

Table 18e. Ezetimibe and total ezetimibe exposure ratios in animals vs humans are shown in the Table below by the sponsor (volume 1.10, 5C, page 93). Note that AUC (0-24 hrs) exposures of 712 ng.h/ml were used for human studies in adults and adolescents. This Table shows the exposure ratios in repro tox and CAC studies. In adolescents, the AUC exposures of ezetimibe (free drug) were 71 ng.h/ml, and in adults were 155 ng.h/ml, however a combined mean for total ezetimibe in adults and adolescents was 712 ng.h/ml. Also note that sponsor states (in the foot note) that exposure multiples for combination toxicities could not be calculated because NOELs for combination tox studies could not be defined.

Table 29 Animal-to-Human Exposure Ratios Based on Mean AUC(0-24 hr) Values for Ezetimibe and Total Ezetimibe in Mice, Rats, Pregnant Rats, Pregnant Rabbits, and Dogs

Species (Sex)	Study (Dose)	AUC(0-24 hr)		Animal-to-Human Multiple			Study No.
		Ezetimibe (ng-hr/mL)	Total Ezetimibe (ng-hr/mL)	Ezetimibe		Total Ezetimibe	
				Adolescent	Adult	Adolescent/Adult	
Mouse (M)	Oncogenicity (500 mg/kg)	366	113133	2.36	5.16	159	99334 ^a
Mouse (F)	Oncogenicity (500 mg/kg)	305	155697	1.97	4.30	219	
Rat (M)	Oncogenicity and Toxicity NOEL ^a (1500 mg/kg)	130 ^b	10124	0.84	1.83	14.2	96453, ¹³ 99237 ¹⁴ 00037 ¹¹
Rat (F)	Toxicity NOEL (250 mg/kg)	130 ^b	9474	0.84	1.83	13.3	
Rat (F)	Oncogenicity (500 mg/kg)	130 ^b	11741	0.84	1.83	16.5	
Rat (Pregnant)	Embryo-Fetal (1000 mg/kg)	47.8	4929	0.31	0.67	6.92	99373 ¹⁵
Rabbit (Pregnant)	Embryo-Fetal (1000 mg/kg)	72.4	113094	0.47	1.02	159	99291 ¹⁷
Dog (M,F)	Toxicity NOEL (1000 mg/kg)	704	6554	4.54	9.93	9.21	97110 ²³

a: NOEL = No-observed effect level
 b: Mean exposure for male and female rats (average exposure over dose range of 50 to 2000 mg/kg, including SN 97003).

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Exposure multiples for combination (ezetimibe/statin) therapy were not calculated since a "no observed effect level" (NOEL) could not be defined in any combination toxicity study.

Distribution:

Distribution of the drug after single and multiple doses in rats:

The distribution of the drug was determined after oral (single or multiple) and iv dosing of ¹⁴C- SCH 58235 in SD rats (1 rat/sex/time point). After single oral administration most of the activity was observed in the GI tract (small and large intestinal wall, stomach wall) and liver. In addition the drug radioactivity was seen in kidneys (in both sexes), spleen/lung/fat (males), and in pancreas/bladder wall (females). In SD rats the tissue/blood ratio ranged from 0.9 (in the fat) to 32 (in the small intestine). In long Evans rats the tissue plasma ratio ranged from 0.2 (in testes) to 41 (in the liver). Following multiple dosing for 7, 14 or 21 days the distribution was not significantly different than what was observed after a single dose. Peak levels were observed at 4 hrs post dose in the GI system, liver, and lungs in both sexes, and in myocardium/pancreas in males, and in ovaries in females. However, in female SD rats, the drug accumulated in the ovary after 21 days of administration, see Tables 19-20. Following iv administration the

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volume of distribution area for the parent drug was large (6.73 L/kg), indicating that the drug was extensively distributed in various tissues. Sponsor explains that since the plasma conc of the conjugated drug was higher, there was relatively poor distribution of the conjugated drug into tissues.

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Table 19. Distribution of ¹⁴C-SCH 58235 in tissues of male SD rats

Table 22 Mean Concentrations of Radiocarbon in Tissues of Male Sprague Dawley Rats After Single Oral (Gavage) and 21 Days of Administration of 10 mg ¹⁴ C-Ezetimibe/kg								
Tissue	Tissue Concentration (ng-equiv/g) ^a							
	Day 1				Day 21			
	4 hr	T:B ratio (4 hr) ^b	10 hr	24 hr	4 hr	T:B ratio (4 hr) ^b	10 hr	24 hr
Plasma ^c	79.2	0.495 ^d	49.4	ND	52.8	0.541 ^d	32.3	ND
Cardiac Blood	99.9	1.00	NI	NI	65.6	1.00	NI	NI
Vena Cava	45.6	0.46	80.9	NI	NI	NC	NI	NI
Hepatic Vasculature	NI	NC	37.7	NI	55.9	0.85	18.3	NI
Liver	1220	12.2	381	57.8	1130	17.3	367	120
Kidney In Toto	116	1.16	206	NI	183	2.79	68.7 ^e	28.8
Kidney Cortex	148	1.48	NI	NI	NI	NC	NI	NI
Kidney Medulla	39.7	0.40	NI	NI	NI	NC	NI	NI
Lung	118	1.18	76.9	NI	83.7	1.28	BLQ ^e	BLQ
Adrenal Gland	NI	NC	NI	NI	195 ^f	2.97	96.9 ^e	NI
Fat	91.5	0.92	NI	NI	21.9 ^e	0.33	NI	BLQ
Myocardium	NI	NC	NI	NI	11.8 ^f	0.18	NI	NI
Esophagus	291	2.91	NI	NI	376 ^f	5.72	NI	NI
Pancreas	NI	NC	945 ^e	NI	NI	NC	NI	BLQ ^f
Skin	NI	NC	NI	NI	42.6	0.65	BLQ	NI
Spleen	87.2	0.87	353 ^f	NI	NI	NC	NI	6.61 ^e
Stomach Wall	858	8.59	243	NI	3480	53.1	35.9	NI
Small Intestine Wall	3220	32.2	2650	1250	4440	67.7	1680	347
Large Intestine Wall	509	5.09	4080	269	678	10.3	2290	476

a: Observations were based on at least 3 representative sections taken from a single animal.

b: Tissue:cardiac blood concentration ratios at 4 hr

c: Mean plasma concentrations of drug-derived radioactivity from a different dose group (n=4).

d: Blood:plasma concentration ratios determined at 4 hr from pooled blood and plasma (n=4 rats).

e: Observations were based on a single section taken from one animal.

f: Observations were based on 2 sections taken from one animal.

BLQ = Below lower limits of quantitation

NC = Not calculable

ND = Not determined; no sample taken

NI = Not identified; tissue could not be visualized in the section or radiocarbon signal could not be localized.

Bladder, bladder basin, brain, thymus, testis, and muscle had levels of radiocarbon that were NI, 0.00, or BLQ at all time points. Predominant amount of radiocarbon present in stomach, small and large intestinal contents through 48 hr, and BLQ by 168 hr.

Table 20. Distribution of ¹⁴C-SCH 58235 in tissues of female SD rats

Table 23 Mean Concentrations of Radiocarbon in Tissues of Female Sprague Dawley Rats After Single Oral (Gavage) and 21 Days of Administration of 10 mg ¹⁴ C-Ezetimibe/kg									
	Tissue Concentration (ng-equiv/g) ^a								
	Day 1				Day 21				
	4 hr	T:B ratio (4 hr) ^b	10 hr	24 hr	4 hr	T:B ratio (4 hr) ^{b,c}	10 hr	24 hr	168 hr
Plasma ^d	68.7	0.539 ^e	20.4	ND	38.1	0.587 ^e	39.8	ND	ND
Cardiac Blood	16.4	1.00	NI	NI	NI	NC	NI	NI	NI
Vena Cava	25.7	1.56	71.7	NI	NI	NC	NI	NI	NI
Hepatic Vasculature	NI	NC	70.5	NI	93.0	NC	BLQ	NI	NI
Bladder	6030	367	NI	NI	NI	NC	407 ^f	NI	NI
Bladder Basin	2460	149	NI	NI	NI	NC	613 ^f	NI	NI
Liver	449	27.3	478	10.6	1080	NC	320	83.4	NI
Kidney In Toto	70.8	4.31	291	NI	114	NC	NI	13.2	NI
Lung	BLQ	0.00	57.7	NI	65.6	NC	NI	NI	NI
Ovary	NI	NC	NI	NI	231 ^f	NC	224	211	108
Adrenal Gland	NI	NC	NI	NI	136 ^f	NC	BLQ ^f	NI	NI
Fat	BLQ	NC	NI	NI	NI	NC	NI	BLQ	57.0
Muscle	0.49 ^g	0.03	NI	NI	NI	NC	NI	BLQ ^f	NI
Pancreas	115	7.01	443	NI	NI	NC	NI	70.7	NI
Skin	NI	NC	NI	NI	45.1	NC	NI	NI	65.8
Spleen	41.2	2.51	7.41 ^g	NI	NI	NC	8.48	22.6	NI
Stomach Wall	363	22.1	181	NI	380	NC	BLQ	NI	NI
Small Intestine Wall	5010	305	628	184	3310	NC	834	454	NI
Large Intestine Wall	2960	180	3380	449	1320	NC	209	638	NI

a: Observations were based on at least 3 representative sections taken from a single animal.
b: Tissue:cardiac blood concentration ratios at 4 hr
c: Ratio could not be calculated because cardiac blood was not measurable.
d: Mean plasma concentrations of drug-derived radioactivity from a different dose group (n=4).
e: Blood:plasma concentration ratios determined at 4 hr from pooled blood and plasma (n=4).
f: Observations were based on 2 sections taken from one animal.
g: Observations were based on a single section taken from one animal.
BLQ = Below lower limits of quantitation
NC = Not calculable
ND = Not determined; no sample taken
NI = Not identified, tissue could not be visualized in the section or radiocarbon signal could not be localized.
Kidney cortex and medulla, brain, thymus, myocardium, and esophagus had levels of radiocarbon that were NI at all time points. Predominant amount of radiocarbon present in stomach, small and large intestinal contents through 48 hr, and BLQ by 168 hr.

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Distribution of the drug during pregnancy:

The tissue distribution of the ^{14}C - SCH 58235 (10 mg/kg) was also examined in pregnant SD rats on gestation day 18 (n=3/time point). Sponsor states that the tissue distribution of radioactivity was similar in pregnant vs in non-pregnant rats and most of the radioactivity was concentrated in the GI tract. However, in pregnant rats, most of the radioactivity remained in the GI tract and contents, and higher amounts were observed in pregnant rats vs in non pregnant animals (see Tables 20 and 20a). Some radioactivity was also observed in liver (1411 ng-equiva/g), kidneys (110 ng-equiva/g), lungs (27 ng-equiva/g), heart (18 ng-equiva/g), ovaries (27ng-equiva/g), and bladder (229 ng-equiva/g) at 4 hrs after dosing. The radioactivity was below the limit of quantification in the pooled tissues and blood of fetuses up to 48 hrs post dosing.

Table 20a. Distribution of ^{14}C -SCH 58235 in tissues of pregnant SD rats

Table 4 Mean Concentrations of Radioactivity in Tissues (ng eq/g of tissue) of Pregnant Rats Following a Single 10 mg/kg Oral Dose of ^{14}C -Ezetimibe Suspension						
Mean Concentration [ng eq/g of tissue (%CV)]						
	Time (hr)					
	1	2	4	8	24	48
Liver	1016 (24)	939 (8)	1411 (41)	622 (49)	112 (42)	0 (-)
Large Intestines	71.9 (87)	0 (-)	1538 (91)	6723 (48)	354 (78)	45.7 (173)
Large Intestine Contents	0 (-)	0 (-)	94368 (31)	155435 (42)	6844 (24)	1495 (90)
Small Intestines	14574 (31)	8243 (34)	9815 (45)	3686 (45)	500 (31)	35 (173)
Small Intestine Contents	335398 (36)	247568 (33)	144655 (68)	38810 (83)	4071 (41)	442 (81)
Stomach	40473 (74)	42408 (46)	17435 (22)	948 (73)	70.7 (173)	0 (-)
Stomach Contents	207183 (87)	146842 (20)	70587 (20)	10327 (109)	925 (173)	0 (-)
Cecum	0 (-)	72.0 (173)	15259 (78)	13839 (7)	860 (7)	228 (94)
Cecum Contents	0 (-)	265 (173)	159701 (35)	161633 (49)	3382 (75)	736 (73)
Kidneys	47.4 (91)	73.2 (3)	110 (16)	22.9 (173)	0 (-)	0 (-)
Lungs	593 (173)	0 (-)	27.2 (173)	0 (-)	0 (-)	0 (-)
Heart	29.3 (173)	0 (-)	17.8 (173)	0 (-)	0 (-)	0 (-)
Brain	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Placenta	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Uterus	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Amnions	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Mammary Gland	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Amniotic Fluid	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Ovaries ^a	0 (-)	0 (-)	27.1 (87)	0 (-)	0 (-)	0 (-)
Bladder ^a	139 (75)	107 (35)	229 (81)	63.7 (100)	0 (-)	0 (-)
Carcass	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Blood ^a	32.1 (27)	37.4 (16)	47.2 (23)	17.4 (78)	0 (-)	0 (-)
Plasma ^a	53.4 (25)	60.4 (14)	79.5 (24)	29.9 (78)	5.59 (88)	0 (-)

a: Reported as ng eq/g
 --: Not calculated
 Reference Notebook No. 43396, p. 321.

However fetal exposure is confirmed in the segment II/III studies in rats when 1000 mg/kg/day was given to pregnant rats by gavage from implantation through early and late gestation (GD 6-20). Sponsor states that the fetal exposure to the drug on GD20 (1.2 $\mu\text{g}\cdot\text{h}/\text{ml}$) and its glucuronide (17.5 $\mu\text{g}\cdot\text{h}/\text{ml}$) was consistent with passive placental transfer followed by glucuronidation and limited clearance of the drug in the fetus (Rane A et al, Drugs and fetal metabolism, Clin Pharmacol Ther 14(4):662, 1973; Lucier GW, Perinatal development of conjugate enzyme system, Environ Health Perspective 18:25, 1976). Sponsor states that there is decreased glucuronidation capability, or glucuronide formation in the fetus, and/or greater placental transfer of the parent drug relative to the conjugate. However, this has not been determined, and is based on above quoted references in the literature (ref 143 and 144, vol 1.10, page 119, under metabolism technical summary). The exposures of the drug (1.2 $\mu\text{g}\cdot\text{h}/\text{ml}$) and glucuronide (17.5

µg.h/ml) in the rat fetal blood in the above study suggest that the glucuronide is present in higher levels than the drug in rat fetus, like in maternal rat blood on GD20 (drug 4.2 µg.h/ml, glucuronide 8.1 µg.h/ml), but we do not know if it was transferred as a parent drug or a glucuronide.

In rabbits, when the drug was given on GD 7-22, the rabbit fetus was exposed to both the drug (0.17 µg.h/ml) and glucuronide (4.5 µg.h/ml) on GD22, the maternal values on GD22 were higher for the glucuronide (drug 0.12 µg.h/ml, glucuronide 157.8 µg.h/ml). Sponsor states that the AUC values of the free drug were similar in the dam (0.12 µg.h/ml) and fetus (0.17 µg.h/ml) on GD22, but note that the AUC values of total drug were higher in the dam (158-181 µg.h/ml) than in rabbit fetus (4.7 µg.h/ml).

