CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-445

PHARMACOLOGY REVIEW(S)

NDA 21-445

Review completed: September 12, 2002

PHARMACOLOGY/TOXICOLOGY COVER SHEET

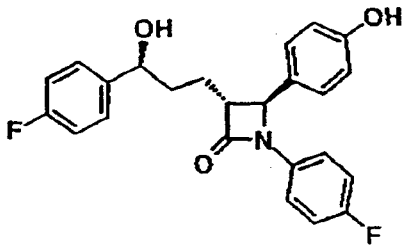
NDA number: NDA 21-445
Review number: 1
Sequence number/date/type of submission: December 27, 2001, original application, 3/4/2002, 4/26/02, 5/16/02, 7/3/02, 7/9/02, 8/5/02 (amendments to the original application).
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: MPS Singapore CO., LLC, Singapore
Manufacturer for drug substance: Schering-Plough LTD, Singapore Branch, Singapore

Reviewer name: Indra Antonipillai, Ph.D. Pharmacology Reviewer.
Division name: Division of Metabolic and Endocrine Drug products,
HFD #: HFD# 510
Review completion date: August 30, 2002

Drug:

Trade name: Zeita (10 mg tablets). It is a cholesterol absorption inhibitor.
Generic name (list alphabetically): Ezetimibe
Code name: SCH-58235 (tablets 10 mg).
Chemical name: 1-(4-fluorophenyl)-3®-(3-(4-fluorophenyl)-3(S)-hydroxypropyl)-4(S)-4-hydroxyphenyl)-2-azetidione
CAS registry number: 163223-33-1
Mole file number: N/A
Molecular formula/molecular weight: $C_{24}H_2O_3NF_2$/409.5

Structure:



SCH 58235

Indra Antonipillai, Ph.D.

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/temeck/koch
Review code:
File name: nda21445 (zeita)

Relevant INDs/NDAs/DMFs: IND —— (SCH 58235), IND ——————
Drug class: Inhibitor of cholesterol absorption (—— inhibitor).
Indication: Lipid lowering.
Clinical formulation: SCH 58235 10 mg tablets also contain the following inactive ingredients:

	mg/tablet
SCH 58235, ——	10
lactose monohydrate	
microcrystlline cellulose	
sodium lauryl sulfate	
croscarmellose sodium	
providone —— USP	
magnesium stearate	
——	
Total weight	100

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Physical chemical properties: SCH 58235 is a white crystalline powder with melting point of 163°C, and particle size —— It has 3 chiral centers and 8 possible isomers. Moisture level is dependent on relative humidity —— —— SCH 58235 is soluble in water ——— , and is freely soluble in organic solvents like methanol or acetone

The drug tablet is in an ——— ——— ——— no other forms have been found. An increase in the moisture at 6-months at 40°C/75% RH was accompanied by some conversion to the ——— ——— However, this process does not change the drug's dissolution, particle size or stability. As indicated SCH 58235 (or ezetimibe) has 3 chiral centers, and 8 possible isomers, and all 8 isomers have been synthesized and assigned stereochemical configuration ——— verified by a ——— procedure, which shows the ——— impurities in the drug substance are in amounts greater than — (but less than — , see Table below, which shows the acceptance limits of these impurities.

Following are the acceptance limits for impurities in the ——— drug substance (SCH 58235):

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Test	Acceptance Limits	Procedure Reference
Related Compounds:		

According to ICH Q3A guidance document, from pharm/tox aspect above impurities need to be qualified if present in amounts > 0.1% in the drug substance. Therefore sponsor has conducted special 1-month bridging toxicity studies in rats & dogs and genotox studies with added impurities to the drug. For details see the special toxicity study section. In the drug product, the degredants and impurities should be <1%, or these would need to be qualified. The total ——— impurities in the drug product were ——— and did not have to be qualified.

Route of administration: oral

Proposed use: The drug is indicated alone (monotherapy) or in combination with an HMG-CoA reductase inhibitor (combination therapy), as an adjunctive therapy to diet for the reduction of elevated LDL-cholesterol in patients with primary hypercholestrolemia (heterozygous familial and non-familial). The recommended dose of zeita is 10 mg/day, it can be administered any time of the day with or without food. It is also indicated as an adjunctive therapy for the reduction of elevated sitosterol and camposterol levels in patients with primary homozygous familial sitosterolemia. In addition, zeita is indicated in homozygous familial hypercholestrolemia (HoFH) patients with an HMG-CoA reductase inhibitor (approved for HoFH), for reduction of LDL-cholesterol as an adjunct to other lipid lowering treatments.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise

Executive Summary

1. Recommendations

A. Recommendation on approvability

Pharmacology recommends approval of this drug for monotherapy for the proposed indication, but preclinical studies do not support safety of ezetimibe combination with statins. Generally, a NOAEL could not be established. The toxicity profile appears to be that associated with statins. In some cases a metabolic interaction is demonstrated. However the toxicity appears at lower duration and exposure than statin monotherapy. Clinical data submitted on ezetimibe in combination with statins of 3-month duration do not demonstrate a safety concern

B. Recommendation for Nonclinical Studies:

The preclinical studies are adequate to support the safety of 10 mg/day dose for monotherapy, and no further studies are recommended. The preclinical studies in general have adequately assessed for 10 mg/day combination therapy with statins, but due to low or non-existent safety margins in rats and dogs to humans, this combination is not recommended for approval, based on animal studies alone. Combination of ezetimibe and statin produces equal, or greater toxicity than statin alone, which is well established and monitorable. Additionally clinical data on the combination of a short duration (3-months) do not show cause for concern according to the medical officer.

[ ]

C. Recommendation on Labeling: see the labeling section on page 392

II. Summary of Nonclinical Findings

1A. Brief Review of Nonclinical studies For Monotherapy (ezetimibe):

Preclinical studies include toxicology studies in rats, dogs and mice with durations of single dose up to 12 months, 2-year carcinogenicity studies in rats and mice, genotoxic studies, reproductive toxicity studies in rats and rabbits, and special toxicology studies. Ezetimibe monotherapy revealed some toxicity at exposures >10X human dose in heart, lymph nodes, kidney and bone marrow.

In 6 to 12-month chronic toxicity studies in rats and dogs at high doses (rats 1500 and 500 mg/kg/day in males and females respectively and dogs 300 mg/kg/day), the main target organs of toxicity were heart (**rats**: mononuclear cellular infiltration in 8/15 vs 5/15, **dogs**: mononuclear cellular infiltration of minimal severity in 1/4 dogs vs 0/4 controls), and lymph nodes (**rats**: accumulation in plasma cell in 8/15 vs 4/15 controls, **dogs**: lymphoid mesenteric hyperplasia, mesenteric & mandibular hemorrhage/erythrophagocytosis in 8/8 vs 4/8 controls). These histopath changes were not examined at low-mid doses in these studies, therefore it is unknown if there was a dose related trend in any of these findings. The heart toxicity was also seen in one month bridging studies, which were conducted with ezetimibe containing ——— impurities (as recommended by ICHQ3A guidelines) as

these were present in the drug substance in amounts ——— In 1-month bridging studies, heart and lymph nodes were again a target organ in **rats** (heart: mononuclear cellular infiltration in 2/10 rats vs 0/10 controls, lymph nodes: hyperplasia in 1/10 vs 0/10 in controls) and **dogs** (heart: 1/4 males had an enlarged heart with thickening of the right ventricular wall, nodularity of the left arterio-ventricular valve, and altered shape of the aortic valve, ¼ females had mononuclear cellular infiltration vs 0/4 controls, lymph nodes: brown pigment accumulation in 4/8 vs 0/8 in controls, and lymphoid hyperplasia in 2/8 vs 0/8 controls). In addition toxicity was noted in the kidney and spleen in rats and dogs. **The tissue distribution** studies of ezetimibe showed the presence of radioactivity in the myocardium after multiple dosing (up to 21 days) in male rats, but not after a single dose in either sex. Ezetimibe is a weak ACAT inhibitor, as indicated earlier that some ACAT inhibitors have previously produced cardiotoxicity in monkeys (multiple areas of fibrosis and mononuclear cellular infiltration, and PVC's), dogs (heart murmurs) and rats (degeneration/infiltration of mononuclear cells/myocarditis). The relevance of these findings in humans is unknown. **The above toxicities in heart and lymph nodes were observed at exposure levels of about 20X and 8-10X the human exposures** in rats and dogs respectively, based on human dose of 10 mg/day.

Ezetimibe produced malformations/variations in segment II study in rat fetuses (small size heart, short filamentous tail, vestigial right kidney/dilated renal pelvis, anasarca and increased skeletal variations at 500 mg/kg/day), and rabbit fetuses (agenesis of tail/ shortened tail, excencephaly, omaphalocele and increased skeletal variations at 250 mg/kg/day-1000 mg/kg/day), and in segment III study in rat fetuses (missing tail in F1 & F2 pups at 300 mg/kg/day). In rabbits maternal toxicity was observed at 1000 mg/kg/day (increased resorptions & altered sex distribution). The maternal Safety factor in repro toxicity studies in rats (segment I, II) was ≈6-8 fold the human exposure. The maternal Safety factor in rabbits in segment II study was much higher (140 fold the human exposure). The safety factor in embryo fetal (developmental) studies in rats and rabbits was 4 and 100 fold respectively the human exposure. Mortalities are of some concern in the repro toxicity studies (as shown in the Table, page 373-374), however the incidences were low and it is unknown at this time if these are drug related, or as sponsor states incidental/accidental. Higher distribution of radioactivity was noted in the GI tract and contents in pregnant vs non-pregnant rats. Also in tissue distribution studies, low levels of radioactivity were observed in the heart in the pregnant rats, not seen in non-pregnant after a single dose. Although ezetimibe radioactivity was below the limit of quantification in fetal tissues and blood in distribution studies, the fetus exposure to ezetimibe was confirmed in segment II/III studies in rats and rabbits. In rats, on gestation day 20, the exposures to total ezetimibe (18.7 µg.h/ml) was 0.5 fold higher in the fetus than in the mother (12.2 µg.h/ml). On the other hand 10X higher levels of total drug were found in rabbits on gestation day 22 (181 µg.h/ml), but the exposure to total ezetimibe in fetuses (4.7 µg.h/ml) was almost 40 times lower than in the mother. Ezetimibe was also transferred through the milk and values of total drug on lactation day 12 in fetus were half (11.3 µg.h/ml) of what is found in the mother (23.1 µg.h/ml). These data suggest that ezetimibe is present in the fetal blood during gestation, can be transferred to the newborn by mothers milk, and may pose a risk to pregnant women and nursing mothers/newborns.

Ezetimibe tested negative in Ames test, chromosome aberration assay in human lymphocytes and mouse micronucleus test, suggesting it does not have mutagenic potential.

In the 2-year oncogenic studies, no significant neoplastic or non-neoplastic tumor findings were observed at doses up to 500 mg/kg/day in mice, and up to 1500 and 500 mg/kg/day in male and female rats respectively. The exposure levels at above doses were about 160-220X and 14X the human exposure in mice (males and female respectively) and rats respectively, based on the human dose of 10 mg/day.

1B. Pharmacologic activity: Zeita (Ezetimibe, SCH 58235) is a lipid lowering drug, which blocks the intestinal absorption of cholesterol. However as indicated earlier its mechansim of action is unknown. Sponsor states that SCH 58235 and like compounds supposedly interfere with cholesterol uptake in to the intestinal wall by an as yet undiscovered mechanism. Ezetimibe is almost completely metabolized (via first pass) to a phenolic glucuronide (SCH 60663), which also takes place in the intestine. In rats, glucuronide was found to be more potent than the parent drug in inhibiting the absorption of cholesterol (90% vs 70% with the parent compound). After ezetimibe administration, the drug moves quickly from the intestinal lumen through the intestine wall, where it is glucuronidated, goes into the portal plasma and through the liver into the bile. However, after glucuronide administration, the large majority of compound is associated with the intestinal lumen/wall much more avidly than the drug itself, which may explain why glucuronide is more potent than the drug in inhibiting cholesterol. Glucuronidation is generally considered one of the major detoxification process, which inactivates the drug. It makes the drug more polar, more water soluble, improves its ability to be excreted into bile and/or urine, thereby eliminating it from the body. However this is not true for ezetimibe. The Pk in this case reflect the total drug (i.e ezetimibe + glucuronide). However this may not be unique to ezetimibe, enhanced potency of morphine has been recently reported by formation of 6-glucuronide morphine.

