tails with fused caudal vertebra and reduced numbers of caudal vertebra (2/18 litters in both groups, with 5 & 2 fetuses effected respectively), but also heart malformations (in two fetuses each with MD/HD combo groups). In the MD combo one fetus had ventricular septum defect (membraneous and muscular) and thickened ventricular valve with one small and one enlarged atrial chamber. In the HD combo 2 fetuses had multiple heart malformations, one fetus had a small heart with a ventricular septum defect (membranous) and a small atrial chamber and the other fetus had a small atrial chamber. At HD combination, skeletal findings such as scoliosis, scrambled lumbar vertebra, hemivertebrae, fused and/or bifurcated ribs were present. NOAEL not established for maternal toxicity based on transient food consumption and fecal changes, according to the sponsor. However, maternal NOAEL was1000 mg/kg SCH 5832 +1 mg/kg simvastatin due to premature deaths of 2 dams in the HD combination. Developmental NOAEL was 1 mg/kg simvastatin + 1000 mg/kg SCH 58235 based on increased external and visceral malformation compared to controls.

5. In a segment II teratology study with **drug+atorvastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (25, 50, 100 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or atorvastatin alone (100 mg/kg/day). Maternal exposures of the total drug on GD 17 were 8.6, 21.3, 66.2 µg.h/ml at 1000/25, 1000/50, 1000/100 mg/kg/day respectively. In a

previous rat study of SCH 58235 with 1000 mg/kg/day on GD 15, exposures were 4.9 µg.h/ml (study #96383). Thus exposure to total drug increased by 2-10 fold when SCH 58235 was co-administered with atorvastatin. Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Exposures to atorvastatin free acid, para-hydroxy atorvastatin, and ortho hydroxy atorvastatin were all slightly decreased when high dose of atorvastatin (100 mg/kg/day) was co-administered with SCH 58235. The HD combination significantly decreased the gestation body weight gain (by 10%, 62* g vs 69 g in the control), food consumption (by 8%, 22 vs 24 g/rat/day in controls) in rats, decreased the mean body weights of fetuses, and produced increased incidences of skeletal variations (reduced ossification of sternebrae which sponsor attributes to decreased fetal body weight). Maternal/Developmental NOAEL was 1000 mg/kg SCH 58235 + 50 mg/kg atorvastatin, based on decreased maternal/fetal body weights, maternal FC and increased incidence of reduced ossification of sternebrae.

6. In a segment II teratology study with **drug+atorvastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + atorvastatin (5, 25, 50 mg/k/day) from day 7-19 of gestation. Control animals received the vehicle or atorvastatin alone (50 mg/kg/day). Maternal exposures of the total drug on GD 19 were 124, 132, 149 µg.h/ml at 1000/5, 1000/25, 1000/50 mg/kg/day respectively. In a previous rabbit study of SCH 58235 with 1000 mg/kg/day on GD 19, exposures to total drug were 113 µg.h/ml (study #96385). Thus exposure to the total drug did not significantly increase when SCH 58235 was co-administered with atorvastatin (124-149 in the present study vs 113 µg.h/ml with the drug alone in a previous study). Systemic exposure to unconjugated SCH 58235 was <1% of exposure to total SCH 58235. Coadministration of SCH 58235 with atorvastatin 50 mg/kg increased the exposure to atorvastatin free acid by 1.5 fold (323 vs 214 ng.h/ml) and to ortho-hydroxy atorvastatin by 2.5 fold (1441 vs 584 ng.h/ml), but had no effect on the mean systemic exposure to para-hydroxy atorvastatin (167 vs 152 ng.h/ml). All combination doses produced increased clinical signs (a reduced number and small fecal pellets in 6-8/20 rabbits vs 0-1/20 in vehicle or atorvastatin controls), decreased food consumption in rabbits (by 30, 33 & 53% respectively), and HD combo produced decreased BW gains by 38%. All combination doses produced skeletal malformations (fused caudal vertebra and sternebrae), MD & HD combination (25 and 50 mg/kg atorvastatin + 1000 mg/kg SCH 58235) produced visceral malformations (gallbladder absent, ectopic/misshapen kidneys), and HD combination produced external malformations of kinked tail (1.3% in fetuses and 5.9% in litters) and increased skeletal variations (sternebrae assymetrical). NOAEL could not be established for the maternal toxicity as all combination doses decreased food consumption and produced fecal changes, and HD combo decreased BW gain. Maternal NOAEL and developmental NOAEL were both 1000 mg/kg SCH 58235 + <5 mg/kg/day atorvastatin. Developmental NOAEL was based on increased skeletal malformations (fused caudal vertebra and sternabra) at all combination doses, but increased external (kinked tail) and visceral malformations (gallbladder absent) were observed at MD and/or HD combo. Sponsor's no effect level (NOEL) for both maternal and in utero effects was also < 5 mg/kg/day atorvastatin + 1000 mg/kg SCH 58235.