When drug (1000 mg/kg/day) was given to lactating rats (by gavage), the drug was transferred to nursing pups via milk on LD12, and exposure to conjugated drug was less in the pup (11.2 µg.h/ml) than in the dam (16.7 µg.h/ml), with AUC ratios of 0.02 and 0.7 (pup:dam) for the drug and glucuronide respectively. Lacteal transfer of the drug was also determined in ¹⁴C- SCH 58235 derived milk on day 12-14 postpartum in rats and also in blood samples from nursing pups, where milk:plasma ratio was 0.2-0.7 over 24 hrs post-dose. In rats, higher exposure of the total drug was observed in mothers during lactation (23.1 µg.h/ml) than during gestation (12.2 µg.h/ml), and pups had 50% of the exposures (11.3 µg.h/ml) through mother's milk (23.1 µg.h/ml). Rabbits had almost 15-30 fold higher exposure to the drug on GD 10-22 (158-181 µg.h/ml) than rats during GD 10-20, (6-12 µg.h/ml), however rat fetuses had 4-fold higher exposures to the total drug (18.7 µg.h/ml) than rabbit fetuses (4.7 µg.h/ml), see Table 21. Thus the drug crosses the rat and rabbit placenta during gestation and is transferred into the milk of lactating rats.

Table 21. Toxicokinetic parameters in segment III study in rats, and rabbits with SCH 58235 at one dose of 1000 mg/kg/day

Rat	AUC (0-24 Hrs) µg.h/ml		
	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

Distribution of the drug in red blood cells and its binding to plasma proteins

There is little distribution of the drug radioactivity into red blood cells in animals (mice, rats, dogs) following oral or iv administration of 1-10 mg/kg ¹⁴C- SCH 58235. Blood to plasma ratios of radioactivity ranged from — in mice, — in rats, and — in dogs. SCH 58235 (98-99.8%) was extensively bound to plasma proteins in all

animals tested (mouse, rat, rabbit, dog serum) and in human serum at conc ranging between 5-200 ng/ml. Similarly the glucuronide (84-98%) metabolite was extensively bound to plasma proteins. The extent of ^3H conjugated drug binding to mouse (84-90%) and rat (88-93%) plasma protein binding was similar to human plasma protein binding (87-93%), but it was higher in rabbit plasma (97-98%), and lower in dog plasma (81-85%).

Table 22. The binding of SCH 58235 and its glucuronide to plasma proteins in various species and humans

Table 24 ^3H-Ezetimibe and ^3H-Conjugated Ezetimibe Binding to Plasma Proteins		
Species	Mean % Bound (Range)	
	Ezetimibe ^a	Conjugated Ezetimibe ^b
Mouse	99.4 (99.1-99.6)	(84.4-90.4)
Rat	98.8 (98.4-99.0)	(88.1-92.6)
Rabbit	98.4 (98.1-98.6)	(96.8-98.1)
Dog	99.3 (99.0-99.5)	(81.0-85.3)
Human	99.7 (99.5-99.8) ^{c,d}	(87.3-92.7) ^e
		(87.8-92.0)

a: Mean % radioactivity bound to plasma protein over range of 5 to 200 ng ^3H -ezetimibe/mL plasma (SN 98269); no concentration-dependent effect on binding.

b: Mean % radioactivity bound to plasma protein over range of 2 to 2000 ng ^3H -conjugated ezetimibe/mL plasma (SN 99393); no concentration-dependent effect on binding.

c: Binding at concentrations of 20 ng/mL ezetimibe; binding essentially the same at 200 ng/mL ezetimibe (SN 98270).

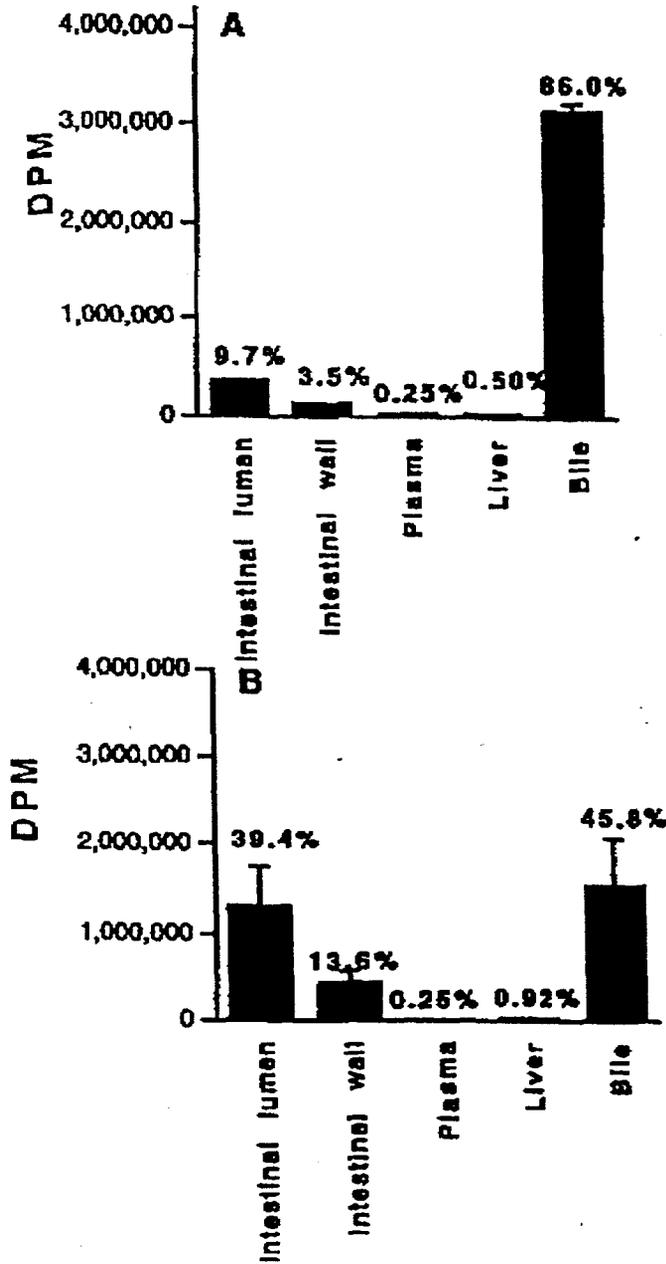
d: Radiochemical purity of ^3H -ezetimibe was _____

e: Mean % radioactivity bound to plasma protein over range of 2 to 2000 ng ^3H -conjugated ezetimibe/mL plasma (SN 99129).

Distribution of the drug (^3H SCH 58235) or its glucuronide metabolite (^3H -SCH 60663) in the bile duct-diverted rats: In the surgically prepared rat model as used before, control bile or bile containing the drug or its glucuronide (radiolabeled + carrier) were delivered intraduodenally (n=5/group), and bile was collected at 1, 2, 2.5 hrs, animals were sacrificed at 2.5 hrs and blood, livers and intestine were analyzed. When glucuronide was delivered, most of the glucuronide was found in the intestinal lumen and wall (39.4% and 13.6% respectively) vs less was found in these tissues when given as a free drug (9.7% and 3.5% respectively), Figure 16. 86% of the drug was found in the bile, and half of it was a glucuronide (45.8%). A time course of the appearance in bile is shown in figures 17-18 for both the drug and glucuronide. The peak appearance of the drug is 1 hr, while of glucuronide is 2 hrs, and less amounts of metabolite are found in the bile (when given as a glucuronide) vs the drug. Also 7 min after dosing, the majority of the

drug remains in the intestinal lumen, and more of the drug is found in the portal plasma, liver and bile when given as a drug vs when given as a glucuronide, see figures 18.

Figure 13. Distribution of ³H drug (A) or its glucuronide metabolite ³H-SCH 60663 (B) in the intestine, plasma, liver and bile, in the bile duct-diverted rat.



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Figure 14. Appearance of ³H drug or its glucuronide metabolite (³H-SCH 60663) in the bile over time in rats.

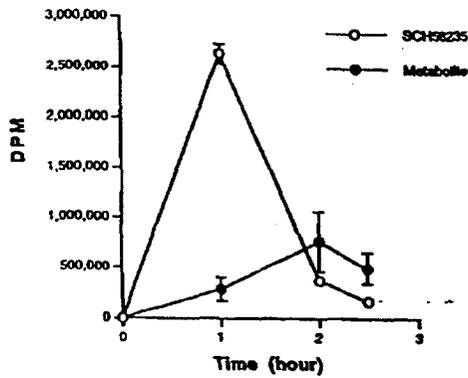
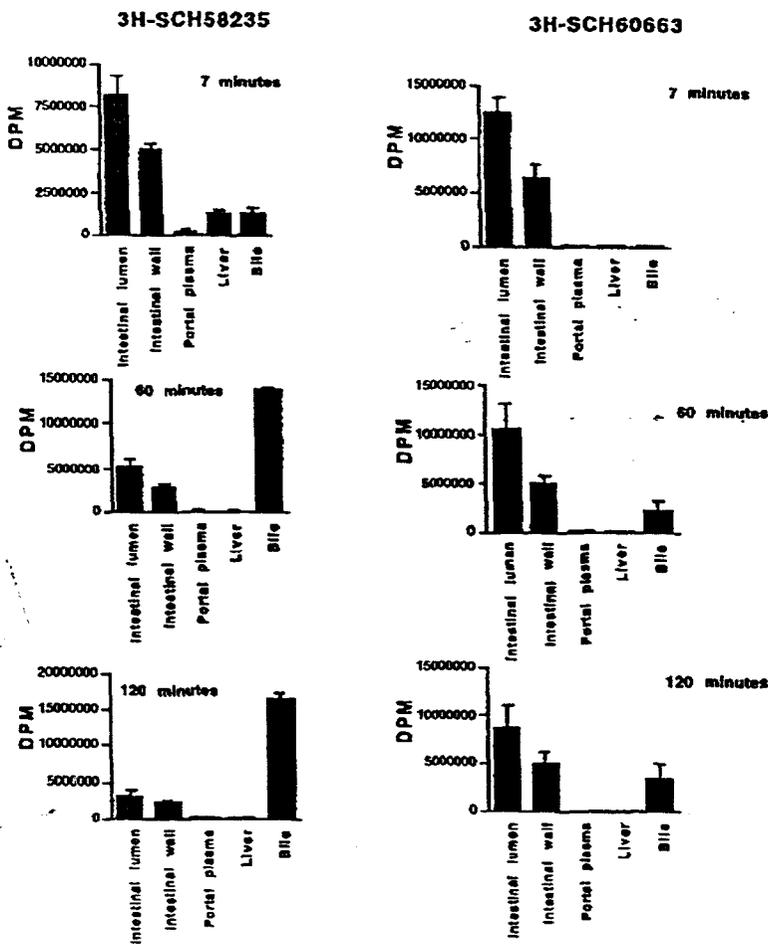


Figure 15. Distribution of ³H drug or its glucuronide metabolite (³H-SCH 60663) after intraduodenal dosing of the two compounds in rats.



Metabolism:

SCH 58235 metabolism has been determined in rats, dogs, mice and human plasma & feces and in biliary excretion studies (in rats and dogs). In all species tested the drug is metabolized almost exclusively via glucuronidation at the 4-hydroxyphenol group to form phenolic glucuronide (or conjugated drug SCH 60663). In vivo, minor amounts of the oxidative metabolite SCH 57871 (<3.2% of dose) were observed in the feces of mice, rats, dogs and humans. Human urine also contained SCH 57871 glucuronide (<1% of dose). Trace amounts of benzylic glucuronide (<1%, SCH 488128) were observed in the pooled human urine (0-72 hr following oral administration) and in the pooled dog bile (0.02%, 0-48 hrs after oral and iv dosing), see Figure 13. Sponsor states that the minor amounts of a rearranged isomeric form of the drug (SCH 59566) results from non-enzymatic rearrangement within the drug molecule in vitro. However percentage of this compound was not minor in rats (16.4% of dose in the female rat after iv dosing), or dogs (6.9% of the dose in the female dog after oral dosing). SCH 59566 was not found in males, see the structure of this isomeric form in Figure 17.

Figure 16. Metabolic pathway of SCH 58235 (ezetimibe or zeita)

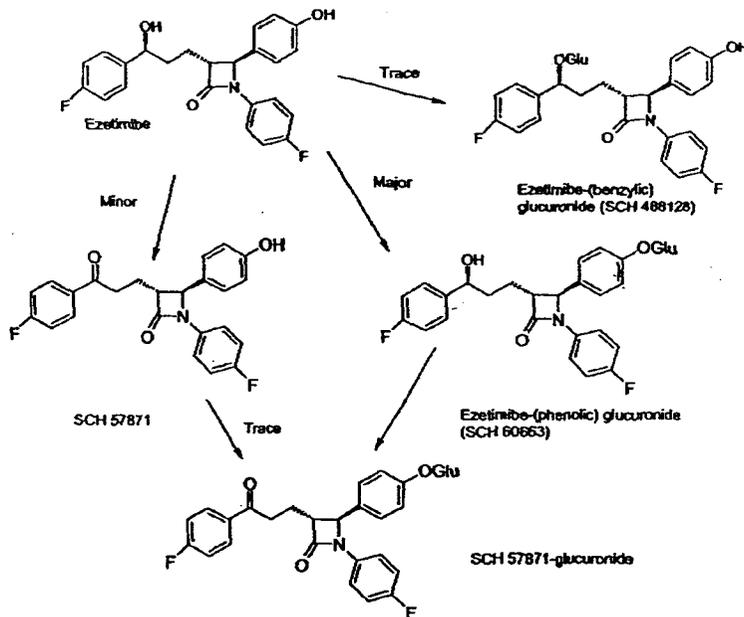
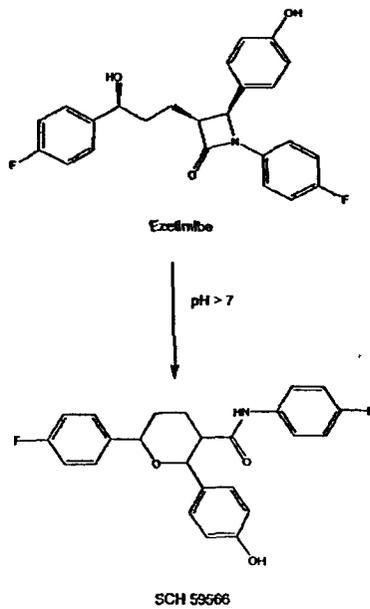


Figure 11 Metabolic Pathway for Ezetimibe

5.C.5.1. In Vivo Metabolism

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Figure 17. In vitro rearrangement of SCH 58235 to SCH 59566.



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In Vitro Rearrangement Pathway for Ezetimibe

Metabolite profiling in eight healthy male volunteers showed systemic exposure to total drug radioactivity to be 93%, with conjugated representing 90% at 0.5 hrs, and 100% by 24 hrs. Sponsor states that the **uncharacterized (unknown) radioactivity peaks in plasma were generally <12% of the total integrated radiocarbon peak area across species**, see appendix1, Tables1-4.