However, as indicated earlier ezetimibe is a weak ACAT inhibitor, and may well be working by that mechanism.

[ ]

As far its pharmacological activity is concerned it inhibits cholesterol in cholesterol fed animals with ED_{50} values 0.5-700 µg/kg. It is more potent in monkeys (0.5 µg/kg) than in other species (dogs had ED_{50} values of 7 µg/kg, rats 30 µg/kg, hamsters 40 µg/kg, and mice 700 µg/kg). These values are below NOAEL in rat and dog toxicity

studies. However, in chronic rat and dog toxicity studies, ezetimibe does not have impressive effects on lowering cholesterol. Note that in pharmacology studies, ezetimibe did not lower cholesterol in animals when placed on a normal chow diet, it only worked when animals were placed on a high cholesterol diet. In humans 10 mg/day (or 167 µg/kg/day) of ezetimibe lowers LDL-cholesterol by 18% and total cholesterol by 12-13%.

1C. Non clinical safety issues relevant to clinical use:
There are no clinical issues with monotherapy. The target organs of toxicity in pre-clinical studies were heart & lymph nodes (in rats/dogs), and kidney and bone marrow (in rats). These target organ toxicities were observed at ≥ 10X human doses, suggesting sufficient safety of margin in humans.

2A. Brief Review of Nonclinical studies For Combination Therapy (Ezetimibe + Statins):
Preclinical studies include single dose toxicology studies in rats and mice, 3-month studies in rats and dogs, genotoxicity studies, and segment II reproductive toxicity studies in rats and rabbits.

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

[ ]

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

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

Ezetimibe + statins (pravastatin, lovastatin, simvastatin and atorvastatin) tested negative in Ames test, chromosome aberration assay in human lymphocytes and mouse micronucleus test, suggesting that the combination does not have mutagenic/clstogenic potential. Ezetimibe also did not show mutagenic/clastogenic potential.

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

effect level' (NOEL) could not be defined in any combination toxicity study. Therefore safety factors in humans are not available. However these toxicities could be monitored in humans, and currently no significant adverse events are seen in the liver, skeletal muscle, or stomach in humans in the clinical studies.

2C. Non clinical safety issues relevant to clinical use with the combination therapy:

The target organs of toxicity in rats were liver, stomach and skeletal muscles (and with certain statins, the spleen, heart and prostate, see individual study). In male rats the lowest combination dose was generally a tolerated dose, but in female rats no NOAEL dose could be generally established as all doses produced toxicity. **In dogs**, the target organ of toxcity was mostly the liver, (and with certain statin combination, the testes, heart, lungs, see individual study), and generally no NOAEL dose could be established (see individual study). Thus the drug ezetimibe in combination with statins has low or non-existent safety margins in rats and dogs to humans. The clinical studies of ezetimibe + statin combination in the current NDA do not seem to show major adverse events related to above toxcities in animals. Therefore from the pharmacology point of view, the proposed application may be approved at the discretion of the medical reviewer.

III. Administrative

A. Reviewer signature: ----------

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B. Supervisor signature Concurrence:----------

Non-concurrence: ----------
(see memo attached)

cc: IND Arch
HFD-510
HFD-510/davisbruno/antonipillai/temeck/stadel/koch
Review code: AP for monotherapy
File name: nda21445-0 (zeita)

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PHARMACOLOGY / TOXICOLOGY REVIEW

Introduction: Zeita (Ezetimibe, SCH 58235) is a lipid lowering drug, which blocks the intestinal absorption of cholesterol.

[ ]

The sponsor states that the current drug (SCH 58235) has increased in vivo potency, better safety profile (lack of hepatic weight gain and P-450 enzyme induction in preclinical studies), reduced systemic exposure, simplified metabolism and little if any PK interaction with lovastatin,

[ ]

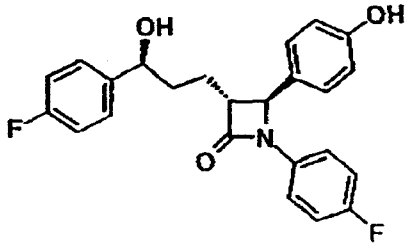
The mechansim of action of SCH 58235 ——————— is unknown. However it may have structure similarities to another ACAT inhibitor (Roth DB et al, J Med. Chem, 35: 1609-1617, 1992) and this may be how it is working. Scientists did an extensive study on the importance of structure in ACAT inhibitory activity in vitro and in vivo (J. Med. Chem, 35, 1609-1617, 1992). They noted that aryl ureas were known to be potent inhibitors, that the methoxy substituted aryl ring connected to the N of an amide bond was a good structure, and that the chain lengths tested (n=6-15) did not affect activity.

[ ]

Sponsor states that the current drug is a weak ACAT inhibitor, and implies that this is not the main mechanism, however they do not offer other suggestions.

The recommended human dose is 10 mg/day. The drug is mainly metabolized to glucuronide in mice, rats, dogs, humans.

The structure of SCH 58235



SCH 58235

I. PHARMACOLOGY:

Primary pharmacodynamics:

In vivo studies

1. **Effects of ezetimibe on hypocholesterolemic activity** in hamsters, mice, dogs, and rhesus monkeys

Seven day studies in cholesterol-fed animals (hamsters 0.5%, rats/mice/dogs 1%, monkeys 0.25%, 4-6/group) were conducted with the drug, given by either gavage (hamsters, rats, mice) or in a diet (monkeys, dogs). The inhibition of hepatic cholesteryl ester accumulation was the primary end point in these animal models, and is utilized as a marker of cholesterol absorption (or as a marker of hypocholesterolemic activity).

Table 1. The ED_{50} values for hypocholesterolemic activity of ezetimbe in various animal models

Species	ED_{50} values (µg/kg/day)
Cholesterol-fed male hamsters	40
Cholesterol-fed female rats	30
Cholesterol-fed male mice	700
Cholesterol-fed dogs	7
Cholesterol-fed rhesus monkeys	0.5

Thus, the drug was found to be most potent in lowering cholesterol in cholesterol-fed monkeys (ED_{50} was 0.0005 mg/kg/day or 0.5 µg/kg) in the above study. In cholesterol-fed rabbits, doses as slow as 0.03 mg/kg/day reduced plasma cholesterol levels. The potency of the drug in inhibiting the cholesterol absorption in the rat is (ID_{50} =1.5 mg/kg) higher than in monkey (ID_{50} =0.5 µg/kg).

Note that in chow-fed or in non-cholesterol fed animals, the drug had no effect on lowering plasma cholesterol levels

A. **Studies in monkeys**: cholesterol fed (0.25%) monkeys treated for 3 weeks daily with the drug SCH 58235 (0.03-1000 µg/kg/day) had significant reductions in cholesterol levels compared to the control animals, in whom the cholesterol actually went up (116-146 mg/dl).

Figure 1. Effects of SCH 58235 on plasma cholesterol levels in cholesterol fed monkeys:

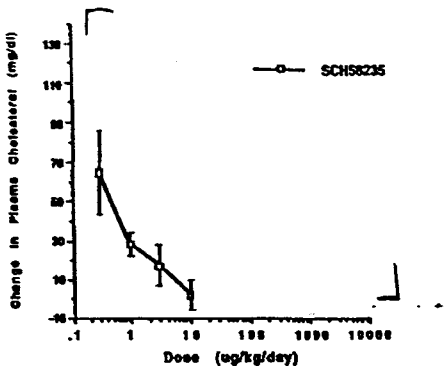
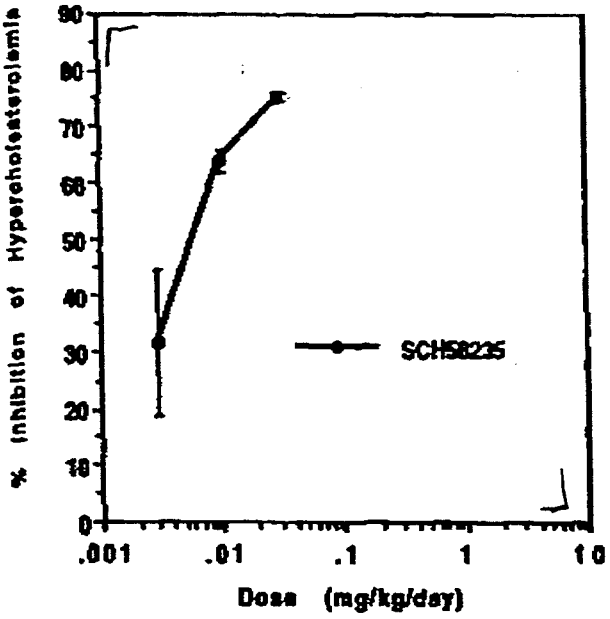


Figure 7 Effect of SCH 58235 and ——— on Plasma Cholesterol Levels in Cholesterol-Fed Rhesus Monkeys.

B. **Studies in dogs**: Similarly both SCH 58235 and ——————— reduced plasma cholesterol in cholesterol- fed (1%) dogs after one week, again SCH 58235 was more potent. This is in contrast to the control dogs where the cholesterol increased from 119 mg/dl to 165 mg/dl, Figure 2.

Figure 2. Effects of SCH 58235 after one week on plasma cholesterol levels in cholesterol fed dogs:



C. **Studies in rabbits**: In rabbits supplemented with 1% cholesterol along with 6% peanut oil for one week, the drug (0.6 mg/kg/day) decreased cholesterol after 1-week. SCH 58235 not only reduced LDL but also VLDL, IDL levels, and HDL levels in rabbits, Table 2. The microscopic evaluation of the aortic samples revealed that while control animals had fatty streak lesions consisting of a few layers of foam cells, the fatty streak lesion development was completely inhibited by the drug. This is shown by aortic esterified cholesterol accumulation, which was decreased by 68% while free cholesterol accumulation was decreased by 55%, Table 3.

Table 2. Effects of SCH 58235 on lipoprotein cholesterol levels in cholesterol fed rabbits:

	Total Cholesterol (mg/dl)				
	Chylomicra	VLDL & IDL	LDL	HDL	Percent Recovery
Control	27	389	178	33	65
	±2	±37	±16	±4	±1
SCH 58235	2[a]	78[a]	114[a]	20[a]	68
0.61mg/kg/day	±1	±20	±22	±3	±2

Values are means±SEM for the terminal timepoint (n=7-8/group). Lipoproteins were separated by [redacted] and lipoprotein content was expressed as mg/dl of plasma ([a]p<0.05 compared to control group). See Notebook #32935, pp. 135, 136.

Table 3. Effects of SCH 58235 on rabit aortic atherosclerosis:

	Tissue Aortic	Cholesteryl Ester (mg/g)	Free Cholesterol (mg/g)
0.5% Cholesterol	Arch	4.92±.96	2.03±0.23
Control	Thoracic	1.25±.39	0.94±0.23
0.5% Cholesterol +	Arch	1.55±.46[a]	0.92±.09[a]
.0012% SCH 58235	Thoracic	0.95±.28	0.57±.07

Values are means±SEM with 8 samples/group ([a]p<0.05 compared to control group). See Notebook #34146, pp. 15-29.

2. **Effects of ezetimibe in combination wth HMG-CoA reductase inhibitors on hypocholesterolemic activity in dogs:**

[redacted]

Therefore, a series of 14-day studies were conducted in dogs with the drug (SCH 58235) + HMG-CoA reductase inhibitors (statins) to show the synergy in plasma cholesterol levels, and PK effects on statins.