7. In a segment II teratology study with **drug+lovastatin in rats**, pregnant animals (n=25/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + lovastatin (10, 25, 50 mg/k/day) from day 6-17 of gestation. Control animals received the vehicle or lovastatin alone (50 mg/kg/day). Maternal exposures of the total drug or lovastatin were not evaluated in this study. The HD combination group had significantly decreased food

consumption (by 9%), and a net body weight change (by 12%). No viable fetuses in 1/23 rats. All combination doses of SCH 58235 + lovastatin produced higher incidences of skeletal variations (sternebra unossified, and focal thickening of ribs compared to vehicle or lovastatin 50 mg/kg/day alone), and skeletal malformations (fused ribs vs none with the vehicle or lovastatin control), sponsor considers these not drug related, because these were single incidences. However these single incidences were seen at all doses vs none in both control groups. Extra thoracic ribs were observed at MD & HD combinations vs lovastatin controls. There is maternal toxicity at all lovastatin doses, which probably accounts for the skeletal variations seen in the combination groups, although there are increased incidences at HD combination. Maternal NOAEL=1000 mg/kg SCH 52835 + 25 mg/kg lovastatin based on statistically significant decreases in FC, and no viable fetuses in one female at 1000 SCH + 50 lovastatin mg/kg group. Developmental NOAEL was1000 mg/kg SCH 52835 + <10 mg/kg lovastatin based on the increased incidence of skeletal variations/malformations observed with all combo doses (1000 mg/kg SCH 52835 + 25 to 50 mg/kg lovastatin compared to lovastatin alone 50 mg/kg/day).

8. In a segment II teratology study with **drug+lovastatin in rabbits**, pregnant animals (n=20/group) were given oral SCH 58235 (1000 mg/kg/day by gavage) + lovastatin (2.5, 10, 25 mg/k/day) from day 7-19 of gestation. Control animals received the vehicle or lovastatin alone (25 mg/kg/day). Maternal exposures of the total drug or lovastatin were not evaluated in this study. In the LD and HD combination groups, 1/20 and 2/20 rabbits aborted vs none in other groups. All combination doses produced increased early resorptions (8-20% vs 1-3 in vehicle and lovastatin control), and increased incidences of post implantation losses (9-21% vs 3-4% in controls). Also decreases in live fetuses at MD and HD combo (126-140 vs 160-176 with vehicle & lovastatin controls), none of these repro data reached the statistical significance. Mid and high dose combination groups had higher incidences of skeletal variations such as sternebra fused (litter incidences 5-6% vs none in controls) and forelimb distal humeral/epiphysis unossified (11-25% vs 5% in controls). Omphalocele at MD and shortened tails at ≥ MD occurred in a single litter. Maternal and developmental NOAEL was 1000 mg/kg SCH 52835 + <2.5 mg/kg lovastatin based on the increased incidences of abortions in mothers at low and high doses, and post implantation losses and early resorptions observed with all combination doses (1000 mg/kg SCH 52835 + 2.5 to 25 mg/kg lovastatin compared to vehicle or lovastatin alone 25 mg/kg/day). Sponsor's NOAEL for maternal and developmental toxicity is 1000 mg/kg SCH 52835 + 2.5 mg/kg lovastatin, based on soft stool findings in 1/25 rabbits at MD and HD combination, and developmental is based on increased resorptions (fetal incidences 5, 2, 13, 33, 18% respectively, litter incidences 2.6, 1, 7.7, 19.6, 12.3% respectively) and abortions at mid and/or high combination doses

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

Repro-tox study	**NOAEL's**	
Segment II study in rats and rabbits with SCH 58235 + pravastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +250 mg/kg/day of pravastatin (or ≈ 16 fold the human AUC exposures,	1000 mg/kg/day of SCH 58235 +250 mg/kg/day of pravastatin (or ≈ 16 fold the human AUC exposures,

	based on 10 mg/day dose of SCH 58235 alone in non-pregnant adult humans).	based on 10 mg/day dose of SCH 58235 alone in adult non-pregnant humans).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of pravastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +5 mg/kg/day of pravastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + simvastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of simvastatin (or ≈ 12 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +10 mg/kg/day of simvastatin (or ≈ 7 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 +1 mg/kg/day of simvastatin (or ≈ 150 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +<1 mg/kg/day of simvastatin (or ≈ 150 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + atorvastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +50 mg/kg/day of atorvastatin (or ≈ 30 fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).	1000 mg/kg/day of SCH 58235 +50 mg/kg/day of atorvastatin (or ≈ 30fold the human AUC exposures, based on 10 mg/day dose in humans of the drug SCH 58235 alone).
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 + <5 mg/kg/day of atorvastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).	1000 mg/kg/day of SCH 58235 + <5 mg/kg/day of atorvastatin (or ≈ 200 fold the human AUC exposures, based on 10 mg/day dose of SCH 58235 alone).
Segment II study in rats and rabbits with SCH 58235 + lovastatin		
	Maternal NOAEL	**Developmental NOAEL**
Segment II study in rats	1000 mg/kg/day of SCH 58235 +25 mg/kg/day of lovastatin (no exposures were available).	1000 mg/kg/day of SCH 58235 +<10 mg/kg/day of lovastatin.
Segment II study in rabbits	1000 mg/kg/day of SCH 58235 + <2.5 mg/kg/day of lovastatin (no exposures were available).	1000 mg/kg/day of SCH 58235 + <2.5 mg/kg/day of lovastatin.

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

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

Expsoure multiples in animals to humans with combination studies (ezetimibe + stain) are given below, using the toxic doses in segment II studies in rats and rabbits.