The contribution of specific UDP-glucuronosyltransferase (UGT) to the formation of conjugated glucuronide and SCH 488128 was examined in human jejunum and liver microsomes. UTG-1A1, 1A3, 2B15 exhibited catalytic activity in the formation of the glucuronide conjugate in liver and jejunum microsomes (SCH 60663), while UGT-2B7 was the major enzyme in human jejunum microsomes for formation of SCH 488128 from SCH 58235. Trace amounts of benzylic glucuronide (SCH 488128) were also observed in human urine and dog bile as indicated earlier.

Epimerization of SCH 58235 in humans

The drug SCH 58235 has three chiral centers but only the benzylic carbon is involved in the biotransformation of this drug to the oxidative (or ketone) metabolite SCH 57871. A method was developed to separate this ketone metabolite from

Also plasma and feces were analyzed for

Sponsor states that above compounds were not detected in the pooled plasma or feces in human subjects, therefore there is no evidence that the compound goes through any chiral inversion following oral administration to humans. Similarly potential conversion of SCH 59566 (rearranged isomer) to the original drug was also examined in human liver. SCH 59566 was not converted to the drug or metabolites of drug in human liver.

1. Biliary excretion and enterohepatic circulation of ¹⁴C-SCH 58235 following a single oral administration to rats (study # P-6683):

In this study ¹⁴C-SCH 58235 (1 mg/kg) was given to two groups of male and female bile duct cannulated rats to determine the biliary excretion and enterohepatic circulation. Donor rats (n=3/sex) received the oral suspension of the drug (25 µCi/rat) and recipient rats (n= 3-4/sex) received the drug (2 µCi/rat) intraduodenally (ID) with pooled donor bile. Bile, urine and feces were examined for up to 48 hrs after the dose administration. The results showed that the oral drug undergoes rapid hepatic and fecal elimination, 49% of radioactivity in males and 72% in females was excreted in bile (0.04% in cage wash and urine), or was eliminated via feces (males 40%, females 24%) as unabsorbed drug. The majority was excreted within 24 hrs. Small % was eliminated via the kidney (males 0.07%, females 4%) and less than 1% in the carcass, after 48 hrs. The major portion of the radioactivity in bile (males 60%, females 84% of the ID administered pooled bile from donor rats) is reabsorbed and re-excreted in bile. The remaining 40% and 16% in males and females (of intraduodenally administered dose) was in feces at 48 hrs. Thus enterohepatic circulation of the ¹⁴C-drug derived radioactivity after oral administration in intact rats is 29% in males, and 60% in females. The majority of the drug was a glucuronide conjugate (95%) in the pooled bile of male and female rats, and no differences in metabolite profiles were observed in sexes.

In vitro studies in liver and kidney slices, hepatocytes, liver and jejunum microsomes showed that the drug was mainly metabolized to a glucuronide in all species including mouse, rat, dog, and human. Similarly in portal vein plasma of rats collected following ID administration of ³H-drug, 95% of radioactivity was associated with the glucuronide, indicating that the drug is extensively conjugated in the intestine.

Following drug interaction studies were reviewed under IND _____ (on 9/29/98 & 4/26/2001)

Drug interaction studies (amendment #143). Sponsor states the following: In rat and dog liver microsomes, the drug competitively inhibits lovastatin metabolism, consistent with its effects on CYP3A-mediated metabolism at toxicological doses. However in recently completed clinical studies, the PK profiles of simvastatin and its hydroxy acid in humans administered with 10 mg of simvastatin for 14 days were not altered by _____ or 10 mg of ezemibe, and no synergic increases in ALT have been noted in normal volunteers. Also pharmacokinetics of the drug + lovastatin (at 20 mg/day), drug + atorva (at 10 mg/day), drug + prava (20 mg/day), drug + cervast (at 0.3 mg/day) were not altered. Similarly in the above clinical studies, levels of hydroxy-lovastatin, orthohydroxy atorvastatin, or hydroxy-cervastatin/O-desmethylcervastatin were not altered. Sponsor states that the drug does not appear to inhibit CYP3A mediated oxidative drug metabolism in vivo, except at high doses in rat/dog studies, but these two drugs have synergistic effects on decreasing serum cholesterol at lower doses in dogs. The substantial increases in ALT in dogs following combo administration was not accompanied by histopath changes in liver or muscle, and were attenuated following mevalonate co-administration. However, all doses increased the ALT values with statin + drug in dogs, and produced liver toxicity (see DFS sign off on IND _____).

In a 3-month toxicity study in mice (study # SN 96456), liver microsomal protein, cytochrome P450 content, and CYT-P450 dependent activities of 7-pentoxoresorufin O-dealkylase (PROD), and 7-ethoxyresorufin O-deethylase (EROD) were examined. The drug had no effect on cytochrome P-450 drug metabolizing enzymes (see DFS sign off on IND _____)

In the current NDA submission following enzyme induction and potential drug interaction studies have been provided (volume 1.10, 5C drug metabolism, pages 93-101):

In a 4-week dietary study in female mice with SCH 58235 (study # SN 94079), when noncertified drug was used (batch number 35497-3-4) at 300 & 1500 mg/kg/day, except for a slight increase in 7-pentoxoresorufin O-dealkylase (PROD) activity at 1500 mg/kg/day (217 vs 160 nmol/min/g liver in controls), no induction of other hepatic drug metabolizing enzymes was observed, even at the highest dose. _____

Table 22a. 4-week tox studies in female mice showing hepatic drug metabolizing enzymes.

Table 2 Female Mouse Liver Benzphetamine N-Demethylase, 7-Ethoxycoumarin O-Deethylase, 7-Pentoxoresorufin O-Dealkylase and 7-Ethoxyresorufin O-Deethylase Activity ^a			
Parameter	Control	SCH 58235	SCH 58235
	0 mg/kg/day	300 mg/kg/day	1500 mg/kg/day
Benzphetamine N-Demethylase (nmol/min/mg mic. prot.)	5.5 ± 1.0	5.5 ± 0.6	7.7 ± 2.2
	(nmol/min/g liver)	160 ± 27	217 ± 52
	(nmol/min/tot. liver)	403 ± 63	531 ± 140
7-Ethoxycoumarin O-Deethylase (nmol/min/mg mic. prot.)	2.2 ± 0.2	2.1 ± 0.2	2.7 ± 0.6
	(nmol/min/g liver)	63 ± 10	79 ± 21
	(nmol/min/tot. liver)	158 ± 17	190 ± 39
7-Pentoxoresorufin O-Dealkylase (pmol/min/mg mic. prot.)	39 ± 11	42 ± 5	85 ± 50
	(pmol/min/g liver)	1120 ± 246	2337 ± 1128 ^b
	(pmol/min/tot. liver)	2847 ± 710	5811 ± 3225
7-Ethoxyresorufin O-Deethylase (pmol/min/mg mic. prot.)	118 ± 15	117 ± 15	150 ± 29
	(pmol/min/g liver)	3439 ± 388	4288 ± 866
	(pmol/min/tot. liver)	8684 ± 835	10427 ± 1890

a: Values are means ± S.D. of data from 4 samples. Each sample is a combination of 2 mice.
 b: Significantly different from control (p ≤ 0.01).
 c: Significantly different from control (p ≤ 0.05).

Data recorded in Schering Notebook No. 33766.

However, sponsor states that above was an exploratory study in mice, in a subsequent 3-month dietary dose range finding study in mice (study # SN 96456) using a certified drug substance (SCH 58235, batch Number SZ-58235-97-X-101, at 100, 500, 2000 mg/kg/day), above finding was not reproduced. In addition no histopath changes in the mouse liver, which are consistent with induction of CYPs (liver weight, hepatocellular hypertrophy) were observed in either study (4-week or 3-month mouse study).

Table 22b. 3-month dose range tox study in mice (study # SN 96456) showing no effect on hepatic drug metabolizing enzymes.

Male	Control	SCH 58235			
Parameter	0 mg/kg	100 mg/kg	500 mg/kg	2000 mg/kg	
7-Pentoxylresorufin O-Dealkylase					
(pmol/min/mg mic. prot.)	64.8 ± 16.5	73.6 ± 15.0	82.6 ± 12.9	81.8 ± 11.5	
(pmol/min/g liver)	1801 ± 530	2014 ± 823	2145 ± 643	2173 ± 144	
(pmol/min/total liver)	2491 ± 648	3037 ± 1146	3077 ± 518	3304 ± 122	
7-Ethoxyresorufin O-Deethylase					
(pmol/min/mg mic. prot.)	50.1 ± 15.8	47.7 ± 48.7	27.1 ± 3.1	39.8 ± 15.3	
(pmol/min/g liver)	1418 ± 588	1448 1808	733 ± 349	1038 ± 324	
(pmol/min/total liver)	1934 ± 653	2153 ± 2619	1038 ± 334	1592 ± 541	

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Female	Control	SCH 58235			
Parameter	0 mg/kg/day	100 mg/kg	500 mg/kg	2000 mg/kg	
7-Pentoxylresorufin O-Dealkylase					
(pmol/min/mg mic. prot.)	113.1 ± 21.6	133.8 ± 17.6	113.4 ± 13.0	117.1 ± 22.6	
(pmol/min/g liver)	3185 ± 623	2752 ± 590	2547 ± 1022	2967 ± 496	
(pmol/min/total liver)	3332 ± 424	3221 ± 761	3120 ± 1476	3371 ± 734	
7-Ethoxyresorufin O-Deethylase					
(pmol/min/mg mic. prot.)	34.3 ± 2.9	37.7 ± 6.8	31.8 ± 4.8	35.4 ± 13.4	
(pmol/min/g liver)	964 ± 68	768 ± 140	707 ± 271	883 ± 265	
(pmol/min/total liver)	1034 ± 228	906 ± 248	869 ± 405	1008 ± 361	

a: Values are means ± S.D. of data from 3 or 4 mice per dose group.
Data recorded in Schering Notebook No. 40350.

Statin studies have shown that atorvastatin, lovastatin, simvastatin (but not pravastatin) undergo CYP3A4 metabolism in the gut and liver, while fluvastatin is metabolized by CYP2C9. Therefore inhibition of CYP-mediated metabolism by SCH 58235 would produce increased plasma conc of the parent statin. The CYP3A mediated metabolism was examined using lovastatin and testosterone as substrates in the dog and rat liver microsomes. SCH 58235 competitively inhibited lovastatin metabolism in the rat and dog liver microsomes at concentration of 100 μ M (\geq 41 μ g/ml). SCH 58235 also inhibited CYP-mediated metabolism of testosterone (with IC₅₀ of 5, 53, 7 μ M in rat, dog, and human liver microsomes respectively). These IC₅₀ values were decreased to 1.6, 2.8, 0.25 μ M, when the drug was preincubated with microsomes prior to incubation with ezetimibe, suggesting that this inhibition was metabolism/mechanism based (volume 1.10, page 95). However sponsor states that these are non-physiological conditions. SCH 58235 has a low absolute bioavailability in rats and dogs (\leq 1.6%), with most of the drug being conjugated in the enterocyte following absorption, therefore inhibition of CYPs by SCH 58235 is not expected in vivo. The clinical studies have not demonstrated any drug interactions showing changes in PK of statins (atorvastatin, lovastatin, simvastatin, fluvastatin) or ezetimibe using therapeutic doses. Therefore, a metabolic based interaction between SCH 58235 and statin is unlikely.

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Sponsor makes a following statement in the volume 1.10, 5C, pages 93-101

- Ezetimibe exposure did not increase with coadministration of the statins, except in pregnant rats dosed with ezetimibe and atorvastatin.
- While exposure to ezetimibe in the rat was the same at ezetimibe doses ≥ 50 mg/kg, increases in exposure to conjugated ezetimibe, the statins, and the hydroxylated statins were seen.
- Increases in exposure to conjugated ezetimibe were seen in mice and rats, but not in rabbits or dogs.
- Increases in exposure to the statins and the hydroxylated metabolites were seen in mice, rats, pregnant rats, pregnant rabbits, and dogs.
- In rats, coadministration of pravastatin [not metabolized by cytochrome P450 (CYP) 3A] produced the same pharmacokinetic interactions, as did coadministration of the CYP3A substrates, atorvastatin, lovastatin, and simvastatin.

Note that in the above statement that even when ezetimibe or statin exposures are not altered with the combination, the exposures to conjugated ezetimibe (which has as much drug activity as the parent drug) and the hydroxylated metabolite of statins are increased. In the 3-month combination (ezetimibe + statin) toxicity studies in rats, the combination generally increased the PK exposure of ezetimibe and/or statin (or their active hydroxy acid). This was generally not seen in 3-month combination toxicity studies in dogs, except for simvastatin and lovastatin (see individual study). This was also seen in 2-week female dog study with lovastatin, where no changes in lovastatin conc. were observed, but 1.3-1.5 fold increase in hydroxy-lovastatin conc were observed (study # SN 94078, volume 1.114). Sponsor explains that multidrug resistance enzyme (MDR1) may play some role in the competitive interaction between ezetimibe and some statins, but organic anion transporting polypeptides (OATPs) with MRP2 may be important in the transport anionic xenobiotics including many types of glucuronide conjugates.

Sponsor explains why there is an increase in exposures of ezetimibe or statin in animals in the following statement (volume 1.10, pages 97-98):

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The potential for ezetimibe to serve as a substrate for MDR1, and inhibit MDR1-mediated efflux of drugs, was evaluated in hamster ovary cell microsomal membranes⁷⁸ and viable whole cells.⁷⁹ An interaction of ezetimibe with MDR1 was demonstrated; V_{max} was estimated as ~ 63 nmol/min/mg (270% of basal activity), and K_m as ~ 21 μ M.⁷⁸ Ezetimibe also inhibited the MDR1-mediated efflux of daunorubicin (IC_{50} ~ 24 μ M, ≈ 9800 ng/mL) in CR1R12 cells, but the maximum inhibition of efflux seen at ~ 80 μ M ezetimibe was only 35% of the inhibition achieved with 5 mM vanadate.⁷⁹ Ezetimibe similarly inhibited daunorubicin and rhodamine 123 transport via human MDR1 (IC_{50} 25.3 and 42.3 μ M, respectively) in NIH-3T3 human MDR1-transfected cells.

Although ezetimibe appears to be a substrate of MDR1, and therefore could potentially inhibit MDR1-mediated drug efflux, pharmacokinetic interactions in laboratory animals were also observed between ezetimibe and pravastatin, a non-MDR1 substrate. No pharmacokinetic interactions were observed when ezetimibe was administered in clinical pharmacology studies with MDR1 substrates (cimetidine,¹³⁴ digoxin,¹⁵¹ simvastatin,¹³⁸ lovastatin,¹³² atorvastatin¹²⁹).

Disposition studies indicated that a carrier-mediated transport mechanism participates in the hepatocyte uptake and biliary excretion of pravastatin.^{152,153} Drug interactions at the excretion level might therefore occur as a consequence of competition for carrier-mediated transport across the hepatic sinusoidal or bile canalicular membrane. Recently, human and rat organic anion transporting polypeptides (OATPs) in the sinusoidal and canalicular membranes have been identified as transporters of pravastatin, lovastatin, simvastatin, atorvastatin, and hydroxylated metabolites of atorvastatin.^{154,155} Little is known about specific organic anion polypeptide transporters in other species (mouse, rabbit, and dog).