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Ezetimibe (at ED_{50} dose of 0.007 mg/kg/day) was given to dogs fed a chow diet alone (containing maltodextrin), or in combination with lovastatin (5 mg/kg/day), fluvastatin (5 mg/kg/day), or pravastatin (2.5 mg/kg/day). Ezetimibe alone, or lovastatin alone had a minimal effect on plasma cholesterol levels in chow fed dogs, but combined caused a ≥ 50% reduction in plasma cholesterol levels, Figure 3 & 4. Similarly pravastatin + ezetimibe caused a 41% reduction in plasma cholesterol levels. Fluvastatin alone reduced cholesterol by 38%, however combined with ezetimibe produced 60% reduction in plasma cholesterol levels, compared to controls. Similarly simvastatin or atorvastatin (1 mg/kg/day) plus ezetimibe produced a significant synergistic 30% reduction in plasma cholesterol levels. The sponsor claims that SCH 58235 did not significantly affect the plasma levels of statins in dogs and the synergistic effect in lowering plasma cholesterol is due to different mechanisms of action of the two drugs. Ezetimibe effects are due to inhibition of biliary cholesterol absorption, while HMG-CoA reductase inhibitors effects are due to inhibition of a compensatory increase in hepatic cholesterol biosynthesis.

Figure 3. Effects of SCH 58235 and lovastatin after 14-days of treatment on plasma cholesterol levels in dogs:

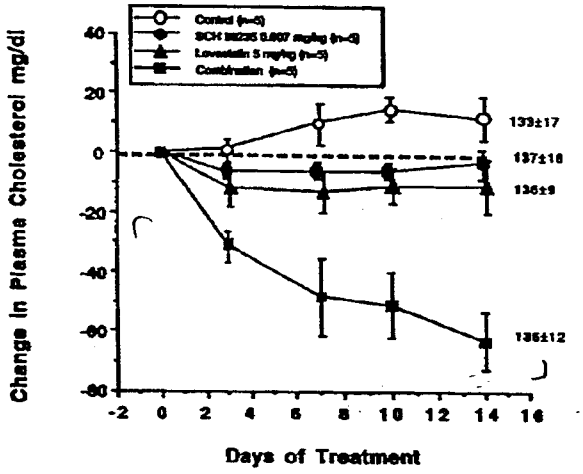
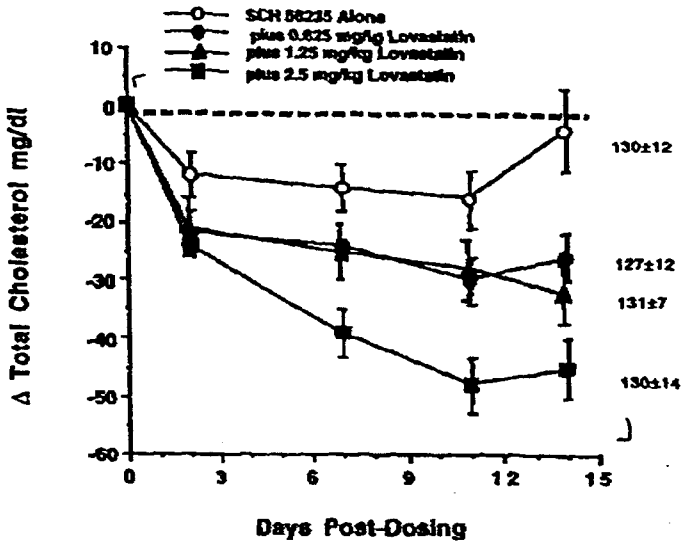


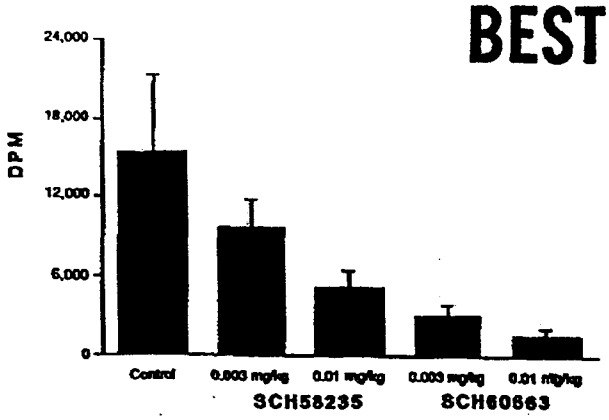
Figure 4. Effects of SCH 58235 and with various doses of lovastatin after 14-days of treatment on plasma cholesterol levels in dogs:



3. **Contribution of metabolite of SCH 58235 to the pharmacological activity of the drug**

The drug is almost completely metabolized (via first pass) to a phenolic glucuronide (SCH 60663, see metabolism section), which mostly occurs in the intestine. In an acute rat study (which is a published study in British J of Pharmacology 129: 1748-1754, 2000), when the drug or its glucuronide (3-10 µg/kg) were given intraduodenally (ID) to rats (n=5, with surgically diverted bile ducts), glucuronide was more potent than the parent compound in inhibiting the absorption of cholesterol. The drug activity was monitored in this study by determining the inhibition of ^{14}C-cholesterol appearance in plasma, the percent inhibition of cholesterol appearance was not provided, but from the figure it appears that the drug (SCH 58235) inhibited the ^{14}C-cholesterol appearance in plasma approximately by 70%, while the glucuronide (SCH 60663) inhibited it by 90% at the same dose of 0.01 mg/kg/day or 10 µg/kg. Since the doses were given in mass equivalents instead of molar equivalents, the study suggests that if equimolar doses would have been given the potency would be greater for the glucuronide conjugate (MW of parent is 409 vs of glucuronide is 585.6 g/mol).

Figure 4a. Effects of the drug SCH 58235 or its glucuronide metabolite (SCH 60663) on ^{14}C
-cholesterol absorption inhibition in the bile duct-diverted rat.



Sponsor states that SCH 58235 and like compounds interfere with cholesterol uptake in to the intestinal wall by an as yet undiscovered mechanism. Generally, glucuronidation makes the drug more polar, more water soluble, improves its ability to be excreted into bile and/or urine, thereby eliminating it from the body. Thus glucuronidation is considered one of the major detoxification process, which inactivates the drug, but it is not true for this class of compounds.

Sponsor states that due to unknown molecular mechanisms and lack of an in vitro assay, it is not known whether the drug itself or the glucuronide form, or both are active moieties. However glucuronidation of the drug improves its activity in two ways 1) the drug is repeatedly delivered back to the site of action via enterohepatic circulation, 2) glucuronidation appears to increase the residence time in the gut. Once the drug is glucuronidated, >95% of the drug is in either in the intestinal lumen or wall, and the glucuronide is excreted at a slower rate into the bile than the drug. Therefore it is unknown whether the glucuronide of the drug has inherent activity per se, or whether the glucuronide simply indirectly enhances the activity by delivering the parent drug back to site of action after enzymatic hydrolysis of the glucuronide moiety. After SCH 58235 administration, the drug moves quickly from the intestinal lumen through the intestine wall, where it is glucuronidated, goes into the portal plasma and through the liver into the bile. However, after glucuronide administration, the large majority of compound is associated with the intestinal lumen or wall. The glucuronide remains in the intestinal lumen associated with intestinal wall much more avidly than the drug itself, which may explain why glucuronide is more potent than the drug in inhibiting cholesterol. **Thus 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 humans SCH 58235 (or ezetimibe) constitutes only 10-20% of the total drug in plasma, while its glucuronide constitute 80-90% of the total drug. Both the drug and glucuronide have long half lives (of approximately 22 hrs).

NDA amendment submitted by the sponsor on 7/9/02 states that the drug had an ED_{50} of 0.04 mg/kg/day (or 40 µg/kg/day) in the 7-day cholesterol fed hamster model as indicated in the pharmacology section. Sponsor states that in the same hamster model, glucuronide (SCH 60663) given once a day by oral gavage in corn oil inhibited the accumulation of hepatic cholesteryl esters with an ED_{50} of 0.17 mg/kg/day (or 170 µg/kg/day), the sponsor provides a reference for this study (Vaccaro WD et al, Bioorg Med Chem Letters 8: 313, 1998) with no details, but explains that it is possible that in an

orally gavaged study with the glucuronide (SCH 60663) in the hamster, the glucuronide was hydrolyzed by intestinal glucuronidases back to the parent compound. Therefore the activity for inhibiting cholesterol absorption in the hamster model could not be separated between ezetimibe and glucuronide.

The second study sponsor quotes is the acute study in the bile duct cannulated rats with ezetimibe and glucuronide (see Figure 4a), where absorption of the cholesterol was significantly inhibited by ezetimibe and glucuronide (British J of Pharmacology 129: 1748-1754, 2000). Sponsor states that this bile duct cannulated rat model inhibits the enterohepatic circulation of ezetimibe, but the phenolic glucuronide may be locally de-glucuronidated in the intestine to ezetimibe. Therefore sponsor has provided a following new study to see if deglucuronidation effects the cholesterol absorption inhibitory activity. It was shown that in rats when an iodinated SCH 60663 (^{125}I-SCH 61209, an iodinated ezetimibe phenolic glucuronide analog) was given alone intraduodenally or with a β-glucuronidase inhibitor (D-glucaro-1, 4-lactone, i.e. GL), two hours later 21% deglucuronidated ^{125}I-SCH 61209 was found in the intestinal mucosal tissue in the control rats. In the presence of GL, deglucuronidation of the ^{125}I-SCH 61209 analog was inhibited by 80%, which remained >90% glucuronidated and localized in the intestine with less than 0.2% of the compound absorbed. In conclusion, inhibition of the glucuronide with GL had no effect on the potency of glucuronide in inhibiting cholesterol absorption. **Thus glucuronide has an intrinsic activity as a potent cholesterol absorption inhibitor and does not require conversion to the parent drug to be active.**

4. **Mechanism of action**: Effects of SCH 58235 on hepatic cholesterol esterification (or on **ACAT activity**):

The sponsor has examined the effects of the drug on esterification of cholesterol in the liver by microsomal acyl CoA:cholesterol acyl transferase (ACAT), which plays an important step in cholesterol storage and lipoprotein biosythesis. Extensive study on the importance of structure in ACAT inhibitory activity in vitro and in vivo is described in a publication (J. Med. Chem, 35:1609-1617, 1992). By esterification, cells can process cholesterol for transport purposes or allow excess cholesterol to be stored within intracellular droplets.

A. **Ex-vivo method**:
They have measured the ACAT activity of the drug ex-vivo in hamster liver homogenates in the presence of ACAT inhibitors by Burrier et al method (biochem. Pharmacol. 47 (9):1545, 1994). This is based on the fact that ACAT inhibitors also reduce cholesterol absorption and cholesterol availability to the liver, and since concentrations of this substrate are not constant and need to be accounted in an ex-vivo measurements, the sponsor uses an exogenous addition of cholesterol to saturate the enzyme with respect to the substrate, followed by a conventional ACAT assay.
The hamsters were given 0.5% cholesterol chow for 7 days without, or with 10 mg/kg/day of SCH 58235 (or 250-fold the IC_{50} of 40 μg/kg for this compound in the cholesterol fed hamster). The positive control; ACAT inhibitor (CL277082, 100 mg/kg/day), and a a negative control (or 1 g/kg cholestyramine, a known cholesterol absorption inhibitor at high doses) were also included in the study. All 3 drugs inhibited plasma cholesterol and hepatic cholesteryl ester accumulation over 7 days Table 4, however SCH 58235 did not have an effect on ACAT activity, while CL277082 did,

Figure 5, but note that only doses of 10 mg/kg were used for SCH 58235, while ten times higher doses (100 mg/kg/day) of the positive control ACAT inhibitor CL277082 were used. Sponsor concludes that SCH 58235 does not act directly or indirectly to alter hepatic ACAT activity. This is because SCH 58235 does not accumulate in the liver, but all of the drug passes through the liver for biliary excretion. Glucuronide metabolite of SCH 58235 was not tested in this model. Also, note that these studies are done on cholesterol fed animals.