Animal to human exposure multiples of total ezetimibe and statins with the combination (ezetimibe 1000 mg/kg/day + statin), based on 7-14 day PK studies in humans.

	Human AUC (0-24h)	**Rat exposures (AUC_{0-24h}) & toxic doses**			**Rabbit exposures (AUC_{0-24h}) & toxic doses**		
	Human ng.h/ml	Toxic doses mg/kg/d	Rats µg.h/ml	Exposure multiples	Toxic doses mg/kg/d	Rabbits µg.h/ml	Exposure multiples
1) Pravastatin	70	1000/500	(1000/ 125,250,500 mg/kg/d) 2, 4.8, 18.2	260X	100/25	(1000/ 5, 25, 50 mg/kg/d) 1.2, 4.2, 10.0	60X
T. ezetimibe	711		9.8, 11.2, 30	42X		139, 135, 145	200X
2) Simvastatin	8	1000/25	(1000/ 5, 10, 25 mg/kg/d) 18, 43, 163* ng.h/ml	20X	1000/5	(1000/ 1, 5, 10 mg/kg/d) 1, 11, 20* ng.h/ml	1X
OH-simvastatin	6		0.9, 2.4, 9.3	1500X		0.13, 1.4, 1.8	233X
T. ezetimibe	---		3.6, 4.7, 8.1	11X		99, 104, 114	146X
3) Atorvastatin	22	1000/100	(1000/ 25, 50, 100 mg/kg/d) 0.85, 6.2, 7.9	350X	**1000/25**	(1000/ 5, 25, 50 mg/kg/d) 32, 199, 323* ng.h/ml	9X
OH-atorvastatin	19		1.2, 7.0, 7.7	400X		0.13, 0.62, 1.4	32X
T. ezetimibe	707		8.6, 21.3, 66	93X		124, 132, 149	186X
4) Lovastatin	21	1000/<10	(1000/ 10, 25, 50 mg/kg/d)[a]	N/A	**1000/<2.5**	(1000/ 2.5, 10, 25 mg/kg/day)[a]	N/A
OH-lovastatin	23						
T. ezetimibe	687						

Doses used for rat and rabbit studies are given in parenthesis.
*- values in ng.h/ml
[a] –No exposures were available in that study
NA-Not available

C. Safety Evaluation on Monotherapy And Recommendations: In 6 to 12-month chronic toxicity studies in rats and dogs at high doses (rats 1500 and 500 mg/kg/day in males and females respectively and dogs 300 mg/kg/day), the main target organs of

toxicity were heart (**rats**: mononuclear cellular infiltration in 8/15 vs 5/15, **dogs**: mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls), and lymph nodes (**rats**: accumulation in plasma cell in 8/15 vs 4/15 controls, **dogs**: lymphoid mesenteric hyperplasia, mesenteric & mandibular hemorrhage/erythrophagocytosis in 8/8 vs 4/8 controls). These histopath changes were not examined at low-mid doses in these studies, therefore it is unknown if there was a dose related trend in any of these findings. The heart toxicity was also seen in one month bridging studies, which were conducted with ezetimibe containing [redacted] impurities (as recommended by ICHQ3A guidelines) as these were present in the drug substance in amounts [redacted] In 1-month bridging studies, heart and lymph nodes were again a target organ in **rats** (heart: mononuclear cellular infiltration in 2/10 rats vs 0/10 controls, lymph nodes: hyperplasia in 1/10 vs 0/10 in controls) and **dogs** (heart: 1/4 males had an enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve, ¼ females had mononuclear cellular infiltration vs 0/4 controls, lymph nodes: brown pigment accumulation in 4/8 vs 0/8 in controls, and lymphoid hyperplasia in 2/8 vs 0/8 controls). In addition toxicity was noted in the kidney and spleen in rats and dogs. **The tissue distribution** studies of ezetimibe showed the presence of radioactivity in the myocardium after multiple dosing (up to 21 days) in male rats, but not after a single dose in either sex. Ezetimibe is a weak ACAT inhibitor, as indicated earlier that some ACAT inhibitors have previously produced cardiotoxicity in monkeys (multiple areas of fibrosis and mononuclear cellular infiltration, and PVC's), dogs (heart murmurs) and rats (degeneration/infiltration of mononuclear cells/myocarditis). The relevance of these findings in humans is unknown. **The above toxicities in heart and lymph nodes were observed at exposure levels of about 20X and 8-10X the human exposures** in rats and dogs respectively, based on human dose of 10 mg/day.