The canalicular multispecific organic anion transporter/multidrug resistance associated protein 2 (cMOAT/MRP2) plays a major role in the transport of anionic xenobiotics, including many types of glucuronide conjugates, across the bile canalicular membrane,^{156,157} and biliary excretion of pravastatin is mediated mainly by cMOAT/MRP2 in normal rats.¹⁵³ Cis-inhibition studies in a cell expression transport system indicated that the human (hOATP2) and rat analogue (roatp1)

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transport other statins in addition to pravastatin, including lovastatin, simvastatin, and atorvastatin,¹⁵⁵ and all of these statins are also excreted in bile.¹⁴⁹ Pravastatin transport in a OATP expression system was inhibited by the other statins, with inhibition by simvastatin \cong lovastatin > atorvastatin > pravastatin.¹⁵⁵ Estradiol-17 β -D-glucuronide, simvastatin acid, and bromosulfophthalein were also found to inhibit the transport of pravastatin in human hepatocytes, presumably through inhibition of hOATP2.¹⁵⁴

The possibility exists that the increases in exposure to conjugated ezetimibe and/or the statins and their hydroxylated metabolites, observed in laboratory animals at high doses of statin and ezetimibe, are related to competitive interactions with organic anion transport protein(s). In the clinic, however, the coadministration of therapeutic doses of potential OATP substrates (simvastatin,¹³⁸ lovastatin,¹³² atorvastatin,¹²⁹ or pravastatin¹³¹) did not lead to any notable change in the pharmacokinetic profiles of ezetimibe, conjugated ezetimibe, statin, or the hydroxylated metabolites of simvastatin, lovastatin and atorvastatin.

In humans ezetimibe and ezetimibe glucuronide constitute 10-20% and 80-90% of the total drug in plasma respectively. In vivo when ezetimibe or its glucuronide was given to healthy subjects for 14 days with or without 4 g of cholestyramine (the bile acid sequestrant), the systemic exposure to ezetimibe and conjugated ezetimibe was reduced to 20% and 49% respectively compared to relative exposure observed when ezetimibe was given alone, suggesting that there is interaction of ezetimibe with bile acid sequestrant.

One also needs to consider the mechanistic interaction between ezetimibe and statins. Glucuronidation is generally considered one of the major detoxification process, which inactivates the drug. However this is not true for ezetimibe, its major metabolite phenolic glucuronide (SCH 60663) of ezetimibe is more potent in inhibiting cholesterol than the ezetimibe itself (0.01 mg/kg of SCH 58235 inhibited the ¹⁴C-cholesterol appearance in plasma approximately by 70%, while the glucuronide inhibited it at the same dose by 90%). Furthermore glucuronide has an intrinsic activity as a potent cholesterol absorption inhibitor and does not require conversion to the parent compound to be active. Glucuronidation is a common metabolic pathway for the open acid forms of statins. Statins and gemfibrozil (GFZ) are used widely for treatment of hypercholesterolemia and hyper-triglyceridemia, and there have been reports of increased risk of myopathy including rhabdomyolysis when two are co-administered. This has been linked to the PK component of two drugs, however glucuronidation has been shown to play a part in this interaction. For example GFZ might serve as a competitive inhibitor of UDP-glucuronosyltransferase (UGT) isoforms, and thereby may increase the active and hydroxy acid forms of statins. In humans, not only P-450 mediated oxidative metabolism catalyzed by CYP3A (atorvastatin) and CYP2C (cerivastatin, fluvastatin) subfamilies has been the major pathway for metabolism of statins, but also glucuronidation (non-CYP3A-mediated) oxidation or lactone hydrolysis play an important role in this PK interaction. For example GFZ has the potential to modulate the PK of other statins by inhibition of of statin hydroxy acid glucuronidation. Thus various statins can exhibit differential susceptibility to the inhibitory effects of GFZ on their metabolic

clearance via glucuronidation and/or via non-CYP3A-mediated oxidative pathway. How this interaction between ezetimibe glucuronide (which has the major drug activity in this case) and statins will play a role in chronic studies in humans is unclear at this time, since PK interactions between these two drugs were not observed in the short term 2-week studies in humans.

In summary the drug is subjected to presystemic metabolism, mainly to phenolic glucuronide (SCH 60663). The drug is primarily metabolized in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary excretion. Minimal oxidative metabolism (a phase I reaction) has been observed in all species evaluated. **The glucuronide metabolite (SCH 60663) is at least as potent (or more potent) than the parent compound (SCH 58235) in inhibiting the absorption of cholesterol.** In 2-week to 3-month studies in animals (mostly in rats, and some in dogs), exposure to total ezetimibe and/or statins (or to their hydroxylated metabolites) was increased when two drugs were co-administered. Sponsor states that these were most likely not attributable to in vivo metabolic CYP-based interactions between ezetimibe and the statins, however this was only shown to be true in mice and has not been fully established in rats and dogs. Sponsor also explains that multidrug resistance enzyme (MDR1) may play some role in the competitive interaction between ezetimibe and some statins, but organic anion transporting polypeptides & MRP2 may be important with many types of glucuronide conjugates. In humans ezetimibe and ezetimibe glucuronide constitute 10-20% and 80-90% of the total drug in plasma respectively. Both the drug and glucuronide have drug activity and are slowly eliminated from plasma with significant enterohepatic recycling. The drug and glucuronide have half lives of approximately 22 hrs.

Excretion

Following administration of a single oral gavage (mouse, rat, dog), or iv dosing (rat, dog) or \rightarrow dosing (in humans) with ^3H or ^{14}C -SCH 58235, the drug was eliminated primarily in feces (>76%) and small amounts (<1-11%) in urine up to 168-336 hrs post-dose, see Table 23. Substantial amount of radioactivity was found in feces after iv dosing (in rats and dogs) which is consistent with extensive biliary excretion of the drug. No sex differences in excretion of drug were observed in dogs, however higher excretion of radioactivity was observed in urine of female rats than in male rats. This difference in extent of urinary excretion was consistent with sex related differences in plasma levels of glucuronide relative to total drug and excretion of mainly glucuronide drug in urine.

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 rats after oral dosing of ^{14}C -SCH 58235, 93-98% of the drug was excreted after 168 hrs, and most of it was in feces. Similarly in dogs after oral dosing, 87% of the total drug was excreted within 336 hrs, and mostly in feces.

In bile duct cannulated rats when ^{14}C -SCH 58235 was given by gavage, 49-72% was recovered in bile, in contrast in dogs only 8-18% of the dose was recovered in bile, indicating decreased intestinal absorption of the drug in the dog compared to the rat.

The enterohepatic recycling of total radiocarbon was 29% in male rats, and 60% in female rats after ID dosing of bile collected from donor rats with ¹⁴C-SCH 58235. Thus the fecal excretion of the drug in rats is attributed to both absorbed drug & metabolite that has undergone biliary excretion and reabsorption, and unabsorbed drug. However in dogs, there is poor intestinal absorption of the drug, and much of the fecal excretion in dogs is due to unabsorbed drug, see Table 23a and 23b.

Table 23a. Excretion of SCH 58235 in mice, rats and dogs

Table 28 Mean Percent of Administered Dose Recovered in Urine, Feces, and Bile Following Single-Dose IV or PO (Gavage) Administration of ¹⁴C- or ³H-Ezetimibe to Rodents and Dogs

Species, Strain	Sex	Route	Dose (mg/kg) (isotope)	Time (hr)	Urine	Feces	Bile	Total Recovery ^a	Study No.
Mice, CD-1	M	PO	5.0 (¹⁴ C)	168	7.61	78.2	-	86.3	95406 ₆
	F				5.81	80.5	-	86.8	
Rats, Long-Evans	M	PO	1.0 (¹⁴ C)	336	0.07	76.2	-	76.3 ^b	95439 ₆₂
	F				0.58	90.7	-	91.3 ^b	
Rats, Sprague Dawley	M	IV	1.0 (¹⁴ C)	168	0.616	98.1	-	98.8 ^b	95405 ₁₄
	F				3.87	92.2	-	96.3 ^b	
	M	PO	5.1 (³ H)	24	0.51	-	97	97.5	D-27056 ₅₇
	M		5.1 (³ H)	24	0.38	-	62	ND	
	M		510 (³ H)	24	0.81	-	11		
	M	PO	1.0 (¹⁴ C)	48	0.525 ^c	18.6	56.9	81.6	97050 ₅₆
	M				0.053 ^c	32.0	40.1	82.4	97262 ₅₆
	F		3.03 ^c	20.9	62.7	87.1			
	M		ID	5 ^d mL	48	0.385 ^c	34.1	53.8	87.9
	F	0.996 ^c				15.3	81.2	97.6	
	F (GD 18)	PO	10 (¹⁴ C)	48	0.711	89.3	-	90.0	99050 ₅₈
	M	PO	1.0 (¹⁴ C)	168	0.052	97.0	-	97.0 ^b	95405 ₁₄
	F				1.48	85.2	-	86.8 ^b	
	M	PO	1.04 (¹⁴ C)	168	0.26	93.6	-	93.9	95404 ₅₈
	M				10 (¹⁴ C)	168	0.05	93.1	-
F	Day 1	0.99	91.1	-	92.1 ^b				
M	168	0.00 ^a	95.2	-	95.2 ^b				
F	Day 21	0.92	95.0	-	96.1 ^b				

Table 23b. Excretion of SCH 58235 in mice, rats and dogs continued

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Table 28 Mean Percent of Administered Dose Recovered in Urine, Feces, and Bile Following Single-Dose IV or PO (Gavage) Administration of ¹⁴C- or ³H-Ezetimibe to Rodents and Dogs

Species, Strain	Sex	Route	Dose (mg/kg) (isotope)	Time (hr)	Urine	Feces	Bile	Total Recovery ^a	Study No.
Dogs, Beagle	M	IV	5.0 (¹⁴ C)	336	1.65	78.2	-	80.9 ^b	95407 ²⁶
	F				1.70	85.5	-	88.5 ^b	
	M	IV	4.5 (¹⁴ C)	144	1.83	1.17	99.0	102	99292 ⁶⁷
	F				7.36	1.94	88.1	98.1	
	M	PO	5.0 (¹⁴ C)	336	0.618	87.6	-	90.7 ^b	95407 ²⁶
	F				0.682	85.5	-	86.7 ^b	
	M	PO	3.9-5.0 (¹⁴ C)	96	0.721	91.0	8.19	100 ^f	99292 ⁶⁷
	F				3.08	78.6	17.8	100 ^f	
Human ^g	M	PO	20 mg 100 µCi (¹⁴ C)	24	6.08	13.2	-	19.3	C97-136 ¹²⁰
				48	9.06	49.9	-	59.0	
				240	11.3	77.7	-	89.0	

- a: Total recovery [feces, urine, cage washes/debris and carcass; or bile, urine, cage washes and carcass].
 - b: Total recovery = urine, feces and cage wash/debris only.
 - c: Urine and cage wash
 - d: 5 mL pooled bile from donor rats (administered 1 mg/kg ¹⁴C-ezetimibe) administered by bolus injection into duodenal cannula.
 - e: <0.005% administered dose
 - f: Recovery in excreta normalized to total excreted radioactivity due to low recovery/sporadic defecation.
 - g: Total recovery = urine and feces
- ND = Not determined

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IV. GENERAL TOXICOLOGY WITH MONOTHERAPY

Acute toxicity studies: In acute oral toxicity studies in mice (n=5/sex), rats (n=5/sex) and dogs (n=2/sex), the maximum doses (3000-5000 mg/kg/day) were well tolerated with the drug, as no mortality was observed, clinical signs were seen in mice (discolored feces in both sexes in mice at 5000 mg/kg/day), and in dogs (abnormal stool and emesis). In intraperitoneal (ip) studies, at 2000 mg/kg mortality in mice (n=5/sex/dose) was observed within 24 hrs in females (5/5 died at 2000 mg/kg/day) and within 3 days in males (3/5 died at 2000 mg/kg/day). In ip studies in rats (n=5/sex/dose), mortality was observed in 1/5 female rats after 7 days of dosing at 2000 mg/kg. The drug decreased mean BW gain in mice (2000 mg/kg) and rats (1000-2000 mg/kg) in ip studies. Clinical signs in mice at 2000 mg/kg/day ip doses (in both sexes) included scant feces, hypoactivity, tremors, urogenital staining. Clinical signs in rats at 2000 mg/kg ip doses in both sexes included distended abdomen, dehydration, scant/loose feces, hypoactivity, tremors, urogenital staining. Macroscopic observations following ip dosing showed pyrogranulomas, adhesions containing residual compound like material in abdomen cavity of mice and rats, and enlarged or pale spleen, suggesting irritation and inflammation produced by the drug.

1) Oral dosing

Table 23c: Clinical signs in acute oral tox study in dogs with ezetimibe

Table 1 Acute Oral Toxicity Study of SCH 58235 in Dogs		
INDIVIDUAL CLINICAL OBSERVATIONS - AN. NO. (DAYS)		
CLINICAL SIGN	DOSE LEVEL (mg/kg)	
	0 (CONTROL)	3000 (SCH 58235)
Emesis, capsules : dog redosed	9F (1)	
Abnormal Stool: drug-like material		14F (1,2) 3M (2), 4M (2), 12F (2)
Emesis: White froth		4M (7)

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2) IP dosing

Studies in mice

Table 23d: Acute IP tox study in mice with ezetimibe

Table 1 Acute Intraperitoneal Toxicity Study of SCH 58235 in Mice							
SUMMARY OF ANTEMORTEM OBSERVATIONS							
OBSERVATION	DOSE (MG/KG):	0 (CONTROL)		1000 (SCH 58235)		2000 (SCH 58235)	
	SEX:	M	F	M	F	M	F
Mortality		0/5	0/5	0/5	0/5	3/5	5/5
Nothing Remarkable		5/5	5/5	5/5	5/5	0/5	0/5
Ear, left: red		0/5	0/5	0/5	0/5	1/5	0/5
Feces: scant		0/5	0/5	0/5	0/5	1/5	0/5
Hypoactive		0/5	0/5	0/5	0/5	1/5	2/5
Tremors soft		0/5	0/5	0/5	0/5	1/5	0/5
Urogenital Staining: yellow		0/5	0/5	0/5	0/5	1/5	0/5
Unkempt		0/5	0/5	0/5	0/5	1/5	0/5
MEAN BODY WEIGHT GAINS (g)							
Days 1-8		6.7	4.6	6.5	4.9	1.4	D
Days 9-15		1.3	1.3	1.0	1.2	0.2	D
D = Dead							