Table 4. Effects of the drug on plasma cholesterol and hepatic cholesteryl ester levels in hamster:

	Plasma cholesterol levels (mg/dl)	Hepatic cholesterol levels (mg/g)
Control	237 (±17)	21.11 (±0.97)
SCH 58235 (10 mg/kg)	103* (±8)	1.35* (±0.15)
CL277082 (100 mg/kg)	128* (±15)	1.33* (±0.19)
Cholestyramine (1 g/kg)	156* (±18)	5.47* (±1.02)

Values are means + SEM; * $p < 0.05$ compared to control group. See Notebook

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Figure 5. Effects of the drug on hamster hepatic ACAT activity

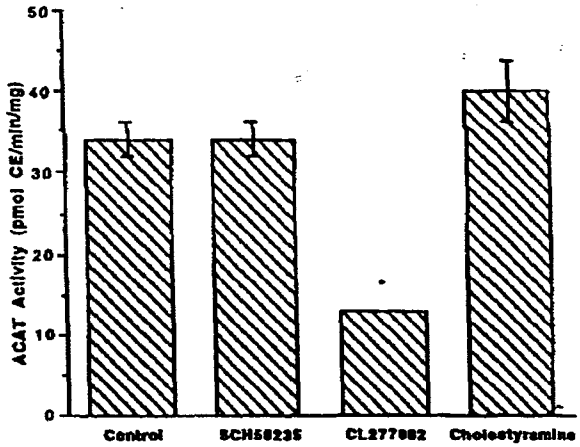


Figure 27 Effect of SCH 58235 on hamster hepatic ACAT activity

Animals were treated with a 0.5% cholesterol-containing diet for 7 days. The indicated animals also received CL277082 (100 mg/kg), SCH 58235 (10 mg/kg) and cholestyramine (1 g/kg). After 7 days, the livers were removed and homogenized for ACAT activity analysis. Incubations contained 1 mg of exogenous cholesterol added in 2 ml of acetone. Activity measurements were made in duplicate with 5 animals in each group. The asterisk indicates $p<0.05$ as compared to the control group. The standard error for the CL277082 group was too small to be visible on this graph.

B. **In vitro study**

1. **Effects of the drug on rat liver microsome ACAT activity**: The in vitro ACAT activity of the drug (and some reference compounds) was examined in the rat liver assay by modified method of Tabas et al (J. Clin. Inv. 79:418, 1987). The rat liver microsomes were prepared by the method of Burrier and Brecher (Biochem. Biophyphys. Acta 879: 229, 1986). In this assay SCH 58235 had an IC_{50} value 18 µM.).

   The other known inhibitors of ACAT such as SA58035, PD128042 and CL277082 had lower IC_{50} values (0.24, 6, and 5 µM respectively), while the glucuronide metabolite of the drug (SCH 60663) was not a very effective inhibitor of ACAT activity, Table 5. It appears that the drug SCH 58235 is 3-times less potent as ACAT inhibitor than the two other known ACAT inhibitors (18 vs 5-6 µM), suggesting that SCH 58235 is a less potent inhibitor of ACAT than the ACAT inhibitors used by sponsor (in an in vitro rat liver microsome assay).

   However the sponsor states that the drug is 3-75 fold less potent than known ACAT inhibitors.

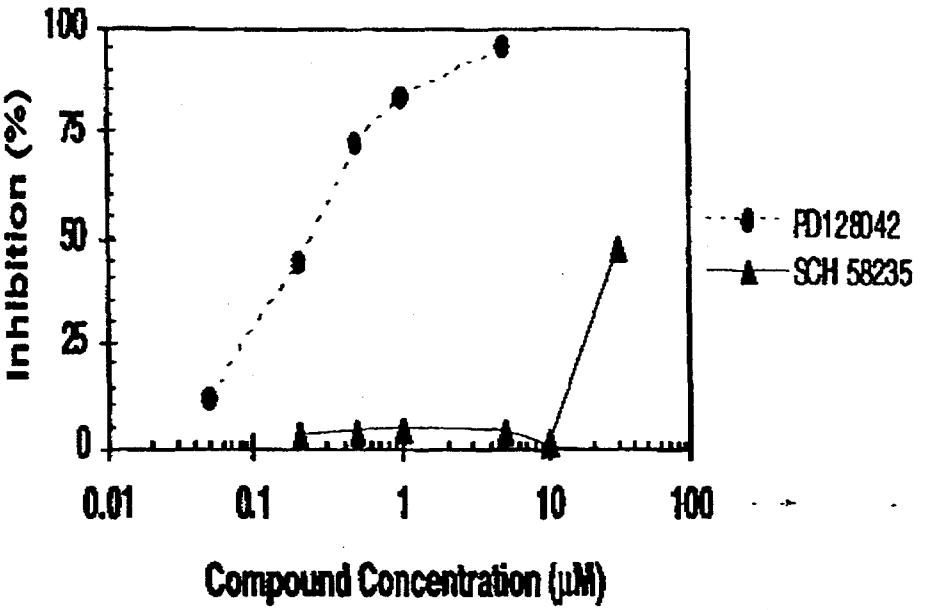
Table 5. Effects of the drug in rat liver microsome ACAT activity:

Compound	Number of Assays	Inhibition of ACAT Activity (IC_{50} or % Inhibition)
SCH 58235	2	18 ± 7 µM
SCH 60663	1	8% at 50 µM
SA58035	3	0.24 ± 0.13 µM
PD128042	2	6 ± 2 µM
CL277082	3	5.0 ± 2.6 µM

Assays were performed with duplicate incubations with doses spanning the IC_{50} range up to a maximal 50 µM concentration. The enzyme activity in the absence of a test compound was about 2000 dpm and background counts in the presence of denatured enzyme were less than 100 dpm. The number of independent assays performed is also shown. The values represent the mean ± std. dev.

2. **Effects of SCH 58235 on cholesterol esterification**: Esterification of cholesterol can be influenced by a number of factors including trafficking of cholesterol or the delivery of cholesterol or fatty acids to the endoplasmic reticulum, which can mimic the activity of ACAT inhibitors. Therefore the ability of ezetimibe to inhibit esterification of cholesterol was assayed using HepG2 cells. Sponsor explains that in this assay the known ACAT inhibitor PD128042 had an IC_{50} value of 0.25 µM, the ezetimibe was ineffective (with IC_{50} values of 0 µM). However from the figure 6 it looks like the drug has an $\approx IC_{50}$ value of 50 µM.

Figure 6. Effects of the drug on ACAT activity in HepG2 cells



3. Effects of SCH 58235 in CaCo-2 and IEC-6 cells: CaCo-2 cells are derived from a human colon adenocarcinoma, while ICE-6 cells are non-transformed enterocytes of rat origin. Both above cell types are surrogate models of absorptive intestinal enterocytes. In both cell types the PD128042 drug was more effective than SCH 58235 (in CaCo-2 cells IC_{50} values were <0.05 µM vs 3 µM with the drug, in ICE-6 cells IC_{50} values were 2.5 µM vs 40 µM with the drug), Figures 7 & 8.

Figure 7. Effects of the drug on ACAT activity in CaCo-2 cells

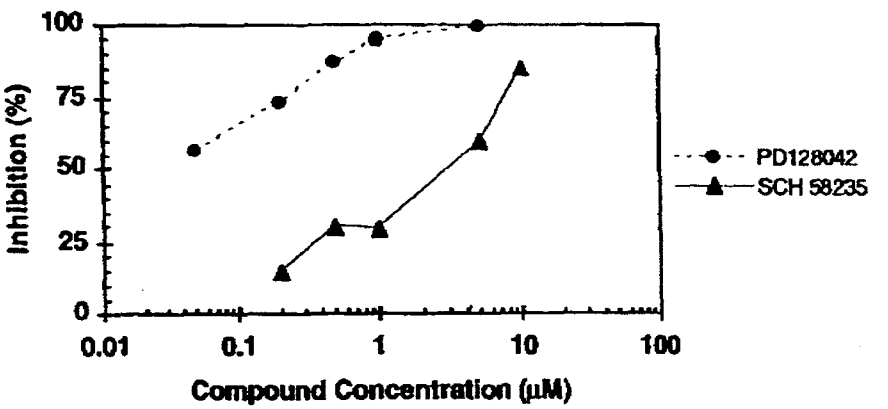


Figure 8. Effects of the drug on ACAT activity in ICE-6 cells

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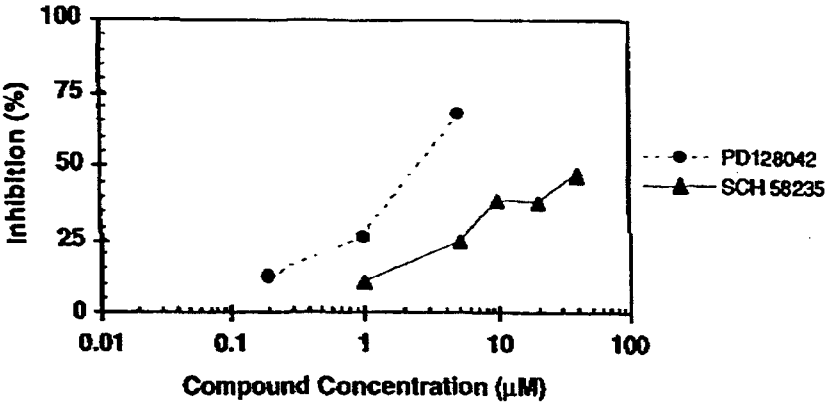


C. **Effects of SCH 58235 on 7-a-hydroxylase, HMG-CoA reductase activity**: In rat liver microsomes, the drug (at 5-15µM) does not significantly affect the activity of cholesterol 7-a-hydoxylase (1451-1541 vs 1338 dpm in controls). SCH 58235 did not inhibit rat liver microsomal HMG-CoA reductase ativity (50 µM of the drug inhibited 16% of HMG-CoA reductase, while 1 µM of lovastatin inhibited this activity by 96%). In HepG2 cells which are well accepted in vitro model of hepatocyte function, cholesterol biosynthesis was inhibited by lovastatin (had IC_{50} values of 0.05 µM), while SCH 58235 had almost no inhibitory activity

D. **Effects of SCH 58235 on pancreatic chlolesteryl esterase activity**: Since pancreatic cholesterol esterase can synthesize cholesteryl esters in a mechanism similar to but distinct from ACAT, the effect of the drug on this enzyme was examined in the purified pancreatic cholesteryl esterase preparation. SCH 58235 had a stimulatory effect on pancreatic chlolesteryl esterase activity (2616 vs 1752 dpm in controls), while the positive control (Way121898) showed an inhibitory effect (543 vs 1752 dpm in controls).

E. **Effects of SCH 58235 on Cholesteryl ester transfer protein (CETP):** This enzyme facilitates the transfer of plasma cholesteryl ester between high density and low density lipoproteins, SCH 58235 (5-50 µM) did not have as much effect on CETP in CETP assay [redacted] 939-1196 vs 1323 cpm in controls) compared to the positive control mAb TP2 (68 vs 1603 cpm in controls)

Sponsor states that SCH 58235 works by a novel mechanism, as the drug inhibits both the total cholesterol and esterified cholesterol after an intraduodenal administration of ^{14}C-cholesterol. In contrast, the drug did not interfere with cholesteryl ester hydrolysis in the intestine.

F. **Effects of SCH 58235 on Inhibition of ^{14}C-cholesterol absorption**: The appearrance of orally administered ^{14}C cholesterol was used to measure the cholesterol absorption in animals. Animals were gavaged with the drug (for eg. hamsters received 0.03 mg/kg/day of SCH 58235 or corn oil), one hr prior to 1 mci of ^{14}C-cholesterol with 0.1 mg of unlabeled cholesterol. Using this method, sponsor states that it selectively inhibits the absorption of ^{14}C-cholesterol in hamsters by 76%, rats by 84%, and in inbred mice by 67%, Table 6. Sponsor did not use a specific ACAT inhibitor to show that it does not inhibit the absorption of cholesterol in this model.