Ezetimibe produced malformations/variations in segment II study in rat fetuses (small size heart, short filamentous tail, vestigial right kidney/dilated renal pelvis, anasarca and increased skeletal variations at 500 mg/kg/day), and rabbit fetuses (agenesis of tail/ shortened tail, excencephaly, omaphalocele and increased skeletal variations at 250 mg/kg/day-1000 mg/kg/day), and in segment III study in rat fetuses (missing tail in F1 & F2 pups at 300 mg/kg/day). In rabbits maternal toxicity was observed at 1000 mg/kg/day (increased resorptions & altered sex distribution). The maternal Safety factor in rats in repro toxicity studies in rats (segment I, II) was ≈6-8 fold the human exposure. The maternal Safety factor in rabbits in segment II study was much higher (140 fold the human exposure). The safety factor in embryo fetal (developmental) studies in rats and rabbits was 4 and 100 fold respectively the human exposure. Mortalities are of some concern in the repro toxicity studies (as shown in the Table, page 373-374), however the incidences were low and it is unknown at this time if these are drug related, or as sponsor states incidental/accidental. Higher distribution of radioactivity was noted in the GI tract and contents in pregnant vs non-pregnant rats. Also in tissue distribution studies, low levels of radioactivity were observed in the heart in the pregnant rats, not seen in non-pregnant after a single dose. Although ezetimibe radioactivity was below the limit of quantification in fetal tissues and blood in distribution studies, the fetus exposure to ezetimibe was confirmed in segment II/III studies in rats and rabbits. In rats, on gestation day 20, the exposures to total ezetimibe (18.7 µg.h/ml) was 0.5 fold higher in the fetus than in the mother (12.2 µg.h/ml). On the other hand 10X higher levels of total drug were found in rabbits on gestation day 22 (181 µg.h/ml), but the exposure to total ezetimibe in fetuses (4.7 µg.h/ml) was almost 40 times lower than in the mother.

Ezetimibe was also transferred through the milk and values of total drug on lactation day 12 in fetus were half (11.3 µg.h/ml) of what is found in the mother (23.1 µg.h/ml). These data suggest that ezetimibe is present in the fetal blood during gestation, can be transferred to the newborn by mothers milk, and may pose a risk to pregnant women and nursing mothers/newborns.

Clinically relevant issues: Although heart and lymph nodes findings were consistent with the drug in a number of toxicity studies (one & 6-month rat/dog), these were observed at **20X and 8-10X the human exposures** in rats and dogs respectively, (based on human dose of 10 mg/day), which suggests sufficient safety of margin in humans, and these are not seen in clinical studies.

Recommendations: Therefore pharmacology recommends approval of ezetimibe for monotherapy for the proposed indication.

D. Safety Evaluation on Combination Therapy And Recommendations: In 3-month studies in rats, the combination of SCH 58235 + statins generally increased the exposure to both the drug (ezetimibe) and/or statin (or their active hydroxy acids) and toxicity could be explained based on a metabolic interaction of the two drugs. The target organs of toxicity in rats were liver, stomach and skeletal muscles (and sometimes the spleen, heart and prostate, see individual study). In male rats the lowest combination dose was generally a tolerated dose, but in female rats no NOAEL dose could be generally established as all doses produced toxicity. Generally the NOAEL dose for the combination was lower than that established for either ezetimibe or statin monotherapy. **In contrast in dogs**, the combination of SCH 58235 + statins did not generally increase the exposure to ezetimibe or to statin (except for simvastatin and lovastatin, see individual study), so the toxicity could not be explained based on the metabolic interaction of the two drugs. However, toxicity was still seen in dogs in the liver, (and sometimes in testes, heart, lungs, see individual study). In dogs, generally no NOAEL dose could be established with the combination as all doses produced toxicity in the liver, except for lovastatin (see individual study). **The increases in liver enzymes (ALT and/or AST, CGT) were generally seen with the combination in both rats and dogs, but increases in ALT were synergistic in dogs.** Based on chronic dog ezetimibe monotherapy studies, the toxicity observed with the combination appears to relate more to the statin component. In a 12-month dog ezetimibe monotherapy, NOAEL was 100 mg/kg/day, in the rat 6-month study it was 250 mg/kg/day (toxicity in 6-month rat study was bone marrow hyperplasia in males/mononuclear cell infiltration in heart, and glomerular nephropathy and adrenal hypertrophy/vacuolation in females). Generally, an increase in incidences/severity of the toxicity is observed in the target organ with the combination; but new toxicities have not been identified. However, body weight gain decreaments are seen in ezetimibe + statin combination that are not observed with monotherapy of either component.

[ ]

In **segment II study in rats** the combination of SCH 58235 + statins generally increased the exposure of both the drug and statins (or their acive hydroxy acids) by ≈ 2X and produced skeletal variations and other effects (see individual study). In the rat reduced skeletal ossification seen in pravastatin and atorvastatin combination may be

related to the maternal toxicity with the combination. However other statin combinations including those in rabbits show additional malformations, some of which appear independent of concurrent maternal toxicity. In the **segment II study in rabbits** the combination of SCH 58235 + statins did not generally increase the exposure of both the drug or the statins (except for atorvastatin) and produced malformations in tails, caused fused caudal vertebra, and produced visceral malformations of gallbladder, kidney and heart (see individual study)