Table 23e: Macroscopic observations in acute IP tox study in mice with ezetimibe

Table 2 Acute Intraperitoneal Toxicity Study of SCH 58235 in Mice							
SUMMARY OF POSTMORTEM OBSERVATIONS							
OBSERVATION	DOSE (MG/KG):	0 (CONTROL)		1000 (SCH 58235)		2000 (SCH 58235)	
	SEX:	M	F	M	F	M	F
No Visible Lesions		5/5	5/5	4/5	2/5	0/5	0/5
Abdominal Cavity: Small amount of compound-like matter, multifocal		0/5	0/5	0/5	1/5	1/5	0/5
Abdominal Cavity: Moderate amount of compound-like material		0/5	0/5	1/5	0/5	0/5	0/5
Abdominal Cavity: pyogranulomas, mild		0/5	0/5	0/5	0/5	1/5	0/5
Adhesion: mild		0/5	0/5	0/5	0/5	1/5	0/5
Spleen: enlarged, minimal		0/5	0/5	0/5	0/5	1/5	0/5
Spleen: enlarged, mild		0/5	0/5	0/5	0/5	1/5	0/5
Spleen: pale, mild		0/5	0/5	0/5	0/5	1/5	3/5
Spleen: pale, moderate		0/5	0/5	0/5	0/5	3/5	2/5
Subcutis: small amount of compound-like material		0/5	0/5	0/5	2/5	0/5	0/5
Thoracic Cavity: pyogranulomas, minimal		0/5	0/5	0/5	0/5	1/5	0/5

Studies in rats

Table 23f: Acute IP tox study in rats with ezetimibe

Table 1 Acute Intraperitoneal Toxicity Study of SCH 58235 in Rats							
SUMMARY OF ANTEMORTEM OBSERVATIONS							
OBSERVATION	DOSE (MG/KG):	0 (CONTROL)		1000 (SCH 58235)		2000 (SCH 58235)	
	SEX:	M	F	M	F	M	F
Mortality		0/5	0/5	0/5	0/5	0/5	1/5
Nothing Remarkable		5/5	5/5	5/5	5/5	3/5	3/5
Abdomen: distended		0/5	0/5	0/5	0/5	1/5	1/5
Dehydrated		0/5	0/5	0/5	0/5	0/5	1/5
Feces: scant, loose		0/5	0/5	0/5	0/5	0/5	1/5
Feces: soft		0/5	0/5	0/5	0/5	0/5	1/5
Hypoactive		0/5	0/5	0/5	0/5	1/5	2/5
Swelling: hard, lower left abdomen		0/5	0/5	0/5	0/5	1/5	0/5
Thin		0/5	0/5	0/5	0/5	1/5	1/5
Unkempt		0/5	0/5	0/5	0/5	1/5	0/5
Urogenital Staining: yellow		0/5	0/5	0/5	0/5	0/5	1/5
MEAN BODY WEIGHT GAINS (g)							
Days 1-8		75.0	48.2	50.6	36.8	59.1	42.2
Days 9-15		45.3	25.2	44.5	18.9	55.5	20.5

Table 23g: Macroscopic observations in acute IP tox study in rats with ezetimibe

Table 2 Acute Intraperitoneal Toxicity Study of SCH 58235 in Rats							
SUMMARY OF POSTMORTEM OBSERVATIONS							
OBSERVATION	DOSE (MG/KG):	0 (CONTROL)		1000 (SCH 58235)		2000 (SCH 58235)	
	SEX:	M	F	M	F	M	F
No Visible Lesions		5/5	5/5	0/5	0/5	1/5	1/5
Abdomen: distended, mild		0/5	0/5	0/5	0/5	0/5	1/5
Abdomen: lower, wet with urine		0/5	0/5	0/5	0/5	0/5	1/5
Abdominal Cavity: compound-like material, minimal		0/5	0/5	5/5	5/5	0/5	1/5
Abdominal Cavity: compound-like material, moderate		0/5	0/5	0/5	0/5	0/5	1/5
Abdominal Cavity: compound-like material, small amount, multifocal		0/5	0/5	0/5	0/5	1/5	0/5
Abdominal Cavity: pyogranulomas, mild		0/5	0/5	0/5	0/5	0/5	2/5
Abdominal Cavity: pyogranulomas, moderate		0/5	0/5	0/5	0/5	1/5	0/5
Abdominal Viscera: adhesions, moderate		0/5	0/5	0/5	0/5	0/5	1/5
Adhesions: mic		0/5	0/5	2/5	4/5	2/5	3/5
Compound Granuloma: retroperitoneal		0/5	0/5	0/5	0/5	1/5	0/5
Lungs: dark red, severe		0/5	0/5	0/5	0/5	0/5	1/5
Spleen: enlarged, minimal		0/5	0/5	0/5	0/5	1/5	0/5
Thoracic Cavity: compound-like material		0/5	0/5	1/5	4/5	0/5	0/5
Thoracic Cavity: serosanguinous fluid, severe		0/5	0/5	0/5	0/5	0/5	1/5
Compound-like material, subcutis moderate						1/5	

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ACUTE (SINGLE DOSE) STUDIES: Following were the maximal non-lethal doses in mice, rats and dogs:

Oral dosing

MICE (oral gavage): >5000 mg/kg/day in both sexes

RATS (oral gavage): >5000 mg/kg/day in both sexes

DOGS (oral): >3000 mg/kg/day in both sexes

Intraperitoneal (ip) dosing

MICE (ip): 1000 mg/kg/day in both sexes

RATS (ip): 1000 mg/kg/day in females, >2000 mg/kg/day in males

In conclusion when the drug was administered orally, it was not acutely toxic. When administered ip, it was moderately toxic in mice and rats, the cause of mortality was severe irritation and inflammation produced due to ip administration.

Multiple dose toxicity studies: 3-Month rat and dog studies were previously reviewed under the IND (see appendix).

1. Study title: A 6-month Dietary Toxicity Study of SCH-58235 in Rats (Study No. SN 96453)

Key study findings: SCH 58235 following a 6-month dietary administration in rats (males 250, 750, 1500 mg/kg/day, females 50, 250, 500 mg/kg/day) produced increases in plasma AST levels (by 2-fold) at a high dose in females. At a high dose it produced toxicity in males (bone marrow hyperplasia in 3/15 vs 0/15 in controls, lymph nodes accumulation of plasma cells in 8/15 vs 4/15 controls, in heart mononuclear cellular infiltration in 8/15 vs 5/15 rats, myocardial degeneration in ventricles in 1/15 vs 0/15 controls, and glomerular nephropathy in 3/15 vs 2/15 controls) and females (adrenal vacuolation, hypertrophy & pigment accumulation in 4/15 vs 0/15 controls, kidney glomerular nephropathy in 8/15 vs 1/15 controls, and myocardial degeneration in ventricles in 1/15 vs 0/15 controls). Note that histopath changes were not examined at low-mid doses. The NOAEL in the 6-month rat study may be 750 mg/kg/day in males and 250 mg/kg/day in females.

Study no: SN 96453

Volume #, and page #: 1.25, page 1

Conducting laboratory and location: Schering-Plough Research Institute

Date of study initiation: 3/23/1998

GLP compliance: Yes

QA report: yes (X) no ()

Drug lot #, and % purity: 97-58235-X-03

Formulation/vehicle: (meal)

Methods (unique aspects):

Dosing:

Species/strain: Sprague-Dawley rats/Crl:CD (SD)BR VAF/PLUS

#/sex/group or time point (main study): 15/sex/dose

Satellite groups used for toxicokinetics or recovery: Additional 15/sex/dose for TK study.

Age: Approximately 6-8 weeks of age

Weight: Males 226-284 g, females 169-216 g.

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Doses in administered units: Males 0, 150, 750, 1500 mg/kg/day, females 0, 50, 250, 500 mg/kg/day. Dose selection was based on a 3-month dietary (study # P-6290) and 2-week PK (study # P-6666) study in rats, where plasma levels plateaued at 1500 and 500 mg/kg/day in males and females respectively. Therefore high doses of 1500 and 500 mg/kg/day were chosen for males and females respectively in the current study.

Route of administration: Dietary for 4 consecutive weeks

Observations and times:

Clinical signs: Once daily

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Prior to treatment, and once during weeks 13 and 26.

Hematology: In main study animals, during weeks 13 and 26.

Clinical chemistry: In main study animals, during weeks 13 and 26.

Urinalysis: 4-hour fasting and 16 hr non-fasting samples were collected from the main study animals at the conclusion of treatment,

Gross pathology: At sacrifice.

Organs weighed: Organs weighed are listed in the Table 24

Table 24. Tissues collected for organ weights in the 6-month dietary rat tox study

Organs Weighed	
Adrenal Glands	Pituitary Gland*
Brain	Prostate Gland (Ventral)
Epididymides	Salivary Gland - Mandibular
Heart	Spleen
Kidneys	Testes
Liver	Thymus or Remnant
Lungs (plus Bronchi)	Thyroid Gland/Parathyroid Gland*
Ovaries	Uterus with Cervix
at Post-mortem	

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Histopathology: This was performed at sacrifice in control and high dose animals, listed in the histopathology Table 25.

Table 25. Tissues collected for histopath evaluation in the 6-month dietary rat tox study

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Tissues Collected	
Animal Identification ^a Gross Findings Adrenal Glands Aorta - Thoracic Bone - Femur and Sternum Bone Marrow Section - Sternum Bone Marrow for Cytology - Sternum ^b Brain Epididymides Esophagus Eyes Harderian Glands Heart Intestine, Large (cecum, colon) ^c Intestine, Small (duodenum, ileum, jejunum) Kidneys Liver Lungs plus Bronchi Lymph Nodes (mandibular and mesenteric) Mammary Glands - Inguinal ^c	Ovaries Pancreas Parathyroid Gland Peripheral Nerve (Sciatic) Pituitary Gland Prostate Gland Salivary Gland - Mandibular Seminal Vesicles Skeletal Muscle - Biceps Femoris Skin - Inguinal Spinal Cord - Thoracolumbar Spleen Stomach (glandular and nonglandular) Testes Thymus Thyroid Gland Tongue Trachea Urinary Bladder Uterus (plus Cervix) Vagina
<p>a: Collected but not processed.</p> <p>b: Bone marrow cytology specimens were prepared for all toxicity rats sacrificed prior to and at the scheduled necropsy, but were not evaluated due to lack of changes in the peripheral blood.</p> <p>c: Examined histopathologically in male rats when present in routine section.</p>	

Toxicokinetics: During weeks 4 and 25, at 0, 0.5, 2, 4, 8 and 12 hrs. Conjugated and unconjugated drug was measured by _____ by _____

Results:

Mortality: One female in the control group (on day 37), and one female at 500 mg/kg/day (on day 156) were sacrificed due to moribund conditions. Also one male at 150 mg/kg/day (on day 163) was found dead. These deaths were attributed to plasma analysis and were considered incidental

Clinical signs: No drug related effects were observed.

Body weights: No significant treatment related effects on body weights or weight gains in males or females were observed compared to controls. On day 176 the mean BW in males were decreased by 3% at a high dose (681, 699, 694, and 658 g at 0, 150, 750, 1500 mg/kg/day). Mean BW values in females were 364, 357, 360, 374 g respectively.

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

Ophthalmoscopy: Sponsor states that no drug related effects were observed during weeks 13 and 26. However, sponsor has not provided any summary data. The individual data tend to show increases in corneal crystals or chromodacryorrhea in eyes (shedding of bloody tears) of males in week 26 with increased doses compared to controls (0/15, 2/15, 1/15, 3/15). No drug related histopath findings in eyes were observed

Hematology: On day 86 in males, neutrophils count was decreased at 750 mg/kg/day (1.9, 1.9, 1.3*, 1.7 10^3 /UL respectively, $p < 0.05$), and in females it was increased at 500 mg/kg/day (0.66, 0.87, 0.72, 0.94* 10^3 /UL respectively). No other treatment related effects on hematology parameters were observed.

Clinical chemistry: In males, albumin (4.7, 4.6, 4.5, 4.4* g/dl respectively) and phosphorous (9.3, 8.8, 8.7, 8.4* g/dl respectively) were decreased at a high dose, while cholesterol was increased at a high dose in males (83, 80, 81, 105 mg/dl respectively). In females, AST (aspartate aminotransferase) was increased at a high dose by almost 2-fold (143, 143, 129, 246 IU/L at 0, 50, 250, 500 mg/kg/day), sponsor states that this was due to increases in 2/15 female animals. In females, phosphorous (7.1, 6.6, 6.2*, 7.2 g/dl respectively) was decreased at a mid dose, while cholesterol was increased at a high dose again (96, 99, 106, 111 mg/dl respectively). Glucose was increased in females (109, 129, 117, 136* mg/dl respectively).

Urinalysis: On day 85, 4-hour urine volume was decreased in both sexes (males 3.5, 3.6, 2.6, 2.2* ml, females 4.5, 4.1, 3.8, 2.7* ml respectively).

Organ weights: Absolute spleen weight was increased in females at a high dose (0.57, 0.58, 0.55, 0.63* g respectively). Relative spleen weight was increased in males (0.13, 0.14, 0.14, 0.15* % respectively). However, no significant drug related histopath changes were observed in spleen

Gross pathology: Drug related findings were observed in lungs (LN-mandibular enlarged, males 0/15, 2/15, 0/15, 2/15, females 0/15, 0/15, 0/15, 1/15), in liver (in males enlargement at a high dose in 1/15 vs 0/15 in control rats, in females deformity at a mid dose in 1/15 vs 0/15 in controls) and in skeletal muscle (mass in 1/15 males at a high dose vs none in controls). No histopath findings were associated with the above gross findings in lungs, liver or skeletal muscle.

Histopathology: These were performed only in control and high dosed animals. In males histopath findings were observed in the bone marrow (hyperplasia in megakaryocyte in 1/15 rats and myeloid hyperplasia in 2/15 rats vs none in controls), heart (mononuclear cellular infiltration in 8/15 rats vs 5/15 controls) and in lymph nodes (accumulation in plasma cell in 8/15 vs 4/15 in controls). In females, in the adrenal (4/15 rats had vacuolation/hypertrophy or pigment accumulation in cortex vs none in controls), and kidney (increased glomerular nephropathy was observed in 8/15 vs 1/15 control females). while myocardial degeneration in ventricle (of minimal severity) was observed in both sexes (1/15 M+ 1/15 F vs none in control groups). However, sponsor claims that no histopath findings were observed in the 6-month study in rats.

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Table 26- Histopathology data with SCH 58235 (males at 0, 150, 750, 1500 mg/kg/day, females 0, 50, 250, and 500 mg/kg/day) in the 6-month dietary toxicity study in rats:

	(n=15/sex/dose for controls and high dosed rats)	
	Males	Females
Adrenal		
Vacuolation, cortical*	1/15, ne, ne, 0/15	0/15, ne, ne, 1/15
Hypertrophy, focal, cortical*	1/15, ne, ne, 1/15	0/15, ne, ne, 2/15
Pigment accumulation in cortex*	0/15, ne, ne, 0/15	0/15, ne, ne, 1/15
Bone Marrow		
hyperplasia in megakaryocyte*	0/15, ne, ne, 1/15	0/15, ne, ne, 0/15
hyperplasia, myeloid**	0/15, ne, ne, 2/15	0/15, ne, ne, 0/15
Kidney		
Glomerular nephropathy, membranous**	2/15, ne, ne, 3/15 ^a	1/15, ne, ne, 8/15
Lymph nodes		
Accumulation in plasma cell, medulla**	4/15, ne, ne, 8/15	2/15, ne, ne, 3/15
Thymus		
Atrophy, cortical/extravascular erythrocyte*	0/15, ne, ne, 1/14	0/15, ne, 1/1, 1/15
Heart		
Mononuclear cellular infiltration*	5/15, ne, ne, 8/15	3/15, ne, ne, 2/15
Fibrosis**	4/15, ne, ne, 4/15	0/15, ne, ne, 0/15
Myocardial degeneration in ventricle*	0/15, ne, ne, 1/15	0/15, ne, ne, 1/15

ne = Not examined.