Table 6. Appearance of plasma ^{14}C-cholesterol in hamsters, rats and mice:

	Plasma [^{14}C]-Cholesterol (DPM/ml)		
	Hamsters	Rats	Mice
Control	1250 ± 150	1518 ± 265	4495 ± 908
SCH 58235	302 ± 36*	241 ± 61*	1488 ± 345*

SCH 58235 was administered to hamsters at a single dose of 0.03 mg/kg, rats at a dose of 0.1 mg/kg, and mice at a dose of 1 mg/kg prior to 1 mCi of [^{14}C]-cholesterol. After 2 hours, the distribution of radioactivity in plasma was assessed. The data are presented as the Mean ± SEM, n=4-5/group. The (*) next to data point indicates p<0.05 as compared to the control animals. See

To determine whether the uptake of other lipids was also inhibited by the drug, a hamster model was used, where sponsor states that the movement of orally dosed ^{3}H-triglyceride in the presence of single dose of the drug (SCH 58235 3 mg/kg, 2-hrs after administration) was assessed through the intestine into the plasma. This experiment showed no difference between the control and SCH 58235 treated absorption of the ^{3}H-triolein or ^{14}C- oleate. This suggests that the drug does not inhibit pancreatic lipase or cholesterol esterase. Note that the drug significantly decreased (by 92%) the absorption of ^{3}H- cholesterol derived from cholesteryl ester, Table 7. However, sponsor concludes that the drug has no effect on absorption of triglycerides, fatty acids, or bile acids in a hamster model

Table 7. The Effect of SCH 58235 on the Absorption of Triglycerides and Cholesteryl Esters in Hamsters.

	Plasma (DPM/ml)		
	[^{3}H]-triolein	[^{3}H]-cholesterol	[^{14}C]-oleate
Control	11806 ± 1541	6491 ± 742	6438 ± 232
SCH 58235	9027 ± 1109	537 ± 135*	6919 ± 1156

SCH 58235 was administered to hamsters at a single dose of 3 mg/kg for triglycerides and at a dose of 10 mg/kg for cholesteryl esters prior to 1 mCi of [^{3}H]-trolein or [^{3}H]-cholesteryl-oleate and cholesteryl-[^{14}C]-oleate. After 2 hours, the appearance of radioactivity in plasma was assessed. The data are presented as the Mean ± SEM, n=4-5/group. The (*) next to data point indicates p<0.001 as compared to the control animals. See Notebook #33242, pp.86-87,

Similarly, male SD rats (n=5/group) were gavaged with the drug (0.3 mg/kg SCH 58235 or corn oil), 1-hr prior to 1 mg of unlabeled carrier + 1 mCi of either ^{3}H- progesterone, ^{3}H-ethinyl estradiol, ^{3}H-sitosterol or ^{14}C-cholesterol in corn oil (0.25 ml). Cholesterol absorption was monitored in plasma by measuring the appearance of orally administered ^{14}C-cholesterol over 4 hrs. Sponsor states that the drug did not inhibit progesterone or ethinyl estradiol, see Table 8. However as seen in Table 8, the drug increases the absorption of ^{3}H-ethinyl estradiol in the rat intestine, the significance of this finding is unknown. It tends to decrease the absorption of labeled progesterone in the intestine by 26% (4794 vs 6458 dpm/10 cm in controls), while it clearly decreased cholesterol in the plasma, liver and intestine.

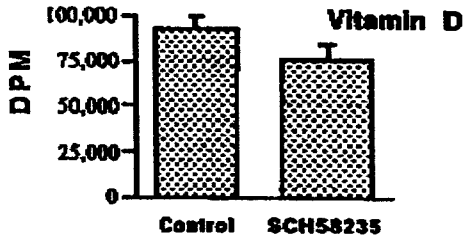
Table 8. Appearance of plasma ^{14}C-cholesterol and other sterols in rats :

	[^{14}C]-cholesterol	[^{3}H]-progesterone	[^{3}H]-ethinyl estradiol	[^{3}H]-sitosterol
Control Plasma (DPM/ml)	1177 ±256	885 ±81	936 ±86	710 ±33
SCH 58235 Plasma (DPM/ml)	384* ±62	1118 ±158	878 ±79	712 ±41
Control Liver (DPM/g)	7599 ±1301	4888 ±450	8416 ±1117	5202 ±561
SCH 58235 Liver (DPM/g)	2495* ±378	7087 ±2227	7546 ±706	3294* ±194
Control Intestine (DPM/10cm)	121890 ±18030	6458 ±2146	3794 ±756	11272 ±1183
SCH 58235 Intestine (DPM/10cm)	52035* ±4688	4794 ±541	8178* ±1650	10781 ±2071

SCH 58235 was administered to rats at a single dose of 0.3 mg/kg one hour prior to 1 mCi of [^{14}C]-cholesterol, [^{3}H]-progesterone, [^{3}H]-ethinyl estradiol, or [^{3}H]-sitosterol. After 4 hours, the distribution of radioactivity in plasma, liver and intestinal wall was assessed. The data are presented as the mean ± SEM, n=5/group. The (*) next to data point indicates p<0.05 as compared to the control

G. **The effect of the drug on bile acid excretion** was examined in anesthetized bile duct cannulated rats (n=5/group). The drug with and without bile was administered into the duodenum, and 1-hr later solution of ^{3}H-taurocholate sodium was delivered to duodenum. This study shows that the drug increases the secretion of radioactivity into the bile, but the difference was not significant. Similarly when anesthetized fasted rats received the buffered drug (10 mg/kg) followed by an hour later of ^{3}H-retinol (vitamin A), or ^{3}H-vitamin D, no significant effects on radioactivity were noted in levels of vitamin A or D. However sponsor states that the drug does not increase bile acid excretion and has no effect on fat soluble vitamins A & D, see Figure 9

Figure 9. Effect of the drug SCH 58235 on vitamin A, vitamin D and bile acid (taurocholic acid) in rats:



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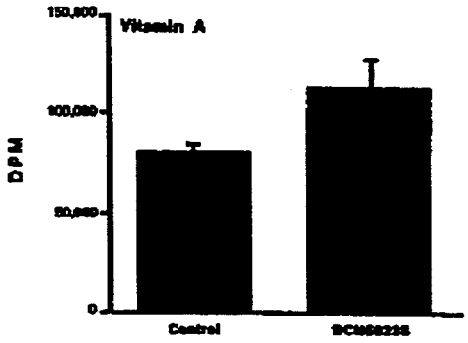

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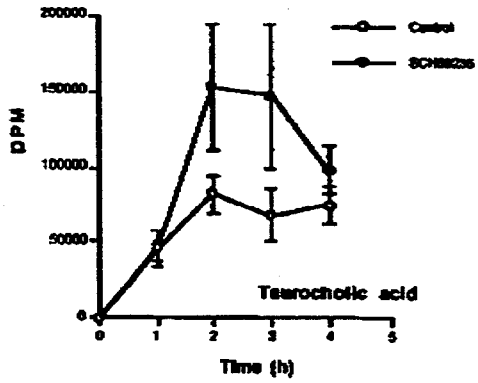
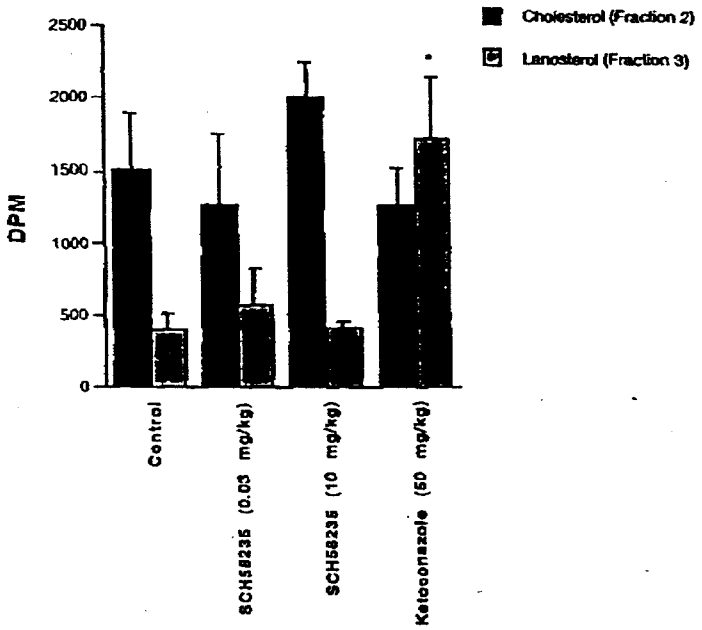


H. **Effects of the drug on synthesis of cholesterol and its precursors in the rat:**

Anesthetized fasted rats (intraduodenally cannulated, n=5/group) received the drug at low or high doses (0.03 and 10 mg/day) or ketoconazole (50 mg/kg/day, as a positive control). One-hr later 10 mCi of ^{3}H-mevalonate in sodium taurocholate was delivered to duodenum, rats were sacrificed 2.5 hrs later. This study showed that the drug either at low pharmacological doses, or at high doses did not have an effect on synthesis of cholesterol or its precursors from mevalonate, while the positive control ketoconazole produced accumulation of lanosterol in the liver and intestine, figure 10.

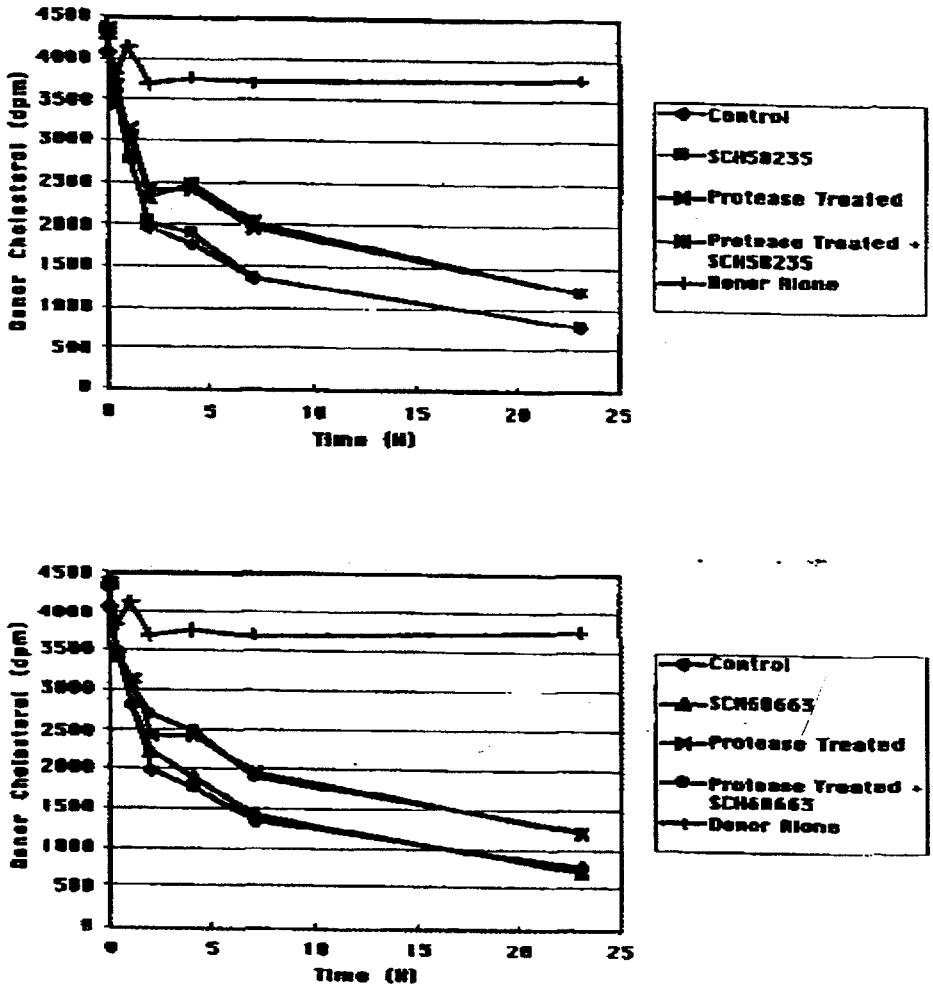
Figure 10. Effects of the drug on synthesis of cholesterol and its precursors from ^{3}H-mevalonate in the rat intestine:



I. Effects of the drug SCH 58235 and its glucuronide metabolite SCH 60663 on the movement of cholesterol from micelles into intestinal brush border membranes:

Brush border membranes (BBM) were prepared using small intestines of rabbits, according to the method by Hauser et al (Biochim Biophys Acta 602: 567, 1980). Test compounds (20 µM) were added to BBM in DMSO. Unilaminar phospholipid vesicles containing radiolabled ^{14}C-cholesterol were also prepared. BBM were then incubated with ^{14}C-cholesterol vesicles at a ratio of 10:1 (acceptor/donor). This method supposedly measures the rate at which cholesterol is transferred to an isolated BBM preparation. Protein mediated uptake is demonstrated by the impaired uptake of cholesterol by BBM which is protease treated. Sponsor claims that using this method, when BBM are treated with papain (protease treated), a 30% loss of membrane associated protein occurs, i.e the cholesterol transfer rate was reduced vs when treated with the drug or the glucuronide. Sponsor states that the drug localizes at the brush border of the small intestine and selectively inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and increase in clearance of cholesterol from the blood. No comparisons are provided as to how ACAT inhibitors fit here. See figure 11.

Figure 11. Effects of the drug SCH 58235 and its glucuronide metabolite SCH 60663 on the transfer of ^{14}C-cholesterol from unilamellar phospholipid vesicles to rabbit intestinal BBM



C. Contribution of glucuronide to the drug activity, and mechansim of action

1. As indicated earlier the drug is almost completely metabolized (via first pass) to a phenolic glucuronide (SCH 60663), which mostly occurs in the intestine. The molecular weight of the drug and its glucuronide are 409.4 and 585.6 Da respectively. Therefore plasma conc, cmax, and AUC values of glucuronide can be obtained by multiplying values by ezetimibe equivalents by 1.43. The glucuronide administration studies have shown that this metabolite has a greater action on inhibition of cholesterol than the drug itself.

Sponsor states that when the drug or its glucuronide was given intraduodenally (ID) to rats (with surgically diverted bile ducts), it was at least as potent as the parent compound in inhibiting the absorption of cholesterol. The sponsor gives two references here (both

references are in jacket 1.11). First reference is study P6311(which is a pharm/tox summary), and the second is a reference 7 which is a published study in British J of Pharmacology 129: 1748-1754, 2000. In this published study, bile duct and ID cannulated rats (n=5) were given the drug or glucuronide alone (at 0.01 and 0.003 mg/kg) intraduodenally. Five minutes later the unlabeled and ^{14}C-cholesterol (1 µCi/dose) was delivered directly into the intestine, at 1.5 hrs rats were sacrificed and plasma was analyzed for the radioactivity, which supposedly monitored the inhibition of ^{14}C-cholesterol appearance in plasma. In this study glucuronide (SCH 60663) was more potent that the parent compound in inhibiting ^{14}C-cholesterol appearance in plasma when delivered into the duodenum of bile duct cannulated rats. From the figure 4a, it looks like the drug (SCH 58235) inhibited the cholesterol appearance approximately by 70%, while the glucuronide inhibited it by 90% at the same dose of 0.01 mg/kg. Since the doses were given in mass equivalents instead of molar equivalents, the study suggests that if equimolar doses would have been given the potency would be greater for the glucuronide conjugate (MW of parent is 409 vs of glucuronide is 585.6 g/mol).

b. Mechansim of drug action

Sponsor states that SCH 58235 and like compounds supposedly interfere with cholesterol uptake in to the intestinal wall by an as yet undiscovered mechanism. After SCH 58235 administration, the drug moves quickly from the intestinal lumen through the intestine wall, where it is glucuronidated, goes into the portal plasma and through the liver into the bile. However, after glucuronide administration, the large majority of compound is associated with the intestinal lumen or wall. The studies have also shown that glucuronide remains in the intestinal lumen associated with intestinal wall much more avidly than the drug itself, which may explain why glucuronide is more potent than the drug in inhibiting cholesterol.

Glucuronidation is generally considered one of the major detoxification process, which inactivates the drug. It makes the drug more polar, more water soluble, improves its ability to be excreted into bile and/or urine, thereby eliminating it from the body. However this is not true for this class of compounds. Sponsor states that due to unknown molecular mechanisms and lack of an in vitro assay, it is not known whether the drug itself or the glucuronide form, or both are active moieties. However glucuronidation of the drug improves its activity in two ways 1) the drug is repeatedly delivered back to the site of action via enterohepatic circulation, 2) glucuronidation appears to increase the residence time in the gut. Once the drug is glucuronidated greater than 95% of the drug is in either in the intestinal lumen or wall, and the glucuronide is excreted at a slower rate into the bile than the drug. This suggests that the systemic exposure of the compound will be low. Similarly enhanced potency of morphine has been recently reported by formation of 6-glucuronide morphine. Therefore it is unknown whether the glucuronide of the drug has inherent activity per se or whether the glucuronide simply indirectly enhances the activity by delivering the parent drug back to site of action after enzymatic hydrolysis of the glucuronide moiety. In the hamster, the glucuronide is hydrolyzed by intestinal glucuronidases back to the parent compound.

In summary, the drug is not an inhibitor of known cholesterol metabolizing enzymes including pancreatic cholesterol esterase, cholesterol 7-alpha hydroxylase, cholesteryl ester transfer protein, and sterol carrier protein-2. It had no effect on the synthesis of cholesterol or precursors, it did not inhibit the conversion of HMG-CoA reductase inhibitors-CoA to mevalonate in rat liver microsomes, acetate to cholesterol in a cell culture of hepatocytes or mevalonate to cholesterol in rats. **Sponsor acknowledges**

that SCH 58235 is a weak inhibitor of ACAT activity. SCH 58235 was ≈ 3-times less potent ACAT inhibitor than two of the three standard known ACAT inhibitors in an in vitro assay for ACAT activity (in rat liver microsome ACAT assay CL2777082 and PD128042 had IC_{50} values of 5-6 μM, while SCH 58235 had an IC_{50} value of 18 μM). Sponsor states that (volume 1.9, page 32) in cholesterol fed hamsters, in rat liver microsomes, cell culture models of hepatocytes and in absorptive intestinal enterocytes, SCH 58235 at best was a weak inhibitor of ACAT (with IC_{50} of > 15 μM), however this activity cannot explain the inhibition of cholesterol absorption by the drug. SCH 58235 is almost completely metabolized to a phenolic glucuronide (SCH 60663) and in rats, glucuronide was found to be more potent (90%) than the parent compound (70%) in inhibiting the absorption of cholesterol. However, in vitro rat studies demonstrate that the glucuronide has less ACAT inhibiotry activity than the parent compound (Table 5). Although the glucuronide can be hydrolyzed by intestinal glucuronidases back to the parent compound. The sponsor explains that where examined glucuronide also had no effect on above enzymes, proteins or pathways, and that the drug inhibits cholesterol by a unique mechanism, but no suggestions are offered as to how it may work.

Drug activity related to proposed indication: In cholesterol fed rhesus monkeys, the ezetimibe had an ED_{50} of 0.0005 mg/kg/day (or 0.5 μg/kg/day) in producing hypo-cholesterolemia. The s**ponsor states that 24 μg/kg/day of ezetimibe totally blocks the absorption of cholesterol in cholesterol–fed monkeys.** In rats it inhibited cholesterol with ED_{50} of 30 μg/kg, in hamsters with ED_{50} of 40 μg/kg, in dogs with the ED_{50} of 7 μg/kg, in mice with the ED_{50} of 700 μg/kg. These ED_{50} values are based on the fact that animals were fed high cholesterol diet. However, there were no effects on plasma cholesterol in animals on normal chow diets. The human dose is 10 mg/day (or 0.167 mg/kg/day or 167 μg/kg/day), which blocks LDL- cholesterol only by 18%, and total cholesterol by 12-13%. Therefore by itself the drug is not a very potent inhibitor of total or LDL-cholesterol in humans. Note that in animals it is an effective cholesterol inhibitor, when they are placed on a high cholesterol diet. Also note that the drug is almost completely metabolized to a phenolic glucuronide (SCH 60663) which mostly occurs in the intestine. When the drug or its glucuronide (3-10 μg/kg) were given intraduodenally (ID) to bile duct diverted rats, glucuronide was more potent than the parent compound in inhibiting the absorption of cholesterol, but ED_{50} values of the glucuronide in comparison to drug have not been established.

PHARMACOLOGY CONCLUSIONS: Sponsor's 7-day studies in cholesterol-fed hamsters, rats, mice, dogs, and monkeys showed ED_{50} values in the μg/kg range for lowering cholesterol in plasma. However, there were no effects on plasma cholesterol in animals on normal chow diets. In toxicity studies also the drug did not have impressive effects on lowering cholesterol in animals. In the 6-month rat toxicity study the plasma cholesterol was actually increased at a high dose (500-1500 mg/kg/day) in both sexes, and no significant effects were observed on cholesterol at lower doses. In the 6-month dog toxicity study, plasma cholesterol was lower by 10-16% in males at all doses (30, 100, 300 mg/kg/day), and was lower by 17-28% in female dogs but it was not statistically significant at any dose in female dogs. 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 ^{14}C-cholesterol appearance in plasma approximately by 70%, while the glucuronide inhibited it at the same dose by 90%). Sponsor does not offer any

suggestions as to how ezetimibe works or its mechanism of action. SCH 58235 is a weak ACAT inhibitor, and is ≈ 3-times less potent (with an IC_{50} value of 18 μM) in vitro in the rat liver microsome ACAT assay vs two other standard known ACAT inhibitors (CL2777082 and PD128042 with IC_{50} values of 5-6 μM). In the same in vitro assay glucuronide (SCH 60663) had low ACAT activity (it produced 8% inhibition at 50 μM, see Table 5 in pharmacology section), but we do not know the ACAT activity of glucuronide in vivo or ex vivo. In ex vivo ACAT assay while 100 mg/kg of the positive control (CL277082) were used, only 10 mg/kg of ezetimibe were tested, and at that dose ezetimibe was negative in that ACAT assay (see pharmacology section). However sponsor states that ACAT activity of ezetimibe cannot explain the inhibition of cholesterol absorption by the drug, but no comparable studies of ezetimibe vs ACAT inhibitors (on inhibition of cholesterol absorption) have been conducted to see how these two differ. The molecular weight of SCH 58235 is 409, and at 18 μM (i.e 409 X 18 μM = 7362 μg/1000 ml) or at 7.4 μg/ml it could act as an ACAT inhibitor. It can be seen in Table 16 that these exposures are achieved in the rat and dog (1-6 month) toxicity studies at high doses (of 300-500 mg/kg/day) and may explain the cardiac toxicity in animals, that have been previously seen with ACAT inhibitors. This may suggest that in fact the drug ezetimibe is a weak ACAT inhibitor and works by that mechanism.

II SAFETY PHARMACOLOGY SUMMARY

Both acute and chronic effects of the drug have been examined in the secondary or safety pharmacology studies:

A. The acute effects.

a. Effects of the drug on GI morphology and motility: Ezetimibe (25 mg/kg/day) did not affect intestinal transit time in conscious in rats (47.4 vs 45.2% in controls, vs atropine 18.4%*, $p<0.05$) and did not produce gastric lesions, in contrast oral indomethacin (10 mg/kg/day) caused lesions in 7/8 rats.

b. Effects of the drug on urine volume and sodium conc.:The ezetimibe (25 mg/kg/day) did not affect urine volume (1.2 vs 1.4 ml/5hrs in controls) or sodium excretion (0.147 vs 0.157 meq/5 hrs in controls) in rats

c. Effects of the drug on blood pressure and heart rates: Ezetimibe (25 mg/kg/day, at a single oral dose, n=4/group) had no effect on heart rates or BP in conscious normotensive rats.

d. Effects of the drug on behavior, autonomic and neurologic function: Ezetimibe was evaluated for behavioral, neurological, and autonomic effects in male rats following single oral doses of 3, 10, 30 mg/kg/day, It had drug-related effects on *behavior* (passivity, body elevation), as well as *neurologic* (change in gait, limb position), and *autonomic effects* ('excretions') at 10 mg/kg and 30 mg/kg (all 6/6 rats at 30 mg/kg had diarrhea).

BEHAVIORAL, NEUROLOGICAL, AUTONOMIC EFFECTS IN MALE RATS

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

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

Thus although no clinical signs at doses of 20, 100, 500, and 1500 mg/kg/day were observed in the standard rat toxicity study, a single dose study in male rats showed modest to marked effects on passivity, limb position, and changes in gait.

B. The chronic effects of the drug have been been examined on cardiovascular & respiratory systems (ECG, heart rates, blood pressures, respiration rates, body temperatures) in the 6 and/or 12 month oral gavage toxicity studies in dogs at doses up to 300 mg/kg/day (or 7-9 fold the human doses, based on AUC exposures). In these studies no drug related effects on cardiovascular or respiratory systems were observed. Immunotoxic effects of the drug have not been examined in animals.

In summary, ezetimibe did not affect intestinal transit time in conscious in rats (47.4 vs 45.2% in controls, vs atropine 18.4%*, $p<0.05$) and did not produce gastric lesions, in contrast oral indomethacin (10 mg/kg/day produced) caused lesions in 7/8 rats. The drug did not affect urine volume (1.2 vs 1.4 ml/5hrs in controls) or sodium excretion (0.147 vs 0.157 meq/5 hrs in controls) in rats and had no effect on heart rates or BP in conscious rats. The drug was evaluated for behavioral, neurological, and autonomic effects in male rats following single oral doses of 3, 10, 30 mg/kg/day, It had drug-related effects on *behavior* (passivity, body elevation), *neurology* (change in gait, limb position), and *autonomic effects* ('excretions') at 10 mg/kg and 30 mg/kg (all six rats at 30 mg/kg had diarrhea). Thus although no clinical signs at doses of 20, 100, 500, and 1500 mg/kg/day were observed in the standard rat toxicity study, a single dose study in male rats showed modest to marked effects on passivity, body elevation, limb position, and changes in gait.

III. PHARMACOKINETICS/TOXICOKINETICS (PK/TK)

Following PK/TK studies (absorption, distribution, metabolism, excretion) have been provided with the drug.

Absorption:

1. **Two week TK studies** in mice, rats and dogs: In 2-week studies in mice and dogs (on regular diet) or in rats (in dietary restricted rats who were offered 25% less food/day), the exposure to SCH 58235 (or to its glucuronide metabolite) was higher in fed animals vs in

fasted animals. Also it was higher when animals received the drug in diet vs via gavage, see Table 9.

Table 9. Exposures of the drug and its metabolite in fasted and fed animals, as well as following gavage and dietary route of administration.

Table 7 Exposure Estimates [AUC(0-24 hr)] For Ezetimibe and Conjugated Ezetimibe in Fasted and Fed Laboratory Animals

Species	Dose (mg/kg)	Route of Administration	Sex	Ezetimibe AUC(0-24 hr) (ng-hr/mL)	Conjugated Ezetimibe AUC(0-24 hr) (ng-hr/mL)	Study No.
Mouse	2000	Gavage – Fasted	M	266	101511	97004[3]
		Diet	M	834	340879	
		Gavage – Fasted	F	129	112928	
		Diet	F	529	585421	
Rat	2000	Gavage – Fasted	M	76.7	4039	97003[9]
		Diet	M	106	10509	
		Gavage – Fasted	F	48.7	5594	
		Diet	F	111	15713	
Dog	1200	Gavage – Fasted	M	451	3208	97474[28]
		Gavage - Fed	M	805	102833	

Absorption in Rats:

2. **In a 3-month dietary toxicity** study in rats, day 90 AUC values of the unconjugated drug (SCH 58235) and the total drug (SCH 58235 + glucuronide metabolite) are presented. There was extensive glucuronidation of the drug in rats. The AUC values did not proportionally increase with the doses.

Table 10: Systemic exposures (AUC 0-24 hr) of unconjugated SCH 58235 and total drug (SCH 58235 + glucuronide) on day 90, in a 3-month dietary toxicity study in rats:

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

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

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

3. **In a 6-month dietary toxicity study in rats**, plasma AUC values of the total drug (SCH 58235 + glucuronide metabolite), unconjugated drug (SCH 58235) and the conjugated drug are presented in weeks 4 and 25 in Table 11. Note that in above 6-month dietary toxicity study in rats, higher doses of the drug were used in males (0, 150, 750, 1500 mg/kg/day) than in females (0, 50, 250, 500 mg/kg/day). The increases in

AUC values of the total drug were not dose proportional and values tended to be higher in week 25 (females 6.6, 9.4, 12.7 µg.h/ml, males 6.1, 13.7, 12.5 µg.h/ml respectively) vs in week 4 (females 3.5, 6.5, 10.4 µg.h/ml, males 4.5, 7.8, 8.8 µg.h/ml respectively), suggesting accumulation of the drug by 1.4-2 fold over time. Also the AUC values of the uncnjugated and conjugated drug were higher in week 25 vs in week 4. . There was extensive glucuronidation of the drug in rats. 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 10 mg/day (0.68 µg.h/ml). 10 mg/day is the recommended human dose.

Table 11: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in week 4 and 25 in a 6-month dietary toxicity study in rats:

Dose (mg/kg)	Gender	SCH 58235 AUC[0-24 hr] (ng-hr/mL)					
		Total		Unconjugated		Conjugated	
		Week 4	Week 25	Week 4	Week 25	Week 4	Week 25
50	F	3532	6567	76.1	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

Dogs

4. **In a 3-month oral —— toxicity study in dogs** (3, 30, 100 and 300 mg/kg/day), day 1 and day 90 AUC values of the unconjugated drug (SCH 58235) and the total drug (SCH 58235 + glucuronide metabolite) are presented in Table 12a. The Cmax plasma concentrations are shown in the next Table (12b). The AUC values did not proportionally increase with the doses. The total (drug+glucuronide) and unconjugated drug seem to accumulate by up to 1.5-3 fold on day 90 vs day 1. There was extensive glucuronidation of the drug. No gender differences were noted and there was lot of variability in values

Table 12a. AUC values in a 3-month toxicity study in dogs

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

Dose (mg/kg)	SCH 58235 (ng h/ml)	Day 1		Day 90	
		male	female	male	female
3	unconjugated	33	35	28	47
30		230	240	240	290
100		180	430	340	780
300		580	610	800	900
3	conjugated +	670	750	880	2,400
30	unconjugated	2,600	1,700	3,900	3,400

100 300		3,900 5,200	5,000 6,200	7,200 10,000	8,200 18,000

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

Table 12b. Cmax values in 3-month toxicity study in dogs

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

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

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

5. **In a 6-month oral gavage toxicity study in dogs**, the total (drug+glucuronide) drug accumulated by up to 2-3 fold over 6-months. There was extensive glucuronidation of the drug as noted in earlier studies in both rats and dogs. The AUC values of the total drug in week 25 were higher at low and high doses (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, Table 13. There was less than dose proportional increase in AUC exposures as was observed in previous rat/dog studies.

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

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)[b]
	Female	388	(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)	2678	(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	(36)	4165	(33)[b]
	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

6. **In a 1-year oral gavage toxicity study in dogs**, the total (drug+glucuronide) and unconjugated drug seem to accumulate by up to 2-fold. Again 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, Table 14.

Table 14: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 on day 1, in week 13, week 26 and week 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	6110	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	176	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	336	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	3990	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	3860	34	3420	22	5650	16

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

7. **In a 3-month oral dietary toxicity study in mice**, using doses of 100, 500, and 2000 mg/kg/day, the saturation of absorption was observed only for the parent compound (unconjugated drug) between 500 and 2000 mg/kg/day (AUC males 0.98 and 0.95, females 0.33 and 0.35 μg.h/ml, at 500 and 2000 mg/kg/day resp) but not for the total (parent + glucuronide metabolite) or conjugated drug in mice. The drug was extensively conjugated, Table 15

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Table 15. AUC (0-24 h, μg.h/ml) values of SCH 58235 (unconjugated, conjugated and total, on day 28) in a 3-month dietary TK study in mice.

AUC values: μg.h/ml

Dose (mg/kg/day)	100	500	2000
Unconjugated SCH 58235			
Males	0.272	0.981	0.952
Females	0.079	0.328	0.352
Conjugated SCH 58235			
Males	41.9	215	363
Females	52.5	346	645
Total SCH 58235 (conjugated + unconjugated)			
Males	42.2	216	364
Females	52.5	347	645

8. **Human exposures**:

a. In a single dose human study (M+F, Total n=24, ages 18-45, protocol #P00750), with 5, 10, 20 mg/day of drug, AUC (0-24 hrs) exposures of the total, glucuronide and free drug were as follows

	Total	glucuronide	Free (μg.h/ml)
5 mg/day	0.240	0.218	0.022
10 mg/day	0.440	0.400	0.041
20 mg/day	0.819	0.743	0.076

b. Multiple dose human studies: In a 7-day study with the drug using 10 mg/day (M+F, Total n=19, ages 18-45, protocol #P01382), AUC (0-24 hrs) exposures of the total, glucuronide and free drug were 0.621, 0.547, and 0.074 μg.h/ml respectively. Cmax values of these were 72, 69, and 5 ng/ml respectively. In another 7-day study using 10 mg/day, where drug was given on two separate occasions separated by at least 7 days (M+F, Total n=12, ages 18-45, protocol #P00252), AUC (0-24 hrs) exposures of the total, glucuronide and free drug were 0.637, 0.552, and 0.072 μg.h/ml respectively.

In multiple dose studies, the exposure of total drug (SCH 58235 + glucuronide conjugate) was higher in mice (42-645 μg.h/ml), than in dogs (1-18 μg.h/ml) or rats (1-13 μg.h/ml). Human exposure of the total drug in 2-week study at 10 and 20 mg/day (or at 0.17-0.33 mg/kg/day) were 0.68 and 1.314 μg.h/ml respectively. The AUC exposures of the free drug SCH 58235 (unconjugated) were lower in rats (2-9%), dogs (2-13%), mice (<0.1-0.6%), than in humans (10-20%), Table 16.

Table16. Comparative AUC exposures of the total and free drug (SCH 58235) in rats, dogs, mice & humans

Toxicity Study	AUC values of the total SCH 58235 (drug + glucuronide metabolite) µg.h/ml	AUC values of the free drug (SCH 58235) µg.h/ml (%-total)
Rat		
3-month dietary toxicity study in rats, values in week 13 Males (20, 100, 500, 1500 mg/kg/day) Females (20, 100, 500, 1500 mg/kg/day)	3.1, 4.7, 7.7, 11.0 1.3, 7.3, 12.0, 13.0	0.03, 0.05, 0.08, 0.23 (2-10%) 0, 0.05, 0.15, 0.12 (<1-9%)
6-month dietary toxicity study in rats, values in week 25 Males (250, 750, 1500 mg/kg/day) Females (50, 250, 500 mg/kg/day)	6.6, 9.4, 12.7 6.1, 13.7, 12.5	0.41, 0.28, 0.39 (3-6%) 0.23, 0.43, 0.26 (2-4%)
Dog		
3-month — · toxicity study in dogs, values in week 13 Males(3, 30, 100, 300 mg/kg/day) Females(3, 30, 100, 300 mg/kg/day)	0.9, 3.9, 7.2, 10.0 2.4, 3.4, 8.2, 18.00	0.03, 0.24, 0.34, 0.80 (3-8%) 0.05, 0.29, 0.78, 0.90 (2-5%)
6-month oral gavage toxicity study in dogs, values in week 25 Males (3, 30, 100, 300 mg/kg/day) Females (3, 30, 100, 300 mg/kg/day)	1.9, 3.1, 4.3, 2.2, 2.4, 5.5	0.13, 0.15, 0.41 (7-13%) 0.14, 0.23, 0.47 (7-10%)
12-month oral gavage toxicity study in dogs, values in week 51 Males (3, 30, 100, 300 mg/kg/day) Females (3, 30, 100, 300 mg/kg/day)	2.9, 4.0, 6.6 2.7, 6.1, 6.1	0.27, 0.14, 0.28 (4-9%) 0.22, 0.24, 0.36 (4-8%)
MICE		
3-month dietary toxicity study in mice, Males (100, 500, and 2000 mg/kg/day) Females (100, 500, and 2000 mg/kg/day)	42, 216, 364 53, 347, 645	0.27, 0.98, 0.95 (0.3-0.6%) 0.08, 0.33, 0.35 (<0.1%)
Human		
7-15 day study (with 10 & 20 mg/day)	0.64, 1.314	0.072 at 10 mg/day (11%) 0.167 at 20 mg/day (13%)

In all toxicity studies, the dog and mouse had the greatest exposure to the free drug (Figure 12a), while mouse and pregnant rabbits had the greatest exposure to the conjugated drug (Figure 12b). In rats, the males had higher exposures to the conjugate than females. In mice, the males had higher exposure to both the free and conjugate.

Figure 12a. Exposure to free drug in various species

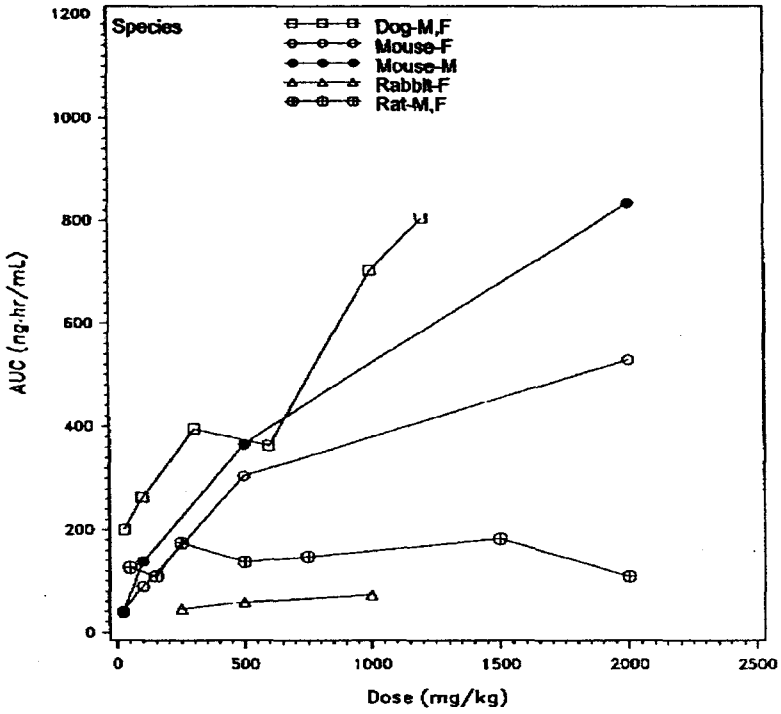

Figure 12b. Exposure to conjugated drug in various species

APPEARS THIS WAY
ON ORIGINAL

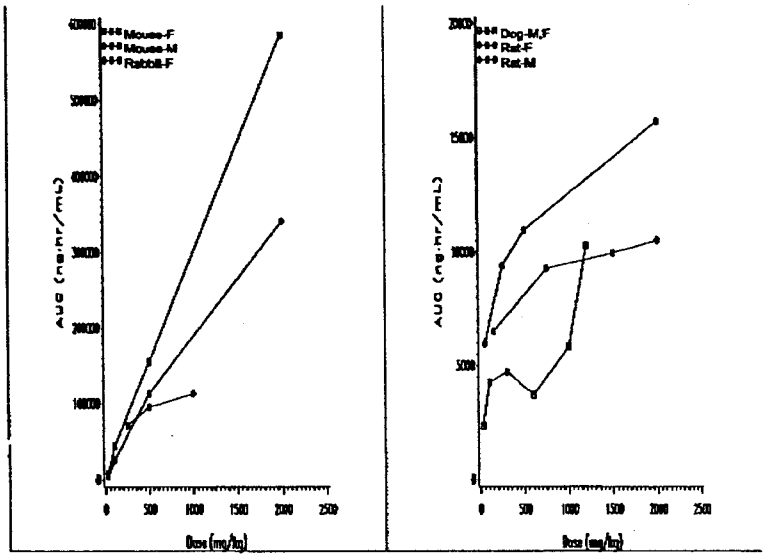

Figure 5 Exposure to Conjugated Ezetimibe Following Multiple-Dose Administration of Ezetimibe to Mice (Diet), Rats (Diet), Pregnant Rabbits (Gavage), and Dogs (Gavage-Fed)

PK/TK summary: Fed animals (mice, rats, dogs) had greater exposure to the drug and its conjugate glucuronide than the fasted animals. In order to increase the exposure of drug in animals, ezetimibe was administered by diet to rats/mice and by gavage in fed dogs in all 1-month to 24-month toxicity/cac studies. In multiple dose studies, the exposure of total drug (SCH 58235 + glucuronide cojugate) was higher in mice (~~~ µg.h/ml), than in dogs (——— µg.h/ml) or rats (—— µg.h/ml). Human exposure of the total drug in 2-week study at 10 and 20 mg/day was 0.68 and 1.314 µg.h/ml respectively. The AUC exposures of the free drug SCH 58235 (unconjugated) were lower in rats (2-9%), dogs (2-13%), mice (<0.1-0.6%), than in humans (10-20%). In the labeling section it is stated that in humans ezetimibe and ezetimibe glucuronide constitute 10-20% and 80-90% of the total drug in plasma respectively

9. Studies with the drug containing ———— impurities:

a. **Rat: One-month bridging dietary toxicity study** in rats were conducted with the drug containing impurities. The impurities were

[ ]

Higher doses were used in males (0, 250, 750, 1500 mg/kg/day) than in females (0, 50, 250, 500 mg/kg/day). The AUC exposures of the drug increased in less than proportional manner, see Table 17a. The values of the total drug in week 4 were not significantly different than in the 6-month toxicity study in rats, Table 17b, suggesting that impurities did not have an effect on drug pharmacokinetics in rats.

Table 17a: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in week 4 in a 1-month dieatry rat toxicity study of the drug + impurities:

Group	Gender	Dose (mg SCH 58235/kg)	Mean SCH 58235 AUC(0-24 hr) (ng·hr/mL)		
			Total	Conjugated	Unconjugated
Low-Dose	M	150	7928	7812	115
	F	50	5312	5237	78.6
Mid-Dose	M	750	11996	11799	207
	F	250	9671	9605	68.7
High-Dose	M	1500	8814	8734	80.5
	F	500	11610	11504	108

Table 17b. AUC (0-24 hrs, μg.h/ml) values of the total drug + impuritiies in the 1-month rat tox study vs in the 6-month rat tox study (at week 4 and week 25 without impurities)

	Males	Females
I-Month rat study		
week 4 (drug+impurities)	7.9, 12.0, 11.6,	5.3, 9.7, 11.6
6-Month rat study		
week 4 (drug with no impurities)	3.5, 6.5, 10.4	4.5, 7.8, 8.8
week 25 (drug with no impurities)	6.6, 9.4, 12.7	6.1, 13.7, 12.5

b. Dog: One-month bridging gavage toxicity study in dogs were conducted with the drug substance containing impurities, using doses of 30, 100, 300 mg/kg/day of ezetimibe. The impurities present in the drug substance were in the range of —— However, slightly higher concentrations of some impurities were used in the toxicity studies than those found in the drug substance (see toxicity section). The AUC values of ezetimibe did not proportionally increase with doses. The exposures were similar in both sexes, Table 18a. The mean (total drug+glucuronide) systemic exposures to the drug in a 6-month dog study (The AUC of parent + metabolite were 2.8, 5.0, 6.4 μg.h/ml respectively) were comparable to the exposures in this 4-week study in dogs (3.8, 5.9, 7.4 μg.h/ml respectively), Table 18b. These studies suggest that impurities did not effect the drug PK in rats/dogs.

Table 18a: Systemic exposures (AUC 0-24 hr) to total, unconjugated and conjugated SCH 58235 in a 1-month gavage dog toxicity study of the drug + impurities:

Dose (mg SCH 58235/kg)	Mean (%CV) SCH 58235 AUC(0-24 hr) (ng·hr/mL)		
	Total	Conjugated	Unconjugated
30[a]	3806 (86)	3555 (85)	347[b] (115)
100[c]	6926 (69)	5587 (72)	341 (40)
300[d]	7345 (38)	6751 (38)	594 (49)
a: N = 6; b: N = 3			
c: N = 7; d: N = 8			

Table 18b. AUC (0-24 hrs, µg.h/ml) values of the total drug + impurities in the 1-month dog tox study vs in the 6-month dog tox study (at week 13 and week 25 without impurities) at same doses.

	Males + Females (µg.h/ml)
I-Month dog study	
week 4 (drug+impurities)	3.8, 5.9, 7.4
6-Month dog study	
week 13 (drug)	2.3, 4.6, 6.1
week 25 (drug)	2.1, 2.8, 4.9

10. Repro tox studies (segment II and III) with SCH 58235 in rats

a. **The segment II studies** were conducted in rats (0, 250, 500, 1000 mg/kg/day from day 6-15 of gestation) and rabbits (0, 250, 500, 1000 mg/kg/day from day 7-19 of gestation). Rabbits had almost 25 fold higher exposure to the total drug on GD 19 (72-113 µg.h/ml) than rats during GD 15 (3-5 µg.h/ml), however embryo fetal NOAEL in both rat/rabbit fetuses was <250 mg/kg/day, (or 4-fold and <100 fold respectively the human exposures).

Table 18c. Tkoxicokinetic parameters in segment II study with SCH 58235 58235 in rats and rabbits at doses of 250, 500, 1000 mg/kg/day/day

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

b. Segment III TK studies were conducted in rats (by gavage on gestation day 6 through lactation day 12) and rabbits (by gavage on gestation day 7 through lactation day 22). Again 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 drug (19 µg.h/ml than rabbit fetuses (5 µg.h/ml). In both rats and rabbits exposure to the conjugated drug accounted for majority of the drug in dams and pups. In rats, the AUC of the total drug (parent + metabolite) at 1000 mg/kg/day on gestation day (GD) 10 and 20 were 5.8 & 12.2 µg.h/ml, and in fetus on GD 20 was 18.7 µg.h/ml. These values on lactation day (LD) 12 in mothers and pups were 23.1 and 11.3 µg.h/ml respectively. Higher exposures of the drug were 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). In rabbits, the AUC of the total drug (parent + metabolite) at 1000 mg/kg/day on gestation day (GD) 10 and 20 were 5.8 & 12.2 µg.h/ml, and in fetus on GD 20 was 18.7 µg.h/ml. These values on lactation day (LD) 12 in mothers and pups were 23.1 and 11.3 µg.h/ml respectively.

Table 18d. Toxicokinetic parameters in segment III studies with SCH 58235 in rats and rabbits at one dose of 1000 mg/kg/day

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