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

Clinically relevant issues with the combination: The main target organs of toxicity with combination therapy in rats were liver, stomach and skeletal muscles (and sometimes the spleen, heart and prostate, see individual study). In dogs, it was mainly the liver (and sometimes testes, heart, lungs, see individual study). In general, NOAELs could not be established for the combination studies in rats/dogs. Note that this lack of safety margins in animals in relation to humans were communicated to the sponsor (in a t-con on 2/4/00) during IND ~~—~~ submission when ezetimibe combination studies with lovastatin and simvastatin in rats and dogs were submitted by the sponosr (see DFS sign off on 4/26/01). In fact it was communicated to the sponsor that "in animal studies, testing of other statins (pravastatin, atorvastatin, etc) in preclinical tox studies may not be helpful, since combination of the drug is being tested at statin doses in humans, that have no safety margin, it is unlikely that additional animal studies with other statins would give a better safety margin. The mechanistic studies of the drug interactions in humans may be more meaningful, using lower or safe doses of two drugs (SCH-58235 + statin)". However in clinical PK studies the combination of the drug (up to 10 mg/day) + approved statin (e.g simvastatin 10 mg/day) did not significantly alter the PK of the drug (SCH 58235) or statin in two week human studies, or produce any additional toxicity. **In the current NDA, sponsor also states that exposures multiples for combination therapy were not calculated since a 'no observed effect level' (NOEL) could not be defined in any combination toxicity study**. Therefore safety factors in humans are not available. However these toxicities could be monitored in humans, and currently no significant adverse events are seen in the liver, skeletal muscle, or stomach in humans in the clinical studies.

Final Recommendation on Monotherapy and on Combination Therapy:
From the preclinical standpoint, approval of this application for monotherapy is recommended, pending acceptable labeling modifications are made. The combination therapy with statins demonstrate exacerbated statin toxicity in rats & dogs. In some cases this can be attributed to a metabolic interaction or species sensitivity (dog sensitivity > human). However with some combinations, statin toxicity is seen at lower

doses than previously described for statin monotherapy. A NOAEL can not be established for any of the combinations in general. However the toxicity profile is well estalished for statins and is clinically monitorable. The appropriate statin warnings should be in place when administered in combination with statins. The approval of this application is left at the discretion of the medical reviewer.

Labeling Review: The following changes in labeling are recommended:

WITHHOLD 8 PAGE (S)

Draft

Labeling

NDA 21-445

X. Appendix

IND [redacted] Pharmacology review of Original IND: 3-Month rat and dog tox studies are reviewed in this submission.

IND [redacted] March 27, 1997

Schering Corporation
Kenilworth NJ

Submission: February 28, 1997

PHARMACOLOGY REVIEW OF ORIGINAL IND

DRUG: SCH 58235 (single enantiomer of optically active cpd; [redacted]

CATEGORY: Lipid Altering (inhibitor of cholesterol absorption)

CLINICAL PLAN: Phase II

RELATED INDs: [redacted]

Elizabeth Barbehenn, Ph.D.

cc: IND Arch
HFD-510
HFD-510/Steigerwalt/Barbehenn
sch58235.#01

SUBMITTED:

CHEMISTRY: (vol 1.2, 1.13, 1.14)
STRUCTURES (Mr: 410):

SCH 58235 has 3 chiral centers (and 8 possible isomers). All 8 have been synthesized.

[redacted]

Particle size: [redacted]

Assay (chemistry)

[redacted]

CLINICAL PLANS: (vol 1.1)
C96-345: **8-week** □pilot□ dose-ranging double-blind, randomized, study in **140 patients at 7 sites** (7 dose groups) at doses of **1, 5, 10, 20, or 40 mg SCH 58235 or placebo or 40 mg lovastatin** (20 pts/group x 7 groups). Drug is to be taken once daily before breakfast. *The only measurement of SCH 59235 will be at the last visit to measure trough levels of parent drug.*

COMPLETED STUDIES: (vol 1.16)
SINGLE, RISING-DOSE STUDY (I96-088): in healthy male volunteers between 18 and 30 years of age
(1, 5, 10, 20, 50 mg) after an overnight fast.
PK: The unchanged drug is □rapidly conjugated and both forms slowly eliminated with evidence of significant enterohepatic recycling□. The effective t1/2 was estimated to be 24 hours (see Table below).
ADR: increases in ALT & AST in one person at 5 mg

MULTIPLE RISING-DOSE STUDY (I96-139): 14 days in healthy volunteers between 18 and 39 years of age (10, 20, 50 mg once daily in the morning after an overnight fast). There were 9/g (tx) and 3/g (placebo).
PK: □not available at this time□

ADRs: increases in ALT & AST (3/9 at 10 mg; 2/9 at 20 mg + 2/3 placebo; and 3/9 at 50 mg. *One person at 50 mg dose was discontinued after 11 days because of a 4-fold increase in ALT which was still 2-fold elevated 13 days postdose.*

PHARMACOLOGY (vol 1.4)
7-DAY STUDIES IN CHOLESTEROL-FED ANIMALS (vol 1.4; p.7)

ED_{50} (mg/kg/day) for accumulation of hepatic cholesteryl esters

Cholesterol-fed male hamster: 0.04
Cholesterol-fed female rat: 0.03
Cholesterol-fed male mice: 0.7
Cholesterol-fed dogs: 0.007
Cholesterol-fed rhesus monkeys 0.0005
In chow-fed animals (no cholesterol), no effect was seen.

Pancreatic lipase activity was increased 50%.