*= minimal severity

**= minimal to mild severity

^a = moderate severity in 1/3 rats

Toxicokinetics: The plasma AUC values are shown in the Table. The total drug+glucuronide seem to accumulate by 1.4-2 fold. There was extensive glucuronidation of the drug in rats. The AUC values of the total drug, as well as unconjugated and conjugated were higher in week 25 vs in week 4. Sponsor states that the values in general were higher in females than in males, but that is not true, in fact the total drug values in week 25 were similar at low and high doses, and higher in males at a mid dose. The AUC values did not proportionally increase with the doses.

The AUC of parent + metabolite at 750 mg/kg/day in male (13.7 µg.h/ml) and at 250 mg/kg/day in female (9.4 µg.h/ml) rats were 7-10 fold, the human AUC at 20 mg/day (1.314 µg.h/ml). The AUC of parent + metabolite at 750 mg/kg/day in male (13.7 µg.h/ml) and at 250 mg/kg/day in female (9.4 µg.h/ml) rats were 14-20 fold, the human AUC at recommended 10 mg/day (0.64 µg.h/ml).

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Table 27: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in week 4 and 25 in a 6-month dietary rat toxicity study:

Dose (mg/kg)	Gender	SCH 58235 AUC(0-24 hr) (ng·h/ml)					
		Total		Unconjugated		Conjugated	
		Week 4	Week 25	Week 4	Week 25	Week 4	Week 25
50	F	3532	6567	78.1	233	3457	6271
250	F	6455	9361	104	427	6359	9582
500	F	10372	12731	95.8	258	10278	10373
150	M	4470	6096	67.7	408	4395	5580
750	M	7795	13663	123	279	7681	13787
1500	M	8802	12464	113	391	8689	13172

Toxicology summary: In a 6-month dietary toxicity study in rats, doses of 0, 250, 750, 1500 mg/kg/day in males, and 0, 50, 250, 500 mg/kg/day in females were used. The increases in AUC values of the total drug were not dose proportional and values were higher in week 25 (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 plasma AST levels in females at a high dose (246 vs 143 IU/L in controls). The target organs of toxicity at a high dose in males are bone marrow (hyperplasia in 3/15 vs 0/15 in controls), lymph nodes (accumulation in plasma cell in 8/15 vs 4/15 controls), heart (mononuclear cellular infiltration in 8/15 vs 5/15 rats, and myocardial degeneration in ventricles in 1/15 vs 0/15 controls), kidney (glomerular nephropathy in 3/15 vs 2/15 controls). In females the target organs at a high dose are adrenal (vacuolation, hypertrophy pigment accumulation in 4/15 vs 0/15 controls), kidney (glomerular nephropathy in 8/15 vs 1/15 controls), heart (myocardial degeneration in ventricles in 1/15 vs 0/15 controls). Note that these histopath changes were not examined at lower doses, therefore 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, but this is based on the absence of histopath evaluation at low and mid doses. The AUC of parent + metabolite at 750 mg/kg/day in male (13.7 µg.h/ml) and at 250 mg/kg/day in female (9.4 µg.h/ml) rats was 14-20 fold the human AUC (0.64 µg.h/ml) at recommended dose of 10 mg/day.

Study title: A 6-Month Oral Toxicity Study of SCH-58235 in Dogs (Study No. SN 96454)

Key study findings: SCH 58235 following a 6-month oral gavage administration in dogs (30, 100, 300 mg/kg/day) produced mild partial to complete loss of pigmentation in the tapetal region of one or both eyes was observed in dogs (1/8, 2/8, 0/8, 1/8 respectively) in ophthalmoscopy exams. The target organs of toxicity at a high dose in males are heart (mononuclear cellular infiltration in 1/4 dogs vs 0/4 controls) and in females spleen (extramedullary hematopoiesis in 2/4 vs 1/4 controls). Since histopath changes were only observed in 1 or 2 animals, the NOAEL dose of the drug in 6-month toxicity study in dogs may be the highest dose of 300 mg/kg/day.

NDA 21-445

Study no: SN 96454

Volume #, and page #: 1.32, page 1

Conducting laboratory and location: Schering-Plough Research Institute

Date of study initiation: Not provided

GLP compliance: Yes

QA report: yes (X) no ()

Drug lot #, and % purity: 98-58235-X-201, 98-58235-X-202, 98-58235-X-207

Formulation/vehicle: 0.4% (w/v) Methylcellulose

Methods (unique aspects):

Dosing:

Species/strain: Dogs, Beagle

#/sex/group or time point (main study): 4/sex/dose

Satellite groups used for toxicokinetics or recovery: Not applicable.

Age: Approximately 5-7 months of age

Weight: Males 5.7-9.3 kg, females 5.4-9.6 kg.

Doses in administered units: Males and females 0, 30, 100, 300 mg/kg/day. Dose selection was based on two 3-month tox studies (study # P-6291 conducted in 1996 & SN97110 conducted in 1999) in dogs.

Route of administration: Oral, gavage at volume of 5 ml/kg for 24 consecutive weeks (182 days)

Observations and times:

Clinical signs: Once daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Prior to treatment, and once during week 26.

Body Temperatures, heart rates, respiration rate: Prior to treatment, and once during weeks 5 and 25.

ECG: Prior to treatment, and once during weeks 4 (group 1 and 3 males, 5 (group 2 and 4 males and all females), and 25.

Hematology: Prior to treatment, and during weeks 12 and 27.

Clinical chemistry: Prior to treatment, and during weeks 12 and 27.

Urinalysis: Prior to treatment, and during weeks 12 and 26.

Gross pathology: At sacrifice on day 183.

Organs weighed: Organs weighed are listed in the Table 28 on day 183

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Table 28. Tissues collected for organ weights in the 6-month dog tox study

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

Histopathology: This was performed at sacrifice in control and high dose animals, listed in the histopathology Table 29.

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Table 29. Tissues collected for histopath evaluation in the 6-month dietary dog tox study

Tissues Collected	
Animal Identification^a Gross Findings Adrenal Glands Aorta – Thoracic Bone – Femur Bone – Rib Bone – Sternum Bone Marrow Section – Rib and Sternum Bone Marrow for Cytology – Rib^b Brain Epididymides Esophagus Eyes with optic nerve Gallbladder Heart Intestine, Large (cecum, colon) Intestine, Small (duodenum, ileum, jejunum) Kidneys Lacrimal Glands Liver Lungs plus Bronchi Lymph Nodes (mandibular and mesenteric)	Mammary Glands - Inguinal Ovaries Pancreas Parathyroid Gland (s)^c Peripheral Nerve (Sciatic) Pituitary Gland Prostate Gland Salivary Gland – Mandibular Skeletal Muscle – Biceps Femoris Skin – Inguinal Spinal Cord – Thoracolumbar Spleen Stomach Testes Thymus Thyroid Gland Tongue Trachea Urinary Bladder Uterus (plus Cervix) Vagina
a: Collected but not processed. b: Bone marrow cytology specimens were prepared for all dogs sacrificed at the scheduled necropsy, but were not evaluated due to lack of changes in the peripheral blood. c: Examined histopathologically when present in routine section.	

Toxicokinetics: On day 1 during weeks 13 and 25, at 1, 4, 8, 12 and 24 hrs. Conjugated and unconjugated drug was measured by _____ by _____

Results:

Mortality: None

Clinical signs: No drug related effects were observed.

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

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

Body Temperatures, heart rates, respiration rate: No treatment related effects were observed on these parameters.

Ophthalmoscopy: The individual data show mild loss of pigmentation in the tapetal region of the eye (right) in 1/4 males at 300 mg/kg/day in week 26 vs not seen prior to the treatment in that male. Similarly in 2/4 females, this was observed in week 26 vs not seen in both females prior to the treatment (one had mild partial bilateral lack of tapetal pigmentation in both eyes, the other had complete lack of tapetal pigmentation in both eyes). 1/4 of control females had a lack of normal tapetal pigmentation in both eyes but it was present in that female prior to the treatment, and then became normal after treatment (unknown how?). **Tapetal** mostly refers to tapetal lucidum which is iridescent

pigment epithelium of the choroid of animals. **Tapetal gives animal's eyes the properties of shining in the dark.** Sponsor states that these findings are incidental. No histopath findings in eyes were observed

Eyes	Pretest	week 26
Males	0/4, 0/4, 0/4, 0/4	0/4, 0/4, 0/4, 1/4*
Females	1/4***, 0/4, 0/4, 1/4	0/4, 2/4**, 0/4, 0/4

*= mild loss of pigmentation in the tapetal region of the right eye

**= mild partial to complete loss of pigmentation in the tapetal region of both eyes

***= Lack of normal tapetal pigmentation in both eyes

Electrocardiograms: No drug related effects were observed.

Hematology: No drug related effects were observed.

Biochemistry: In all treated dogs, cholesterol was lower (males 152, 109, 126, 110 mg/dl at 0, 30, 100 and 300 mg/kg/day on day 183 (decreases of 10-16%). These values in females were 258, 151, 154, 154 respectively (decreases of 17-28%), however in both sexes these were not significantly different from controls. No changes in TG levels were observed. No effects on urine analysis were observed.

Organ Weights: No drug related effects were observed.

Gross pathology: No drug related changes were observed.

Histopathology: These were performed only in control and high dosed animals. In males histopath findings were observed in the heart at a high dose (mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls). A spleen anomaly was observed in males at 100 mg/kg/day (0/4, ne, 1/1, 0/4) and extramedullary hematopoiesis of minimal severity in females (1/4, ne, ne, 2/4).

ne=Not examined.

Toxicokinetics. The plasma AUC values are shown in the Table. The total (drug+glucuronide) drug seem to accumulate by up to 2-3 fold. There was extensive glucuronidation of the drug in dogs. The AUC values of the total drug in week 25 were higher (2062, 2800, 4912 ng.h/ml) vs on day 1 (1639, 3974, 3466 ng.h/ml at 30, 100 and 300 mg/kg/day) in male+ female dogs. No consistent gender differences were noted and there was lot of variability in values. There were less than dose proportional increases in AUC exposures as was observed in rat studies.

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Table 30: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 on day 1, in week 13 and 25 in a 6-month dog toxicity study:

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Dose (mg/kg)	Gender ^a	Total SCH 58235 AUC(0-24 hr) (ng-hr/mL)					
		Day 1		Week 13		Week 25	
30	Male	1910	(23)	3038	(41)	1925	(38)
	Female	1369	(55)	1642	(51)	2198	(51)
	Male & Female	1639	(39)	2340	(53)	2062	(43)
100	Male	3013	(22)	3067	(16)	3192	(51)
	Female	4935	(45)	6076	(55)	2409	(26)
	Male & Female	3974	(46)	4572	(60)	2800	(44)
300	Male	2649	(24)	7338	(39)	4310	(29)
	Female	4283	(12)	4806	(34)	5513	(55)
	Male & Female	3466	(29)	6072	(42)	4912	(46)
Unconjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
30	Male	188	(44)	363	(45)	130	(37)
	Female	112	(36)	336	(52)	137	(28)
	Male & Female	150	(48)	349	(45)	133	(30)
100	Male	186	(24)	263	(9)	149	(15)
	Female	221	(20)	371	(33)	232	(37)
	Male & Female	204	(22)	317	(32)	190	(38)
300	Male	249	(27)	575	(47)	413	(19) ^a
	Female	368	(45)	525	(46)	471	(42)
	Male & Female	309	(43)	550	(44)	446	(34) ^c
Conjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
30	Male	1722	(26)	2676	(42)	1797	(38)
	Female	1258	(57)	1147	(47) ^b	2061	(53)
	Male & Female	1490	(41)	2020	(58) ^c	1929	(44)
100	Male	2827	(22)	2805	(18)	3041	(53)
	Female	4715	(47)	5705	(57)	2178	(25)
	Male & Female	3771	(48)	4255	(63)	2609	(46)
300	Male	2400	(27)	6763	(38)	4165	(33) ^a
	Female	3914	(15)	3998	(30)	5044	(58)
	Male & Female	3157	(31)	5380	(44)	4667	(48) ^c
a: n=4 males or females; n=8 males and females combined b: n=3 c: n=7							

Toxicology summary: In summary, in 6-month toxicity study in dogs (30, 100 and 300 mg/kg/day), the exposure of the total (2.1, 2.8, 4.9 µg.h/ml vs on day 1 1.6, 4.0, 3.5 µg.h/ml at 30, 100 and 300 mg/kg/day), free and unconjugated drug was higher after 6-months suggesting accumulation over time. In ophthalmoscopy exams, 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). Target organs of toxicity may be heart at a high dose in males (mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls) and spleen in females (extramedullary hematopoiesis of minimal severity in females (2/4 vs 1/4 in controls). Since histopath changes were only observed in 1 or 2 animals and was only performed in control and HD, the **NOAEL dose of the drug in 6-month toxicity study in dogs may be 300 mg/kg/day**. The AUC of the parent + metabolite at 100 mg/kg/day in dogs (4.9 µg.h/ml) was 7-fold, the human AUC at 10 mg/day (0.64 µg.h/ml). **No overt toxicity could be established in this study, sponsor should have used higher doses to establish the toxicity in animals.**

Following one year toxicity study in dogs study was reviewed under IND _____
on 4/26/2001

One year Oral Toxicity Study of SCH 5823^a in dogs (Study No. 96455): _____

Sponsor's ID Study #: 96455

Amendment #, Vol. #, and page #: 129, 74.1, page 14.

Conducting laboratory: Schering-Plough Research Institute, Lafayette, NJ).

Date of study initiation and final report: 11/16/1998 and 8/4/200

GLP compliance: Yes

QA Report: Yes (X) No (), Is the evaluation based on a final, QA report: Yes.

Methods: This study examined the effects of SCH-5823~ (at 0, 30, 100 and 300 mg/kg/day) for 1-year in dogs.

Dosing information:

species: Beagle dogs.

#/sex/group or time point: 4/sex/group

age: ≈ 4-7 months old

weight: males 6.4-11.4, females 5.2-9.0 kilograms.

satellite groups used for toxicokinetics: N/A

Dosage groups in administered units: Four groups (4 dogs/sex/group) were given oral SCH-5823~ by gavage (once daily) at doses of 0, 30, 100 and 300 mg/kg/day for 3-months. Control animals received the vehicle only (0.4% (w/v) methylcellulose.

Route, form, volume, and infusion rate (if i.v.): Oral (via gavage).

Drug, lot #: Suspensions of 6, 20, 60 mg/ml had lot #s 98-58235-X-201, 98-58235-X-22, 198-58235-X-07 respectively.

Formulation/vehicle: Formulated in 0.4% (w/v) in aqueous methylcellulose

Times at which Observations are made:

Clinical signs/Physical exams: Daily

Body weights: Prior to dosing, and weekly thereafter.

Food consumption: Daily.

Hematology/Coagulation: Prior to dosing, during weeks 12, 24 and on day 366.

Clinical chemistry: Prior to dosing, during weeks during weeks 12, 24 and on day 366.