ACAT ACTIVITY (vol 1.4)
No effect ex vivo on ACAT levels in hamster liver (vol 1.4; p.82)

ACAT activity in vitro in a microsomal assay (p.111)

	IC_{50} (uM)
SCH 58235	18 7
PD128042	6 2
CL277082	5 3
SA58035	0.24 0.13

BEHAVIORAL, NEUROLOGICAL, AUTONOMIC EFFECTS IN MALE RATS (p.138)

	3.0	10.0	30.0 mg/kg
Passivity	1	2	2
Body elevation	0	2	3
Limb position	0	2	2
Change in Gait	0	1	3
Excretions	0	3	6

Frequency of observations ranked 2 or higher; data at 1 hour postdose in 6 rats/group.
(1= slight; 2= moderate; 3= marked deviation from baseline)

INHIBITION OF UPTAKE (p.134)

Benzodiazepine uptake was inhibited as a f(dose) beginning at 0.1 uM (20%) to 10 uM (60%).
Monoamine uptake was inhibited beginning at 1 uM (20%) to 10 uM (90%).
Thrombaxane A2 and adenosine uptake were inhibited 50% at 10 uM.

ADME:

24 HOUR EXCRETION IN BILE-CANNULATED MALE RATS (vol 1.13)

Rat excreted 97% of radioactivity in the bile after i.v. dose of 5 mg/kg (in 24 hours).
62% of radioactivity in the bile after p.o. dose of 5 mg/kg.
11% of radioactivity in the bile after p.o. dose of 510 mg/kg.
Rats excreted 0.4-0.8% in the urine under these 3 conditions.

IN VITRO INCUBATION WITH LIVER AND KIDNEY SLICES (vol 1.13)

Slices were incubated in vitro with buffer and [^{3}H] SCH 58235 (33 ug/liver except dog was 0.23 ug/liver). The duration of incubation for liver was not provided. The sponsor□s conclusion was that *glucuronide formation was the major pathway. Two unknown metabolites were seen in* kidney along with □considerable inter-subject variability□ in mice after 6 hours at 37^0 C.

$AUC_{0\text{-}24\ hr}$ IN RATS (ng h/ml) (3-month dietary toxicity study)

Dose (mg/kg)	SCH 58235 (ng h/ml)	**Day 90**	
		male	female
20	**unconjugated**	29	0.0
100		46	48
500		80	150
1500		230	120
20	**conjugated + unconjugated**	3,100	1,300
100		4,700	7,300
500		7,700	12,000
1500		11,000	13,000

measurements at 6pm, 12 midnight, and 6 am 3/s/g; CV ranged from 14-110%.

$AUC_{2\text{-}24\ hr}$ IN DOGS (ng h/ml) (3-month ⟶ toxicity study)

Dose (mg/kg)	SCH 58235 (ng h/ml)	**Day 1**		**Day 90**	
		male	female	**male**	female

3	**unconjugated**	**33**	35	**28**	47
30		**230**	240	**240**	290
100		**180**	430	**340**	780
300		**580**	610	**800**	900
3	**conjugated +**	**670**	750	**880**	2,400
30	**unconjugated**	**2,600**	1,700	**3,900**	3,400
100		**3,900**	5,000	**7,200**	8,200
300		**5,200**	6,200	**10,000**	18,000

measurements at 2, 8, 12, and 24 hours postdose in 3-4/s/g; CV ranged from 20-70%.

Cmax DOGS (ng/ml) (3-month ~~—~~ toxicity study)

Dose	SCH 58235	**Day 1**		**Day 90**	
(mg/kg)	(ng /ml)	male	female	male	female
3	unconjugate	2	4	3	3
30	d	20	16	25	18
100		14	34	23	60
300		40	34	84	88
3	conjugated +	40	100	110	190
30	unconjugate	320	170	370	210
100	d	360	370	520	740
300		590	260	1300	1900

measurements at 2, 8, 12, and 24 hours postdose in 3-4/s/g; CV ranged from 20-70%.

HUMAN DATA: UNCHANGED SCH 58235 AFTER ONE DOSE

parameter	**1 mg**	**5 mg**	**10 mg**	**20 mg**	**50 mg**
Cmax (ng/ml)	n.d.	2.2 (44)*	3.3 (45)	4.7 (26)	12 (50)
AUC (ng h/ml)	n.d.	21 (82)	50 (100)	110 (45)	330 (23)
Tmax (h)	n.d.	4.5 (110)	7.0 (38)	11 (66)	14 (100)

HUMAN DATA: TOTAL (UNCHANGED + CONJUGATED) AFTER ONE DOSE

parameter	**1 mg**	**5 mg**	**10 mg**	**20 mg**	**50 mg**

Cmax (ng/ml)	10 (31)*	24 (26)	54 (59)	68 (60)	170 (30)
AUC (ng h/ml)	9.9 (74)	70 (40)	610 (58)	1,023 (23)	2,600 (18)
Tmax (h)	1.2 (23)	1.6 (110)	1.8 (95)	1.3 (140)	1.7 (130)

*coefficient of variation (%) in 6/g

TOXICITY STUDIES

ACUTE (SINGLE DOSE) STUDIES

LD_{50} of MICE (oral gavage): >5000 mg/kg
LD_{50} of MICE (ip): >1000 mg/kg; <2000 mg/kg
LD_{50} of RATS (oral gavage): >5000 mg/kg
LD_{50} of RATS (ip): >1000 mg/kg; <2000 mg/kg
LD_{50} of DOGS (oral): >3000 mg/kg

3-MONTH DIETARY STUDY IN RATS (vol 1.6)
95090. August 1995. Schering-Plough Research Institute.
Batch #s: 35879-020; 95-58235-ZZX-04; 95-58235-ZZX-05.
TREATMENT: Five groups of CD rats (6-weeks old; 20/s/g for controls and HD; 10/s/g for rest) were **given in the diet 0, 20, 100, 500, or 1500 mg/kg/day** for 3 months. Only controls and HD were examined for histopathology (10/s/g) with the other 10/s/g of controls and HD retained for 4 weeks without drug. A separate set of rats (6/s/g) were used for PK measurements. Plasma was obtained at 6:00 pm, midnight, and 6:00 am (end of dark cycle).

RESULTS: There were no drug-related deaths, clinical signs, effects on BW, FC, ophthalmology, hematology, urine chemistry, organ weights, or gross pathology.

CLINICAL CHEMISTRY (week 13; *p<0.05):
ALT (U/L): 48; 66*, 63, 76*, 70* (males) 54; 49, 58, 62, 56 (females)
Cholesterol (mg/dl): 58; 47*, 52, 50, 48* (males) 55; 52, 52, 56, 56 (females)

HISTOPATHOLOGY (10/s/g in controls and 1500 mg/kg):
LUNGS: foam cells in 0 controls and 5 females
LIVER: focal necrosis in 1/10 HD males

□The no-effect dose for systemic toxicity was 1500 mg/kg.□ (p.8)

3-MONTH ORAL ——— TOXICITY STUDY IN DOGS (vol 1.11)
SN95091. August 1995. Schering-Plough Research Institute.
Batch #: 95-58235-ZZX-04
TREATMENT: Five groups of beagle dogs (12-13 months old; 5/s/g for controls and HD; 3/s/g for rest) were **given by ———, 0, 3, 30, 100, or 300 mg/kg/day** for 3 months. □Dogs were

dosesd one hour after a one-hour feeding period to attempt to maximize absorption of SCH 58235 due to its lipophilic characteristics.□ Three/s/g were examined for histopathology with the other 2/s/g of controls and HD were retained for 4 weeks without drug. All dogs were used for PK on days 1 and 90 (immediately prior to dosing, and at 2, 8, 12, and 24 hours postdose).

RESULTS:
There were no deaths, clinical signs, effects on BW, FC, ophthalmology, hematology, urine chemistry, organ weights (no summary data), or gross pathology.

CLINICAL CHEMISTRY (week 13; none significant with *p<0.05):
ALT (U/L): 33; 40, 38, 60, 42 (males) 30; 35, 48, 48, 37 (females)
Cholesterol (mg/dl): 130; 92, 100, 85, 78 (males) 130; 84, 99, 85, 95 (females)

HISTOPATHOLOGY (3/s/g):	**MALES**	**FEMALES**
	0;3,30,100,300 mkd	
LUNG: granuloma foreign body	0; 1,1,1,1	0; 0,0,1,3
granuloma parasitic	0; 0,0,0,1	0; 0,0,1,1
pleural lymphangiect	0; 0,0,0,2	1; 0,0,0,0
acute foreign body pneu	0; 0,0,0,0	0; 3,1,0,0

NON-DRUG-RELATED:		
TESTES: spermatid giant cells	1; 0,1,2,1	
degeneration spermatic cells	3; 1,1,1,2	
EPIDIDYMIDES sperm granuloma	0; 1,1,0,0	
THYMUS atrophy		2; 3,1,2,1
medullary cyst		2; 3,2,2,2
GALLBLADDER lym. foll. hyperplasia	3; 0,0,0,1	1; 0,3,1,1
KIDNEYS mineral/medullary		1; 2,3,2,2
collect. duct vacuol.		0; 2,3,1,0
MAMMARY GLAND lactation		0; 0,1,0,0

□The no-effect dose for systemic toxicity was 300 mg/kg.□ (p.8)

PILOT STUDY IN RATS (TERATOGENICITY) (vol 1.15)
D-27527. August 1996. Schering-Plough Research Institute.
Batch#: 95-58235-ZZX-05
TREATMENT: Four groups of pregnant CD rats (6/g; 10 weeks-old) were given by oral gavage in 0.4% aqueous methylcellulose, **0, 250, 500, or 1000 mg/kg/day on days 6 through 15 of pregnancy**. Dams were killed day 21.

RESULTS: There were no findings in dams or in the fetuses (weight or gross external or visceral changes).

PILOT STUDY IN RABBITS (TERATOGENICITY) (vol 1.16)
D-27494. August 1996. Schering-Plough Research Institute.
Batch#: 95-58235-ZZX-05
TREATMENT: Four groups of pregnant rabbitts (4/g; 6-months old) were given by oral gavage in 0.4% aqueous methylcellulose, **0, 250, 500, or 1000 mg/kg/day on days 7 through 19 of pregnancy.** Dams were killed day 30.

RESULTS: There were no findings in dams or in the fetuses (weight or gross external or visceral changes). □SCH 58235 was not maternotoxic or embryotoxic when given to pregnant rabbits at doses up to and including 1000 mg/kg/day on gestation days 7-19.□

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 ——— 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: DMSO
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.
CHROMOSOME ABERRATION STUDY IN HUMAN LYMPHOCYTES (vol 1.16)
P 6372. March 1996. ———
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).

SUMMARY AND EVALUATION: SCH 58235 [redacted] Schering to develop a drug to lower cholesterol by blocking cholesterol absorption.

[redacted]

PHARMACODYNAMICS: Although 7-day studies in cholesterol-fed hamsters, rats, mice, dogs, and monkeys showed ED_{50} values in the ug/kg range for lowering cholesterol esters in the liver and cholesterol in plasma, there were no effects on plasma cholesterol in animals on normal chow diets (or in the toxicity studies).

TOXICITY: The sponsor tested relatively low doses in rats and dogs for 3 months. In rats, the use of a dietary exposure and a high dose of 1500 mg/kg kept plasma levels low (as opposed to the gavage dosing done with SCH 48461). There was a 40% increase in ALT in all treated male rats. Histopathology was done only in controls and HD rats and showed focal necrosis in the liver of 1/10 HD males as well as foam cells in 5/10 HD females. In dogs, the highest dose tested was only 300 mg/kg, which combined with small numbers (3/s/g), dosing fasted, and high background incidence of histopath findings, prevented statistical significance. (For dosing, the sponsor waited one hour past the one hour feeding time so that dosing could have been done up to two hours postdose.)

Although the sponsor reported no clinical signs in the rat toxicity study at doses of 20, 100, 500, and 1500 mg/kg/day, a single dose study in male rats found drug-related effects on *behavior* (passivity, body elevation), *neurology* (change in gait, limb position), and *autonomic effects* (□excretions□) at 10 mg/kg and 30 mg/kg (all six rats at 30 mg/kg had diarrhea).

Benzodiazepine uptake was inhibited beginning at 0.1 uM; monoamine uptake was inhibited beginning at 1uM and thrombaxane A2 and adenosine at 10 uM.

ADME: The drug was excreted in bile after both i.v. (97%) and p.o. (67%) dosing at 5 mg/kg, although only 11% was excreted when the dose was raised to 500 mg/kg (over 24 hours).
The sponsor stated that in humans, □*unchanged drug is rapidly conjugated and both forms are slowly eliminated with evidence of significant enterohepatic recycling*□ *and thus, a terminal phase half-life cannot be calculated* □*using routine methods*□ *but the estimate was 24 hours.*
We cannot know if there are other metabolites without a radiolabeled study looking at profiles (both lipophilic and hydrophilic).

The drug itself is very lipophilic and, if it is like its predecessor, will show accumulation and long half-lives in tissues as well as in plasma.

The highest doses used in both the rat and dog 3-month toxicity studies were stated to be below the □no-effect dose for systemic toxicity□, but rats were dosed in the diet (vs gavage) and the dogs were not dosed fed. Doses in the 3-month animal studies were based on multiples of the human dose (in mg/kg): this is almost never an appropriate basis as was seen here with the exposure data: the dogs had a Cmax at the highest dose 3x that of people and an AUC 2x human (see below). Furthermore, there is considerable accumulation with time so that one should not compare single dose human data with 3-month exposures in animals.

	Parent Drug Levels (day 1)	
	Cmax (ng/ml)	AUC (ng h/ml)
Male dog (300 mg/kg):	40	580
Man (50 mg):	12	330

[ ]

REPRO TOX STUDIES: The pilot teratogenicity studies in rats and rabbits were negative, but again, doses were low (1000 mg/kg as the high dose and no exposure data), and there were only 6 rats/group or 3 rabbits/group. The Ames test and the chromosome aberration study in human lymphocytes were both negative.

RECOMMENDATION:
If the Medical Officer agrees, the PK data from the multidose study should be submitted before the 8-week study in 140 patients begins (we have only single dose data in humans).

TO BE COMMUNICATED TO SPONSOR:

Lactation was seen in the mammary gland in one treated female dog: please explain.

Since SCH 58235 is a lipophilic drug [redacted] we need a tissue distribution study using radiolabeled drug to determine half-lives in the tissues.

[redacted] profile using radiolabeled drug would provide data on the best animal model for humans.

The doses used in the 3-month dog study were too low to enable us to pinpoint target organs. In addition, by possibly waiting up to two hours post-feeding to dose, plasma exposure was also kept low. Dogs should be 4 to 6 months old at the beginning of the 1-year toxicity study (Redbook, 1993) vs 12 to 13 months old as they were here. Please submit a protocol for a short-term dog study with dogs dosed as done in [redacted] (up to 3,000 mg/kg/day by gavage in methylcellulose).

The histopathology in the 3-month rat study showed control rats with problems in the kidneys, lymph nodes, liver, heart, thyroid, and lungs. Because of this high background incidence, it is difficult to pinpoint target organs and will be an increasing problem as the length of studies and ages of the rats increase. How do you plan to address this issue?

The exposure in the 3-month dietary rat study was low: have you considered a gavage study [redacted]

Before carcinogenicity studies are begun, please submit the range-finding and carcinogenicity protocols.

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9/16/02 12:46:27 PM
PHARMACOLOGIST
The current ezetimibe + statin combination therapy may be approved at the discretion of the medical reviewer, pending labeling modifications are made
Pharmacology recommends the approval of ezetimibe for monotherapy, but for the combination therapy it differs it to the discretion of the medical reviewer

Karen Davis-Bruno
9/16/02 02:08:46 PM
PHARMACOLOGIST
AP pending labeling revisions