Urine analysis: Prior to dosing, during weeks during weeks 12, 24 and on day 365

Ophthalmic Examination: Prior to the treatment, and during week 52.

Electrocardiograph Examinations: ECG were taken prior to dosing, once during week 5, 26 and week 52.

Gross pathology: At sacrifice on day 366.

Organs weighed: *Marked organs in the appended Table were weighed.

Histopathology: At sacrifice from vehicle control and high dose groups, and in any group with gross findings.

Toxicokinetics: Blood was collected from all dogs (in tubes containing sodium heparin, and on ice) on day 1, and during weeks 13, 26, and 51, at 1, 4, 8, 12 and 24 hrs.

Plasma concs. of the free form of the drug and total were determined using

Results:

Mortality: None

Clinical Signs: No drug related effects were observed.

Body weight/Food consumption: No drug related effects were observed on body weights or food consumption.

Ophthalmic Examination: No drug related effects were observed.

Electrocardiograms: No drug related effects were observed.

Hematology: No drug related effects were observed.

Biochemistry: In all treated dogs, cholesterol was decreased by 15, 26 and 31% at 30, 100 and 300 mg/kg/day on day 366 (also similar decreases of 15-29% were noted on day 79 and on day 163). No effects on urine analysis were observed.

Organ Weights: Mean absolute heart weights tended to be lower in males at low-mid doses (98.8, 86.7, 81.3, 90.2 g at 0, 30, 100, 300 mg/kg/day respectively, 2/4 male dogs in low & mid dose groups had heart weights below 81 g), but not so in females (65.1, 67.7, 68.7, 77.9 g respectively). No other drug related effects were observed.

Gross pathology: No drug related changes were observed.

Histopathology: These were performed only in control and high dosed animals. At a high dose of 300 mg/kg/day toxicity was observed in the lymph nodes in **males** (minimal pigment accumulation in mandibular in 3/4 vs 1/4 controls, lymphoid mesenteric hyperplasia of minimal severity in 1/4 vs 0/4 controls, erythrophagocytosis mesenteric minimal to mild in 3/4 vs 0/4 controls) and **females** (minimal pigment accumulation in mandibular in 4/4 vs 2/4 controls, lymphoid mesenteric hyperplasia of minimal to mild severity in 2/4 vs 0/4 controls, minimal mandibular hemorrhage & erythrophagocytosis in 1/4 vs 0/4 controls, erythrophagocytosis mesenteric minimal to mild in 3/4 vs 2/4 controls). In females only in the control group, histopath findings were observed in the heart of 2/4 dogs (these included minimal periarteritis, mild intimal focal hyperplasia, moderate atrial myocardial fibrosis, and mild myocardial degeneration).

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Toxicokinetics. The plasma AUC values are shown in the Table 31. The total (drug+glucuronide) and unconjugated drug seem to accumulate by up to 2-fold. There was extensive glucuronidation of the drug in dogs. The AUC values of the total drug in week 51 were higher (2780, 5010, 6380 ng.h/ml) vs on day 1 (2070, 2700, 3310 ng.h/ml at 30, 100 and 300 mg/kg/day) in male+ female dogs. No gender differences were noted and there was lot of variability in values. The AUC values of unconjugated drug in week 51 were also higher (2470-5650 ng.h/ml vs 1890-3030 ng.h/ml on day 1 at 30-300 mg/kg/day). The AUC values of the free drug in week 51 were 189-315 ng.h/ml vs 16-73 ng.h/ml on day 1.

Table 31. Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 on day 1, in week 13, 26 and 51 in a 1-year dog toxicity study

Dose (mg/kg)	Gender ^a	Total SCH 58235 AUC(0-24 hr) (ng-hr/mL)							
		Day 1		Week 13		Week 26		Week 51	
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
30	Male	1810	51	1470	8	1810	45	2880	58
	Female	2330	15	2530	56	1920	18	2680	42
	Male & Female	2070	34	2000	54	1870	31	2780	47
100	Male	2220	32	2560	19	2320	51	3960	43
	Female	3170	43	4480	30	5180	17	6050	47
	Male & Female	2700	42	3520	39	3750	48	5010	49
300	Male	3350	44	4980	34	4110	21	6640	5
	Female	3260	39	3410	12	3310	22	8110	13
	Male & Female	3310	38	4200	34	3710	23	6380	10
Unconjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)									
30	Male	188	103	147	28	142	77	270	122
	Female	180	38	178	55	116	10	217	42
	Male & Female	184	73	161	43	129	57	243	93
100	Male	173	22	192	28	107	8	136	35
	Female	172	10	342	49	197	31	242	29
	Male & Female	172	16	267	53	152	41	189	42
300	Male	290	39	403	37	338	42	275	32
	Female	265	68	280	25	241	19	355	41
	Male & Female	277	50	341	37	288	38	315	38
Conjugated SCH 58235 AUC(0-24 hr) (ng-hr/mL)									
30	Male	1630	45	1330	10	1670	42	2480	58
	Female	2160	14	2350	57	1800	19	2460	43
	Male & Female	1890	31	1840	56	1740	30	2470	47
100	Male	2050	36	2370	22	2210	53	3750	44
	Female	3000	45	3980	21	4980	17	5810	48
	Male & Female	2530	44	3180	34	3600	49	4780	50
300	Male	3060	50	4580	34	3770	19	5540	18
	Female	3000	37	3130	13	3070	23	5760	16
	Male & Female	3030	41	3660	34	3420	22	5650	16

a: n=4 males or females; n=8 males and females combined.

In summary, in 1-year toxicity study in dogs (30, 100 and 300 mg/kg/day), the exposure of the total, free and unconjugated drug was higher after 1 year suggesting accumulation 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. At a high dose lymph node toxicity was noted in 8/8 vs 4/8 control dogs. These 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), erythrophagocytosis mesenteric minimal to mild (in 6/8 vs 2/8 controls). No effects on any other parameters, including histopath findings in other tissues were observed. **The NOEL dose of the drug in 1-year toxicity study in dogs was 100 mg/kg/day.** The AUC of parent + metabolite at 100 mg/kg/day in dogs (5.01 µg.h/ml) was 8-fold, the human AUC at 10 mg/day (0.64 µg.h/ml).

IV.B. General Toxicology with combination of SCH 58235 + statins

Acute toxicity studies with combination of SCH 58235 + statins:

Acute oral and intraperitoneal (IP) toxicity studies in mice & rats were conducted with lovastatin and simvastatin, using 1:1 ratio of SCH 58235 + statin.

A. Single dose Oral studies of SCH 58235 + statins in rats/mice: Following oral administration of 1000/1000 mg/kg/day of ezetimibe/simvastatin or ezetimibe/lovastatin in rats and mice, no clinical signs, changes in body weights or mortality was observed.

1) Table: Acute oral tox study in mice with ezetimibe + simvastatin (SCH 57098)

Table 1 Summary of Antemortem Observations					
Observation	Group	Control ^a		SCH 58235/SCH 57098	
	SCH 58235 Dose (mg/kg)	0		1000	
	SCH 57098 Dose (mg/kg)	0		1000	
	Sex	M	F	M	F
Mortality		0/5	0/5	0/5	0/5
Nothing Remarkable		5/5	5/5	5/5	5/5
Mean Body Weight Gains (g)					
Days 1-8 ^b		8.3	4.7	8.1	5.3
Days 8-15 ^b		1.7	1.3	2.1	1.4

a: Control mice were each dosed twice with 20 ml/kg of vehicle.
 b: Body weights were collected using the _____ system, which considers Day 0 as the first day of dosing, while clinical observations were collected manually with Day 1 as the first day of dosing. For reporting consistency, body weights are listed as being collected on Days 1, 8 and 15 rather than Days 0, 7 and 14.

2) Table: Acute oral tox study in rats with ezetimibe + simvastatin (SCH 57098)

Table 1 Summary of Antemortem Observations					
Observation	Group	Control ^a		SCH 58235/SCH 57098	
	SCH 58235 Dose (mg/kg)	0		1000	
	SCH 57098 Dose (mg/kg)	0		1000	
	Sex	M	F	M	F
Mortality		0/5	0/5	0/5	0/5
Nothing Remarkable		5/5	5/5	5/5	5/5
Mean Body Weight Gains ^b (g)					
Days 1-8 ^b		80.8	58.4	71.5	48.9
Days 8-15 ^b		51.8	21.4	51.0	18.5

a: Control rats were each dosed twice with 10 ml/kg of vehicle.
 b: Body weights were collected using the _____ system, which considers Day 0 as the first day of dosing, while clinical observations were collected manually with Day 1 as the first day of dosing. For reporting consistency, body weights are listed as being collected on Days 1, 8 and 15 rather than Days 0, 7 and 14.

3) Table: Acute oral tox study in mice with ezetimibe + lovastatin (SCH 48176)

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Table 1 Summary of Antemortem Observations

Observation	Group	Control		SCH 357015	
	SCH 58235 Dose (mg/kg)	0		1000	
	SCH 48176 Dose (mg/kg)	0		1000	
	Sex	M	F	M	F
Mortality		0/5	0/5	0/5	0/5
Nothing Remarkable		5/5	5/5	2/5	5/5
Abnormal Stool, None		0/5	0/5	1/5 ^a	0/5
Dehydrated		0/5	0/5	1/5 ^a	0/5
Rough Haircoat		0/5	0/5	3/5 ^{a,b}	0/5
Mean Body Weight Gains (g)					
Days 0-7		7.8	4.8	7.8	5.0
Days 7-14		2	1.5	1.7	1.0

a: On Day 3, observations of abnormal stool - none, dehydration and rough hair coat were noted in Animal No. 26 and were attributed to the absence of a feeder noted on the same day.

b: On Days 7 through 13, rough hair coat was observed in Animal Nos. 6, 8 and 26 and was considered unrelated of test article administration due to the time of onset.

4) Table: Acute oral tox study in rats with ezetimibe + lovastatin (SCH 48176)

Table 1 Summary of Antemortem Observations

Observation	Group	Control		SCH 357015	
	SCH 58235 Dose (mg/kg)	0		1000	
	SCH 48176 Dose (mg/kg)	0		1000	
	Sex	M	F	M	F
Mortality		0/5	0/5	0/5	0/5
Nothing Remarkable		5/5	4/5	5/5	5/5
Scant feces		0/5	1/5	0/5	0/5
Mean Body Weight Gains (g)					
Days 0-7		78.8	46	70.2	47.6
Days 7-14		56.3	26.1	48	21.4

B. Single dose intraperitoneal (IP) studies of SCH 58235 + statins in rats/mice

5) IP studies in mice with ezetimibe + simvastatin (SCH 57098): Following intraperitoneal (IP) administration in mice of 100/100, 250/250, 500/500, 750/750 mg/kg/day of ezetimibe/simvastatin, mortality was observed at all combinations within 15 minutes to 8 days after dosing in mice (0/10, 1/10, 1/10, 6/10, 7/10 mice at 0/0, 100/100, 250/250, 500/500, 750/750 mg/kg/day of ezetimibe/simvastatin respectively). Clinical signs in mice (in both sexes) included, hunched posture, moribundity, scant feces, hypoactivity (within 15 minutes of dosing), urogenital staining. There was dose-related decrease in BW gain in both sexes at all doses, at the lowest dose decrease was of 15-27%. Maximal non-lethal doses were <100/100 mg/kg/day.

Table: Acute IP tox study in mice with ezetimibe + simvastatin (SCH 57098)

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Observation	Group		SCH 58235/ SCH 57098									
	Control ^a		100		250		500		750			
	SCH 57098 Dose (mg/kg)		0		100		250		500		750	
	SCH 58235 Dose (mg/kg)		0		100		250		500		750	
	Sex:		M	F	M	F	M	F	M	F	M	F
Mortality	0/5	0/5	1/5	0/5	0/5	1/5	3/5	3/5	2/5	5/5		
Nothing Remarkable	5/5	5/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5		
Hypoactive	0/5	0/5	4/5	3/5	5/5	4/5	5/5	5/5	5/5	5/5		
Moribund	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5		
Hunched Posture	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	0/5	0/5		
Scant Feces	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	3/5	2/5		
Urogenital Staining - Yellow/Orange	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	4/5		
Mean Body Weight Gains ^b (g)												
Days 1-8 ^c	8.2 (N=5)	8.6 (N=5)	7.0 (N=4)	5.2 (N=5)	6.0 (N=5)	3.7 (N=4)	5.6 (N=2)	3.9 (N=2)	3.9 (N=3)	NA		
Days 8-15 ^c	1.0 (N=5)	0.7 (N=5)	1.6 (N=4)	0.7 (N=5)	1.4 (N=5)	1.1 (N=4)	0.8 (N=2)	1.7 (N=2)	1.2 (N=3)	NA		

NA: Not applicable, no surviving mice n = number of surviving mice
a: Control mice were each dosed twice with 10 ml/kg of vehicle.
b: Only body weights of surviving mice were used to calculate mean body weight gain.
c: Body weights were collected using the  system, which considers Day 0 as the first day of dosing, while the clinical observations were collected manually with Day 1 as the first day of dosing. For reporting consistency, body weights are listed as being collected on Days 1, 8 and 15 rather than Days 0, 7 and 14

Table: LD-50 values in acute IP tox study in mice with ezetimibe + simvastatin (SCH 57098)

Sex	Estimated LD ₅₀ Value (95% fiducial limits) ^a	Maximum Asymptomatic Dose	Maximum Nonlethal Dose
Male	896 SCH 58235/ 896 SCH 57098	<100 mg/kg SCH 58235/ <100 mg/kg SCH 57098	<100 mg/kg SCH 58235/ <100 mg/kg SCH 57098
Female	387 mg/kg (199-606) SCH 58235/ 387 mg/kg (199-606) SCH 57098	<100 mg/kg SCH 58235/ <100 mg/kg SCH 57098	100 mg/kg SCH 58235/ 100 mg/kg SCH 57098

a: Calculation of the 95% fiducial limits was not possible for male mice based on the pattern of mortality.

6) IP studies in rats with ezetimibe + simvastatin (SCH 57098): Following intraperitoneal (IP) administration in rats of 100/100, 250/250, 500/500, 750/750, 1000/1000 mg/kg/day of ezetimibe/simvastatin, mortality was observed from 250/250 mg/kg/day between days 2, 4 & day 8 after dosing (0/10, 0/10, 7/10, 10/10, 9/10 rats at 0/0, 100/100, 250/250, 500/500, 750/750, 1000/1000 mg/kg/day of ezetimibe/simvastatin respectively). Clinical signs in rats (in both sexes) included, chromorrhoea, hypoactivity, rough hair coat and/or urogenital staining. In surviving rats, thin appearance, moderate dehydration and/or weakness (general or hindquarters) was observed at 500/500 mg/kg/day. Other signs noted were hunched posture, moribundity, scant feces, labored breathing, abdominal swelling at $\geq 250/250$ mg/kg/day. There were dose-related decreases in BW gain in both sexes at all doses, including the lowest dose (by 25% in males & by 12% in females at the lowest dose).

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Table: Acute IP tox study in rats with ezetimibe + simvastatin (SCH 57098):

Table 1 Summary of Antemortem Observations														
Observation	Group		Control ^a		SCH 58235/ SCH 57098									
	SCH 57098 Dose (mg/kg)		0		100		250		500		750		1000	
	SCH 58235 Dose (mg/kg)		0		100		250		500		750		1000	
	Sex:		M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	4/5	5/5	5/5	4/5	5/5
Nothing Remarkable	5/5	5/5	4/5	5/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Thin Appearance	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	3/5	2/5	2/5	3/5	1/5	0/5
Chromohinorrhea	0/5	0/5	0/5	0/5	5/5	2/5	4/5	5/5	5/5	4/5	5/5	4/5	5/5	4/5
Cool to Touch	0/5	0/5	0/5	0/5	0/5	0/5	2/5	2/5	1/5	3/5	0/5	0/5	0/5	0/5
Dehydrated - Moderate	0/5	0/5	0/5	0/5	0/5	0/5	3/5	3/5	0/5	1/5	1/5	0/5	0/5	0/5
Hindquarter Weakness	0/5	0/5	0/5	0/5	0/5	0/5	2/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Hypoactive	0/5	0/5	1/5	0/5	1/5	1/5	5/5	5/5	5/5	4/5	5/5	5/5	2/5	2/5
Labored Breathing	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Loose Stool	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Marbled	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Hunched Posture	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	2/5	2/5	0/5	0/5	0/5	0/5
Prostrate	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Rough Hair Coat	0/5	0/5	0/5	0/5	3/5	0/5	3/5	2/5	2/5	3/5	0/5	0/5	0/5	0/5
Scant Feces	0/5	0/5	0/5	0/5	0/5	0/5	3/5	2/5	3/5	3/5	1/5	2/5	2/5	2/5
Swelling - Abdominal Area	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Urogenital Staining - Brown	0/5	0/5	0/5	0/5	0/5	0/5	4/5	4/5	2/5	3/5	1/5	0/5	0/5	0/5
Weak	0/5	0/5	0/5	0/5	0/5	0/5	2/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5

Table 1 Summary of Antemortem Observations														
Observation	Group		Control ^a		SCH 58235/ SCH 57098		SCH 58235/ SCH 57098		SCH 58235/ SCH 57098		SCH 58235/ SCH 57098		SCH 58235/ SCH 57098	
	SCH 57098 Dose (mg/kg)		0		100		250		500		750		1000	
	SCH 58235 Dose (mg/kg)		0		100		250		500		750		1000	
	Sex:		M	F	M	F	M	F	M	F	M	F	M	F
Mean Body Weight Gains ^b (g)														
Days 1-8 ^c	71.1 (n=5)	49.8 (n=5)	53.5 (n=5)	44.1 (n=5)	19.9 (n=5)	25.1 (n=4) ^d	-17.5 (n=3)	-16.5 (n=2)	32.9 (n=1)	NA	14.5 (n=1)	NA	NA	NA
Days 8-15 ^c	47.7 (n=5)	20.3 (n=5)	29.8 (n=5)	26.8 (n=5)	30.7 (n=5)	53.1 (n=5)	11.3 (n=2)	21.8 (n=1)	NA	NA	39.5 (n=1)	NA	NA	NA

NA: Not applicable, no surviving rats n = number of surviving rats
a: Control rats were each dosed twice with 10 mg/kg of vehicle.
b: Only body weights of surviving rats were used to calculate mean body weight gain.
c: Body weights were collected using the  system, which considers Day 0 as the first day of dosing, while the clinical observations were collected manually with Day 1 as the first day of dosing. For reporting consistency, body weights are listed as being collected on Days 1, 8 and 15 rather than Days 0, 7 and 14.
d: One rat not weighed at this interval.

Table: LD-50 values in acute IP tox study in rats with ezetimibe + simvastatin (SCH 57098)

Acute Intraperitoneal Toxicity Study of SCH 58235 In Combination with SCH 57098 (Simvastatin) in Rats (SN 96412): Study Results			
Sex	Estimated LD ₅₀ Value (95% fiducial limits) ^a	Maximum Asymptomatic Dose	Maximum Nonlethal Dose
Male	492 mg/kg (255-886) SCH 58235/ 492 mg/kg (255-886) SCH 57098	<100 mg/kg SCH 58235/ <100 mg/kg SCH 57098	250 mg/kg SCH 58235/ 250 mg/kg SCH 57098
Female	473 SCH 58235/ 473 SCH 57098	100 mg/kg SCH 58235/ 100 mg/kg SCH 57098	250 mg/kg SCH 58235/ 250 mg/kg SCH 57098

a: Calculation of the 95% fiducial limits was not possible for female rats based on the pattern of mortality.

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7) IP studies in mice with ezetimibe + lovastatin (SCH 48176): In the lovastatin study mice were given intraperitoneal 100/100, 250/250, 500/500, 750/750, 1000/1000 mg/kg/day of ezetimibe/lovastatin. Mortality in mice was observed from the dose of 500/500 mg/kg/day within 24 hrs to 2 days (0/10, 0/10, 0/10, 1/10, 9/10, 10/10 respectively). At two highest doses almost all animals died. Prior to death the clinical signs were similar as seen with above simvastatin study, i.e abnormal stool (none or scant), moribundity and/or prostration, hypoactivity, cool to touch, urogenital staining. No drug related decreases in BW gains were observed up to 500/500 mg/kg/day of ezetimibe/lovastatin, at higher doses the BW changes could not be evaluated as almost all animals died.

Table: Acute oral tox study in mice with ezetimibe + lovastatin (SCH 48176)

Table 1 Summary of Antemortem Observations													
Observation	Group	Control		SCH 357015		SCH 357015		SCH 357015		SCH 357015		SCH 357015	
		C1		T1		T2		T3		T6 ^a		T5	
		SCH 58235 (mg/kg)		SCH 48176 (mg/kg)		100		250		500		750	
		0		100		250		500		750		1000	
	Sex:	M	F	M	F	M	F	M	F	M	F	M	F
Mortality		0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	5/5	4/5	5/5	5/5
Nothing Remarkable		5/5	5/5	5/5	5/5	5/5	5/5	1/5	4/5	0/5	0/5	0/5	0/5
Abnormal Stool, None		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5
Abnormal Stool, Scant		0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5
Cool to Touch		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	4/5	1/5	0/5	0/5
Dehydrated		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	4/5	1/5	0/5	0/5
Hypoactive		0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	3/5	1/5	0/5	0/5
Moribund		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Prostrate		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Urogenital Staining, Minimal		0/5	0/5	0/5	0/5	0/5	0/5	2/5	1/5	4/5	2/5	0/5	0/5
Urogenital Staining, Moderate		0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5
Urogenital Staining, Severe		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Mean Body Weight Gains ^b (g)													
Days 0-7		7.7 (N=5)	6.1 (N=5)	7.0 (N=5)	6.0 (N=5)	6.8 (N=5)	6.5 (N=5)	7.2 (N=4)	5.4 (N=5)	NA	0.9 ^c (N=1)	NA	NA
Days 7-14		1.8 (N=5)	0.3 (N=5)	1.5 (N=5)	1.3 (N=5)	1.3 (N=5)	1.1 (N=5)	1.5 (N=4)	1.7 (N=5)	NA	0.1 ^d (N=1)	NA	NA

NA: Not applicable, no surviving mice.

a: Due to a rounding error, doses for Group T4 were calculated incorrectly and the animals did not receive the intended dose of 750 mg/kg of SCH 58235 and SCH 48176. As a result, Group T6 was added to the study and the 750 mg/kg SCH 58235/SCH 48176 dose level was repeated. Data collected from Group T4 mice are presented in Appendix 3 but were not evaluated.

b: Only body weights of surviving mice were used to calculate mean body weight gain.

c: Days 0-8

d: Days 8-14

Table: LD 50 values in the acute oral tox study in mice with ezetimibe + lovastatin (SCH 48176)

Acute Intraperitoneal Toxicity Study of SCH 357015 in Mice (SN 99009): Study Results			
Sex	Estimated LD ₅₀ Value	Maximum Asymptomatic Dose	Maximum Nonlethal Dose
Male	522 mg/kg SCH 58235/ 522 mg/kg SCH 48176	250 mg/kg SCH 58235/ 250 mg/kg SCH 48176	250 mg/kg SCH 58235/ 250 mg/kg SCH 48176
Female	721 mg/kg SCH 58235/ 721 mg/kg SCH 48176	250 mg/kg SCH 58235/ 250 mg/kg SCH 48176	500 mg/kg SCH 58235/ 500 mg/kg SCH 48176

8) IP studies in rats with ezetimibe + lovastatin (SCH 48176): In the lovastatin study rats were given intraperitoneal 100/100, 250/250, 500/500, 750/750, 1000/1000 mg/kg/day of ezetimibe/lovastatin. Mortality in rats was observed from the dose of 500/500 mg/kg/day within 15 minutes to 6 days (0/10, 0/10, 0/10, 1/10, 5/10, 8/10

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respectively). At the highest dose, all animals died on day 2. Prior to death the clinical signs were abnormal stool (loose, mucoid, none or scant), moribundity and/lacrimation, hypoactivity, rough hair coat, urogenital staining, cool to touch, mild dehydration, nasal discharge. Drug related decreases in BW gains were observed in both sexes, in males at all doses, in females from 250/250 mg/kg/day of ezetimibe/lovastatin, at high doses the BW changes could not be evaluated as almost all animals died.

Table: Acute IP tox study in rats with ezetimibe + lovastatin (SCH 48176)

Table 1 Summary of Antemortem Observations														
Observation	Group		Control		SCH 357015		SCH 357015		SCH 357015		SCH 357015		SCH 357015	
	SCH 58235 Dose (mg/kg)		0		100		250		500		750		1000	
	SCH 48176 Dose (mg/kg)		0		100		250		500		750		1000	
	Sex:		M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	3/5	5/5	3/5	
Nothing Remarkable	4/5	5/5	5/5	5/5	4/5	4/5	3/5	3/5	0/5	1/5	0/5	0/5		
Abnormal Stool, Loose	0/5	0/5	0/5	0/5	0/5	0/5	2/5	1/5	0/5	1/5	0/5	2/5		
Abnormal Stool, Mucoid	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5		
Abnormal Stool, None	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	4/5	2/5	2/5	3/5	
Abnormal Stool, Scant	0/5	0/5	0/5	0/5	0/5	1/5	1/5	2/5	3/5	3/5	3/5	2/5		
Alopecia, Abdominal Area	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
Chromorhinorrhea	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	2/5	0/5	0/5		
Cool to Touch	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5	1/5	0/5	
Dehydration, Mild	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	2/5	0/5	0/5		
Hypoactive	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	0/5	0/5		
Laborated Breathing	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5		
Lacrimation, Left	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	
Moribund	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	
Nasal Discharge, Red	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
Rough Haircoat	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	2/5	1/5	0/5	1/5		
Urogenital Staining, Yellow, Mild	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	3/5	1/5	4/5		
Urogenital Staining, Yellow, Moderate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5		
Mean Body Weight Gains ^a (g)														
Days 0-7	79.6 (n=5)	49.0 (n=5)	64 (n=5)	56 (n=5)	58.2 (n=5)	38.6 (n=5)	35.7 (n=5)	35.2 (n=4)	10.4 (n=3)	16.1 (n=2)	NA	27.0 (n=2)		
Days 7-14	48.6 (n=5)	24.1 (n=5)	48.7 (n=5)	25.1 (n=5)	52.6 (n=5)	31.3 (n=5)	48.3 (n=5)	26.4 (n=4)	35.8 (n=3)	16.2 (n=2)	NA	15.8 (n=2)		

NA = Not applicable, no surviving rats.
a: Only body weights of surviving rats were used to calculate mean body weight gain.

Table: LD 50 values in acute IP tox study in rats with ezetimibe + lovastatin (SCH 48176)

Acute Intraperitoneal Toxicity Study of SCH 357015 in Rats (SN 99007): Study Results			
Sex	Estimated LD ₅₀ Value (95% fiducial limits) ^a	Maximum Asymptomatic Dose	Maximum Nonlethal Dose
Male	758 mg/kg SCH 58235/ 758 mg/kg SCH 48176	100 mg/kg SCH 58235/ 100 mg/kg SCH 48176	500 mg/kg SCH 58235/ 500 mg/kg SCH 48176
Female	771 mg/kg (445-869) SCH 58235/ 771 mg/kg (445-869) SCH 48176	100 mg/kg SCH 58235/ 100 mg/kg SCH 48176	250 mg/kg SCH 58235/ 250 mg/kg SCH 48176

a: Calculation of the 95% fiducial limits was not possible for male rats based on the observed pattern of mortality.

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In conclusion, when **the drug +statins were administered orally** (by gavage) to rats and mice, they were not acutely toxic, and the maximal non-lethal doses were >1000/1000 mg/kg/day of SCH 58235 + lovastatin or simvastatin. When administered IP, it was very toxic in mice and rats. **SCH 58235 + simvastatin** produced mortality in mice, starting at the lowest dose (non lethal doses were <100/100 mg/kg/day), in rats the mortality was seen at 500/500 mg/kg/day (maximal non-lethal doses were 250/250 mg/kg/day). In both mice and rats, the lowest doses produced decreases in BW gain of 15-27%. The clinical signs were chromorrhinrhea, hypoactivity, rough hair coat and/or urogenital staining, thin appearance, moderate dehydration and/or weakness (general or hindquarters), hunched posture, moribundity, scant feces, labored breathing, abdominal swelling. **In the SCH 58235 + lovastatin** study in mice and rats, mortality was seen starting at 500/500 mg/kg/day (maximal non-lethal doses were 250/250 mg/kg/day). No decreases in BW gains were observed in mice, but in rats all doses decreased BW gains. The clinical signs were similar as seen in earlier study with simvastatin, ie abnormal stool (loose, mucoid, none or scant), moribundity and/lacrimation, hypoactivity, rough hair coat, urogenital staining, cool to touch, mild dehydration, nasal discharge. No macroscopic observations following oral or IP dosing were conducted with the combinations. Mortality following acute IP dosing of the combination occurred at lower doses (at 100-500 mg/kg) than following monotherapy of ezetimibe (at 1000-2000 mg/kg) by this route. While in acute oral dosing, testing with the combination (1000/1000 mg/kg in rats and mice) was conducted at lower doses, compared to higher doses with monotherapy (ezetimibe alone, 3000-5000 mg/kg), which did not result in any mortality. Also dogs are more sensitive to the combination, and dog is also a most appropriate model for lipid altering drugs, both in terms of efficacy and toxicity, but dogs were not tested orally with the combination. IP dosing was more toxic, this may be because residual compound like material was seen in the abdomen cavity after this route of administration and appears to have caused severe irritation and inflammation.

ACUTE (SINGLE DOSE) STUDIES with monotherapy and combination therapy:
 Following were the maximal non-lethal doses in mice, rats and dogs:

	Monotherapy (ezetimibe) mg/kg doses	Combination therapy, Ezetimibe+ statin mg/kg doses	
Oral		Ezetimibe+simvastati n	Ezetimibe+lovastatin
mice	>5000	>1000	>1000
rats	>5000	>1000	>1000
dogs	>3000	na	na
IP			
mice	1000	<100 + <100	250-500/250-500
rats	1000-2000	500 + 500	250-500/250-500

na=not available

Multiple dose TOXICOLOGY with combination of SCH 58235 + statins: