Renal and Muscle Toxicity Observed in Animal Studies

Species	Study design	Results	AUC (ng.hr/ml) M/F	Multiple of human exposure			
				80	40	20	10
Rat	160 mg/kg oral 13 weeks	Tubular cell degeneration in 6/7 male and 2/3 female premature killed rats					
	80, 160, 240, 320 mg/kg oral 13 weeks	Tubular cell degeneration in premature killed rats. Males Female 80 2/2 0/0 160 11/12 6/10 240 13/15 5/15 320 10/14 10/15	80: 17100/4390 160: 50500/52400 240: 134000/71800 320: 162000/144000	39/10X 116/120X 307/165X 372/330X	85X 250X 667X 806X	194X 574X 1522X 1840X	427X 1262X 3350X 4050X
Dog	10, 30, 90 mg/kg oral 1 month	1/3 female at 90 mg/kg moribund killed with ↑GOT, LDH, CPK, creatinine, urea nitrogen, total bilirubin, suggestive of renal and muscle toxicity.	10: 1520/1110 30: 3520/3890 90: 19900/23900	4X 8X 46X	8X 18X 99X	17X 40X 226X	38X 88X 498X
Monkey	10, 30 mg/kg oral 6 months	Slight increase of incidence of renal findings in all dose levels, including basophilic change of renal tubule, degeneration of tubular epithelium in cortex, hyalinous crystalline inclusion body in pelvic epithelium	10: 313-352/246-818 30: 1300-4180/733-1080	0.6-2X 2-10X	2-4X 6-21X	3-9X 8-48X	6-20X 18-105X
Rabbit	0.3, 1, 3 mg/kg oral 13 days Reproductive toxicology study	↑ creatinine, urea nitrogen, GOT, GPT, LDH, CPK and necrosis and mineralization (calcium deposition) of the cortical tubular epithelial cells in the kidney, necrosis and mineralization of cardiac and intercostal muscle in the 2/14 killed moribund animals at 3 mg/kg	Exposure data not provided.				

Multiple of human exposure are calculated based on the exposure level where toxicity was observed and the human AUC of 436, 201, 88 and 40 ng.hr/ml for 80, 40, 20, and 10 mg/day, respectively.

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Summary of General Toxicity

Species	Study design	Results	AUC (ng.hr/ml) M/F	Multiple of human exposure			
				80	40	20	10
Mice	20, 60, 200 mg/kg oral 2 weeks	↑ liver wt, hepatocytic hypertrophy at 200 mg/kg					
	20, 60, 200 mg/kg oral 13 weeks	↑ liver wt, hepatocytic hypertrophy at ≥60 mg/kg	20: 145/322 60: 722/969 200: 3080/5610	0.7X 2X 13X	2X 5X 28X	4X 11X 64X	8X 24X 140X
Rat	1000, 2000 mg/kg oral single dose	No apparent toxic effects.					
	15, 50, 150 mg/kg oral 1 month	Liver single cell necrosis, cytoplasmic eosinophilic change at ≥ 50 mg/kg. Reversible.					
	6, 20, 60 mg/kg oral 13 weeks	↑ liver wt, hepatocytic hypertrophy at ≥20 mg/kg, ↑ ALT, AST at ≥ 6 mg/kg.	6: 240 20: 1130 60: 6720	0.6X 3X 15X	1X 6X 33X	3X 13X 76X	6X 28X 168X
	10, 30, 100 mg/kg oral 3 months	Mortality at 100 mg/kg. Liver hypertrophy and forestomach hyperkeratosis at ≥ 30 mg/kg.					
	2, 6, 20 mg/kg oral 6 months	↑ liver wt, hepatocytic hypertrophy at ≥6 mg/kg	2: 149 6: 424 20: 2410	0.3X 1X 6X	0.7X 2X 12X	2X 5X 27X	4X 11X 60X
Dog	1000, 2000 mg/kg oral single dose	Vomiting, abnormal stool, increased ALT, AST and CPK at both doses.					
	10, 30, 90 mg/kg oral 1 month	↑ALT, AST, gallbladder (lamina propia mucosa edema, hemorrhage), eye (retina dysplasia and loss) at ≥30 mg/kg.	10: 1520/1110 30: 3520/3890 90: 19900/23900	4X 8X 46X	8X 18X 100X	17X 40X 226X	38X 88X 498X
	7.5, 15, 30 mg/kg oral 3 months	Ocular opacity at 30 mg/kg. Gallbladder (lamina propia mucosa hemorrhage and inflammatory cell infiltration) at all dose levels.	*7.5: 350/150 *15: 410/440 *30: 1420/1280	6X 8X 27X	15X 17X 59X	35X 40X 140X	85X 100X 350X
	1, 4 mg/kg oral 6 months	No apparent toxic effect.	1: 122 4: 661	0.3X 2X	0.6X 3X	1X 8X	3X 17X
	1, 3, 6 mg/kg oral 12 months	Opacity of cornea at 1 and 6 mg/kg, gallbladder hemorrhage in lamina propria mucosa, hepatocytes atrophy at 6 mg/kg.	1: 237 3: 703 6: 3120	0.5X 2X 7X	1X 3X 16X	3X 8X 35X	6X 17X 78X
Monkey	10, 30 mg/kg oral 6 months	↓ spermatogenic epithelium, giant cell in seminiferous tubulae, vacuolation in seminiferous tubular epithelium, adrenal necrosis of parenchyma at 30 mg/kg.	10: 313-352/246-818 30: 1300-4180/733-1080	0.6-2X 2-10X	1-4X 4-21X	3-9X 8-48X	6-20X 18-105X

AUC in humans are 436, 201, 88 and 40 ng.hr/ml for 80, 40, 20, and 10 mg/day, respectively.

*: C_{max} value, AUC data were not provided.

Based on AUC values, the safety margins at NOAEL are <1 to 2X based on the sponsor proposed MRHD of 80 mg/day. The safety margins will be <1 to 4X based on human dose of 40 mg/day; 2 to 9X based on human dose of 20 mg/day; and 4 to 20X based on human dose of 10 mg/day.

Safety margins based on the sponsor proposed MRHD of 80 mg/day (49 mg/m^2) with AUC of 436 ng.hr/ml.

Species/Toxicity	NOAEL mg/kg	HED (mg/m^2)	AUC (ng.hr/ml)	Safety Margins	
				Based on AUC	Based on mg/m^2
Rat: liver, stomach, kidney	2	12	149	1/3	1/4
Mouse: liver	20	60	M: 145 F: 322	M: 1/3 F: 2/3	1.2X
Dog: gallbladder, liver, eye, kidney	3	60	703	1.6X	1.2X
Monkey: testis, adrenal, kidney	10	120	M: 342 F: 818	M: 0.8X F: 2X	2.5X

Safety margins based on the sponsor proposed MRHD of 40 mg/day (25 mg/m^2) with AUC of 201 ng.hr/ml.

Species/Toxicity	NOAEL mg/kg	HED (mg/m^2)	AUC (ng.hr/ml)	Safety Margins	
				Based on AUC	Based on mg/m^2
Rat: liver, stomach, kidney	2	12	149	¾	1/2
Mouse: liver	20	60	M: 145 F: 322	M: 3/4 F: 1.5X	2X
Dog: gallbladder, liver, eye, kidney	3	60	703	3.5X	2X
Monkey: testis, adrenal, kidney	10	120	M: 342 F: 818	M: 1.5X F: 4X	5X

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Safety margins based on the sponsor proposed MRHD of 20 mg/day (12 mg/m^2) with AUC of 88 ng.hr/ml.

Species/Toxicity	NOAEL mg/kg	HED (mg/m^2)	AUC (ng.hr/ml)	Safety Margins	
				Based on AUC	Based on mg/m^2
Rat: liver, stomach, kidney	2	12	149	1.7X	1
Mouse: liver	20	60	M: 145 F: 322	M: 1.7X F: 3.6X	5X
Dog: gallbladder, liver, eye, kidney	3	60	703	8X	5X
Monkey: testis, adrenal, kidney	10	120	M: 342 F: 818	M: 3.9X F: 9X	10X

Safety margins based on the sponsor proposed MRHD of 10 mg/day (6 mg/m^2) with AUC of 40 ng.hr/ml.

Species/Toxicity	NOAEL mg/kg	HED (mg/m^2)	AUC (ng.hr/ml)	Safety Margins	
				Based on AUC	Based on mg/m^2
Rat: liver, stomach, kidney	2	12	149	3.7X	2X
Mouse: liver	20	60	M: 145 F: 322	M: 3.6X F: 8X	10X
Dog: gallbladder, liver, eye, kidney	3	60	703	17X	10X
Monkey: testis, adrenal, kidney	10	120	M: 342 F: 818	M: 8X F: 20X	20X

Reversibility studies have been conducted in rats and dogs. In rats, animals were allowed to recover for 1 to 2 months after 1 to 6 months of treatment with rosuvastatin. In dogs, animals were allowed to recover for one month after 1 to 12 months of treatment with rosuvastatin. Generally, the rosuvastatin induced liver toxicity appeared to be reversible. However, the toxicity on gallbladder, kidney and eye did not appeared to be fully reversible.

The rosuvastatin-induced toxicity may be directly due to its inhibitory effect on HMG-CoA reductase. When mevalonic acid, the product of HMG-CoA reductase, was co-administrated with rosuvastatin, the cardiac, hepatic, renal or gallbladder toxicity was markedly ameliorated in rats, dogs, and rabbits.

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

In the 2-year oncogenic studies, non-neoplastic alterations included changes in the forestomach (hyperkeratosis and/or hyperplasia and minor erosion and inflammation of the squamous epithelium) and liver (increased foci of alteration) were observed in both species. Neoplastic alterations were limited to hepatocellular adenomas /carcinomas in the mouse at 200 mg/kg/day (10, 21, 48, and 107X human exposure at human doses of 80, 40, 20, and 10

mg/day, respectively), and in the rat, there were an increased number of uterine stromal polyps n females at 80 mg/kg/day (11, 23, 53, and 116X human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively).

Rosuvastatin induced fetal toxicity in rats at 25 mg/kg and rabbits at 3 mg/kg. In rats, both maternal toxicity (reduced body weight and food consumption, liver and renal toxicity) and fetal toxicity (lower number of pups live born, slight low fetal body weight, low incidence of pups with eyes open, and increase in startle amplitude, increases in visceral malformation and skeletal variations, and slightly retarded ossification) were observed at ≥ 25 mg/kg with NOAEL for dams and fetus of 15 mg/kg. In rabbits, severe maternal toxicity (mortality, body weight loss, hypoactivity and debility, and marked histopathologic changes in liver, gallbladder, kidney, heart, and muscle) and fetal toxicity (increase in dead fetuses, decrease in fetal viability index) were observed at 3 mg/kg with NOAEL for dams and fetus of 1 mg/kg. The corresponding exposure levels for rats at 25 mg/kg were 3, 6, 13, and 28X human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. The AUC for pregnant rabbits at 3 mg/kg were not provided. Estimates based on the exposure (C_{max}) in male rabbits at dose of 5 mg/kg, the exposure for female rabbits at 3 mg/kg might be about ½, 1, 3, and 5X human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively.

There was a low distribution of rosuvastatin to fetus in rats (3% or 20% of maternal plasma concentration in fetal tissue or amniotic fluid, respectively) following a single oral dose of 25 mg/kg. Relatively higher distribution in fetal tissue (25% maternal plasma concentration) was observed in 1/4 fetus in rabbits following a single oral dose of 1 mg/kg. However, in the lactating rat, rosuvastatin was found in milk at concentrations up to 3 times those in plasma. Theses data suggested that rosuvastatin have risk to pregnant women and nursing mothers.

Recommendations:

Pharmacology recommends approval of this drug for the proposed indications and the preclinical studies are adequate to support safety for 10 and 20 mg/day. Rhabdomyolysis and acute renal failure were observed at 40 and 80 mg/day in clinical trials and safety margins based on NOAEL in repeated preclinical studies were less than 5 at these dose levels, supporting a clinical risk at these higher dose levels.

Labeling with basis for findings:

CNS Toxicity

C

]

Carcinogenesis, Mutagenesis, Impairment of Fertility

[redacted]

Pregnancy

[redacted]

Where?

X. **APPENDIX/ATTACHMENTS:**

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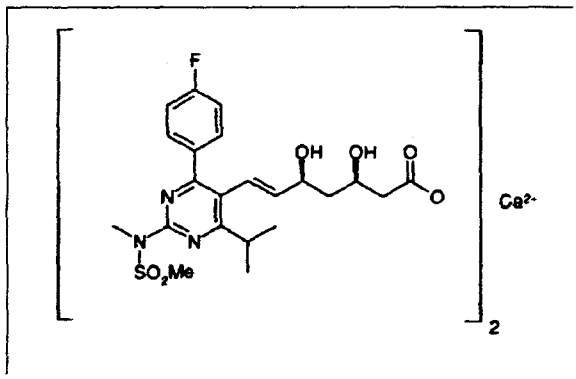
Review and Evaluation of Pharmacology/Toxicology Data of ZD4522

Key Words: cholesterol altering, HMG CoA reductase inhibitor
Reviewer Name: John Zhaolong Gong, Ph.D.
Division Name: Division of Metabolic & Endocrine Drug Products
HFD# 510
Review Completion Date: March 30, 1999

IND Number: 56,385
Serial Number/Date/Type of Submission: SN#000/July 10, 1998/IND, SN005/October, 12 1998, SN006/November 25, 1998, SN009/December 22, 1998, SN011/January 22, 1999, SN014/February 16, 1999, SN015/February 17, 1999, SN016/March 5, 1999, SN020/March 16, 1999
Information to Sponsor Yes (X) No ( )
Sponsor: ZENECA Pharmaceuticals, A Business Unit of Zeneca, Inc., Wilmington, DE 19850.
Manufacturer for Drug Substance: Shionogi & Co., LTD. Japan.
Drug:
Drug name: ZD4522, S-4522
Chemical name: Bis [(E-)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl) amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt
CAS number (if provided by sponsor)
Molecular weight: 1001.16
Structure:



Relevant IND/NDA: Lovastatin (NDA 19,643), Simvastatin (NDA 19,766), Pravastatin (NDA 19,898), Fluvastatin (NDA 20,261), Atorvastatin (NDA 20,702), Cerivastatin (NDA 20, 740)
Drug Class: HMG CoA reductase inhibitor
Indication: primary hypercholesterolemia and mixed dyspipidemias
Clinical Formulation (and components):
Core: ZD4522 (Calcium salt), Lactose, — Microcrystaline cellulose, — — and Magnesium stearate.
Capsule: Ferric oxide yellow, Ferric oxide red, Titanium dioxide, —

Route of Administration: Oral

Proposed Clinical Protocol

The initial phase I trials will be conducted in the United Kingdom and will investigate the tolerability of ZD-4522 and pharmacokinetic profile following single and multiple dosing in Caucasian subjects. A ^{14}C disposition study will also be conducted in the United Kingdom. Subsequent volunteer studies will investigate the effect of food and morning vs. evening administration on the pharmacodynamics and pharmacokinetics of ZD4522 following multiple dose administration. The latter studies will be conducted in the United States.1 40mg maximum dose has been proposed for these studies.

A Phase II study will be conducted in Europe using doses selected following evaluation of early Phase I Japanese and Caucasian data. This early efficacy trial will assist dose selection and will provide a safety data base for subsequent dose response and Phase III comparator trials.

Previous Clinical Experience

A total of 36 Japanese male volunteers have been exposed to ZD4522 in single and multiple dose phase 1 studies and a total of 110 male and 3 females have been exposed in phase I single and multiple dose Caucasian studies. No serious or clinically significant adverse events were observed in any of these studies.

Linear increases in C_{max} and AUC were observed following single dose exposure in Caucasian male volunteers (5,10, 20, and 40 mg; n=4 at each dose) and in Japanese subjects (up to 20 mg). The $T_{1/2}$ approximated 20 hours in these subjects. Food reduced the rate but not the extent of ZD4522 absorption in subjects administered a single 5 mg dose (mean C_{max} 2.4 ng/ml with food and 3.6 ng/ml fasting; n=6 subjects). 7 days repeated dosing at 10, 20 and 40 mg in Japanese subjects and 20, 40, and 80 mg in Caucasian volunteers did not lead to drug accumulation. Serum protein binding was approximately 90% in this studies. In vitro studies with human hepatic microsomes have shown that ZD4522 is neither a substrate for nor an inhibitor of the cytochrome P450 enzyme system.

Single Dose				
5	3.1	5		25.0
10	7.87-8.2	5	15.1	57.9-126
20	6.06-20.5	3-5	16.0-23.2	63.1-209
40	27.4-41.5	3-4	15.2-23.5	275.1-404
80	33.4	4	25.7	308
Steady state				
10	9.38	5	17.4	90.5
20	9.7-22.1	3-5	14.8-15.0	81.8-206
40	37.0-54.3	3-5	17.7-19.0	235-458
80	46.2	5	13.4	329

Introduction/Drug History

ZD4522 was developed by the Japanese pharmaceutical company Shionogi. The data contained in this IND submission have been generated by Shionogi and collated by Zeneca. There has been no marketing experience to date.

ZD4522, a novel member of the statin class of lipid lowering agents, is a synthetic 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor. Since HMG CoA reductase is the major rate-controlling step in the pathway for cholesterol synthesis, statins have proven to be clinically effective in the reduction of plasma levels of LDL and VLDL and are marketed world-wide for the lowering of total cholesterol/LDL-cholesterol levels. First generation statins (lovastatin, pravastatin and simvastatin) are prodrug derivatives of fungal metabolites, whereas ZD4522 is structurally similar to the synthetic second generation statins (super statins, such as, atorvastatin, fluvastatin and cerivastatin).

Studies Reviewed Within This Submission: **Page**

Studies not reviewed within this submission:
None

PHARMACOLOGY

Mechanism of Action

Like other statins, ZD4522 is an inhibitor of HMG CoA reductase. Since HMG CoA reductase is the major rate-controlling step in the pathway for cholesterol synthesis, statins have proven to be clinically effective in the reduction of plasma levels of LDL and VLDL.

Drug Activity Related to Proposed Indication

a. In vitro studies

The effect of ZD4522 on the activity of HMG CoA reductase was studies by measuring the rate of conversion of labeled substrate HMG CoA to the enzyme product mevalonate. Cholesterol synthesis was studied by measuring the rate of conversion of labeled precursor, [^{14}C]-acetate into cholesterol.

Test System	Effects
Rat liver microsomes	HMG CoA reductase inhibition, IC_{50} = 10 nM
Human liver microsomes	HMG CoA reductase inhibition, IC_{50} = 12 nM
Isolated rat hepatocyte	Cholesterol synthesis inhibition, IC50 = 1.1 nM
Human hepatoma cell line	LDL receptor expression increase

b. In vivo studies

ZD4522 was administered orally, precursor [^{14}C]-acetate was given by ip, the incorporation of radioactivity into cholesterol was measured to determine the cholesterol synthesis.

Animal	Dose	Effects
Rat	0.01 – 50mg/kg single dose	Cholesterol synthesis inhibition, selective for liver, ED_{50} = 0.1 – 0.8 mg/kg
Dog	0.01 – 3 mg/kg single dose	HMG CoA reductase inhibition, mevalonate reduction, $ED50_{50}$ < 1 mg/kg
Dog	0.3 – 3 mg/kg/day X 4 days	Serum total cholesterol ↓ by 26% at 3 mg/kg
Dog	0.03 – 0.1 mg/kg/day X 3 months	Serum total cholesterol ↓ by 10% at 0.1 mg/kg
Monkey	12.5 – 50 mg/kg/day X 5 days	Serum total cholesterol ↓ by 28% at 50 mg/kg, VLDL + LDL cholesterol ↓ by 26 – 37% and HDL ↓ by 13 – 25%.
Monkey	3.1 – 6.2 mg/kg/day X 5 days	No effect
Rabbit	3, 10 mg/kg X 6 months	Serum total cholesterol ↓ by 30%.

Summary of Pharmacology

ZD4522 is a potent and competitive HMG CoA reductase inhibitor in vitro and in vivo. In vitro studies showed that ZD4522 inhibits HMG CoA reductase and cholesterol synthesis in rat liver microsomes and isolated hepatocytes, and in human liver microsomes or hepatoma cells. A similar IC_{50} in ZD4522 inhibition on HMG CoA reductase was observed in rat and human hepatic microsomes. In vivo studies indicated that ZD4522 is an effective cholesterol synthesis inhibitor in rat, dog, rabbit and monkey in dose related manner with a marked selectivity for liver over other tissues. Significant lower serum cholesterol was observed in most animal studies.

PHARMACOKINETICS/TOXICOKINETICS

Disposition studies have been carried out in rat and dog, to support toxicological evaluation in these species, using a radiolabelled form of ZD4522. Toxicokinetic data have also been generated in mouse, rat, rabbit, dog and monkey , which are included in toxicology reviews.

Analytical method: — was used in some early studies. The limit of quantification was — ng/ml. RIA was used in later studies. The limit of detection was — and — ng/ml in mouse, rat, dog, and monkey respectively.

Absorption: ZD4522 can be absorbed after oral administration. T_{max} ranged from 0.25 to 6 hour depending on animal species and dose. C_{max} and AUC increased with ZD4522 proportionally at lower doses and more than proportionally at higher doses. The absolute bioavailability is 32% in dogs. The absorption is at least 56% in the rat and 74% in the dog. Fasting, low calcium diet increase absorption. Dog has higher C_{max} and $AUC_{(0-24)}$ than other species.

Distribution: ZD4522 selectively distributed in liver predominantly by active uptake in rat. More than 90% ZD4522 in plasma binds to binding protein, mostly serum albumin in rat, dog and human. Tissue concentrations declined approximately in parallel with plasma concentration after both single and repeated administration, indicating no preferential retention in any tissue, with an apparent half life of about 12 to 20 hours.

Metabolism: Four metabolites in the rat, and 5 in the dog, have been identified by chromatography following oral dosing. Each forms a small proportion of the absorbed dose. ZD4522 appears to undergo beta oxidation forming the pentadienoic acid, pentenoic acid or propenoic acid derivative through dehydration and reduction. The unchanged compound and the pentenoic acid derivative are further conjugated with taurine.

Excretion: The major excretory pathway was via the bile in rat and dog, mostly as unchanged parent compound. The rate of excretion was rapid, with over 80% of dose excreted within 24 hours post dose, and over 99% within 96 hours.

TOXICOLOGY

General Comments:

Toxicology studies have been conducted in rats, dogs and monkeys by oral gavage. The studies ranged from single dose to 12 months.

Study Title: Single Oral Dose Toxicity Study Of S-4522 In Rats

Study No.: B-015-L

Amendment #, Vol #, and Page #: SN000 Vol 3 Page 151 and SN005 Vol 1 Page 1

NOTE: Performed by Research Laboratories of Shionogi & Co, Ltd, Japan. Study period: 2/92-8/92. Final study report dated August 3, 1992. Lot No. 52. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Jcl:SD rats were obtained from ——— ——— Dosing started at 6 weeks of age. S-4522 was administered orally by gavage in 5% aqueous solution of gum arabic.

LD	1000 mg/kg	6	6
HD	2000 mg/kg	6	6

RESULTS:

CLINICAL SIGNS (daily observation):

HD rats exhibited soft stool on day 1, might be caused by bolus administration of test formulation.

MORTALITY:

None.

BODY WEIGHT (checked on day -1, 0, 1, 3, 7, and 14):

1 HDm exhibited weight loss on day 3.

FOOD CONSUMPTION:

No data.

ORGAN WEIGHT:

No data.

GROSS PATHOLOGY (examined at sacrifice):

Lung: red or dark brown spots in 1 LDm, 2 HDm and 1 HDf.
Stomach: dark red sports in forestomach of 1 HDm.
Mesenteric lymph node: brownish coloration in 1 HDm.
Testis: markedly small right testis in 1 LDm.

HISTOPATHOLOGY (examined at sacrifice):

Kidney: 1 HDf exhibited thickening of transitional epithelium of the renal pelvis and slight inflammatory cell infiltration under the pelvic epithelium .
Stomach: 1 HDf had slight eosinophil infiltration in the forestomach.
Jejunum: 1 HDf had slight calcification in the lymph nodule.
Testis: 1 LDm had necrosis of the seminiferous tubule, accompanied by thickening of the tunica albuginea, fibrosis and calcification of the interstitium.

SUMMARY

Title	SINGLE ORAL DOSE TOXICITY STUDY OF S-4522 IN RATS (B-015-L)			
Animal	Rat Jcl:SD strain, 6 weeks old			
Route	Oral gavage			
Dose (mg/kg)	1000		2000	
Sex and # of animal	M	F	M	F
	6	6	6	6
Mortality	None			
Clinical sign	-		Whitish loose feces	
Body weight	-			
Autopsy	-			
Histopathology	-			
Conclusion	Single lethal dose > 2000 mg/kg			

-: No remarkable findings. Empty cell: no data.

Study Title: Single Oral Dose Toxicity Study Of S-4522 In Dogs
Study No.: B-016-L
Amendment #, Vol #, and Page #: SN000 Vol 3 Page 192 and SN005 Vol 1 Page 60

NOTE: Performed by Research Laboratories of Shionogi & Co, Ltd, Japan. Study period: 2/92-9/92. Final study report dated September 25, 1992. Final report amendment dated January 20, 1998. Lot No. 52. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Beagle dogs were obtained from ——— Dosing started at 7 months of age. S-4522 was administered orally by gavage in 5% aqueous solution of gum arabic.

LD	1000 mg/kg	1	1
HD	2000 mg/kg	1	1

RESULTS:
CLINICAL SIGNS (daily observation):
Vomiting: all dogs.
Diarrhea: LDf.
Small amount of mucous bloody stool: HDm.
MORTALITY:
None.
BODY WEIGHT (checked on days –6, -1, 1, 3, 7, and 14):
HDm exhibited weight loss on day 1.
FOOD CONSUMPTION (checked daily):
No dose-related change.
URINALYSIS (checked on days –4, 1, 7, 14):
HDm exhibited positive in glucose and occult blood.
HEMATOLOGICAL EXAMINATION (checked on days –5, -1, 1, 3, 7, 13):
HDm exhibited marked WBC ↑ (due to neutrophils ↑) on day 1.
2 Females exhibited mild WBC ↑ (due to neutrophils ↑) on day 1.
BLOOD CHEMISTRY EXAMINATION (on days –5, -1, 1, 3, 7, 13):

HDm: mild ↑ in GOT, GTP, CPK and ALP; and ↓ in Fe on day 1.
LDf: ↑ in GOT and amylase activity on day 1.
All dogs: ↓ in triglycerides and total cholesterol.

ORGAN WEIGHT:
No data.

GROSS PATHOLOGY (at sacrifice):
HDf: red points in the colon mucosa.

HISTOPATHOLOGY (at sacrifice):
HDm: single cell necrosis in lamina propria mucosa of the duodenum.
HDf: slight hemorrhage in lamina propria mucosa of the duodenum and slight localized cell infiltration in the lung.

SUMMARY

Title	SINGLE ORAL DOSE TOXICITY STUDY OF S-4522 IN DOGS (B-016-L)			
Animal	Beagle dogs, 7 months old.			
Route	Oral gavage			
Dose (mg/kg)	1000		2000	
Sex and # of animal	M	F	M	F
	1	1	1	1
Mortality	None			
Clinical sign	Vomiting, diarrhea (LDf), bloody stool (HDm) on day 1			
Body weight	-	-	↓ on day 1	-
Food consumption	-			
Ophthalmology				
Auditory test				
ECG				
Urinalysis	-	-	Glucose +, Occult blood ++ on day 1	-
Hematology	-	WBC ↑ (neutrophils ↑) on day 1		
Blood chemistry	Amylase activity, GOT ↑ on day 1	-	GOT, GPT, CPK ↑, Fe ↓ on day 1.	-
	Triglycerides and total cholesterol ↓			
Organ weight				
Autopsy			-	Colon: Red points in mucosa
Histopathology			Duodenum: single cell necrosis in the lamina propria mucosa	Colon: slight hemorrhage in lamina propria mucosa
Conclusion	Single lethal dose > 2000 mg/kg			

-: No remarkable findings. Empty cell: no data.

Study Title: One-Month Repeated Oral Toxicity Of S-4522 In Rats
Study No.: B-017-L
Amendment #, Vol #, and Page #: SN000 Vol 4 Page 1

NOTE: Performed by Research Laboratories of Shionogi & Co, Ltd, Japan. Study period: 2/92-1/93. Final study report dated January 29, 1993. Lot No. 52. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: SD rats were obtained from [redacted]. Dosing started at 7 weeks of age. S-4522 was administered orally by gavage in 5% aqueous solution of gum arabic for one month. 6 rats in control and HD were continued for another 30-day recovery study. Dose volume was 5 ml/kg/day for S-4522 treated groups and 10 ml/kg/day for control. CA-1 diet and tap water were provided *ad libitum*.

Control	0	16	16
LD	15	10	10
MD	50	10	10
HD	150	16	16

RESULTS:

CLINICAL SIGNS (observed daily):
No treatment-related changes.

MORTALITY:
None.

BODY WEIGHT (weighed daily):
No treatment-related changes.

FOOD AND WATER CONSUMPTION (every 5 days):
Food consumption decreased slightly (5%) in HDm in the first 2 weeks of administration.
Water consumption did not show dose-related changes.

AUDITORY TEST (tested day -5 or -7, 28 or 29, 29R):
No abnormal finding.

OPHTHALMOLOGIC EXAMINATION (checked day -5 or -7, 23 or 24, 28R):
1 MDm: partial dilatation of the arteriole of the retina on day 23 of administration.

URINALYSIS (checked day 28 or 29, 29R):
No treatment-related changes.

HEMATOLOGICAL EXAMINATION (at necropsy):
HDm, HDf and MDf: Slight shortenings in PT
HDf: Slight shortenings in APTT

BONE MARROW EXAMINATION (at necropsy):
No abnormal finding.

BLOOD CHEMISTRY EXAMINATION (at necropsy):
HDm: triglyceride ↓ and LAP activity slight ↑
MDf and HDf: triglyceride ↓, cholesterol slight ↑ and LAP activity slight ↑
1 HDm (#D101) had high values in GOT, GTP, ALP, total bilirubin and urea nitrogen.
In the recovery period, the changes were reversed.

LIVER BIOCHEMISTRY EXAMINATION (at necropsy):
At the end of S-4522 treatment, male rats exhibited dose-related decreases in triglyceride and cholesterol; female rats exhibited no change in triglyceride, but a slight dose-related increase in cholesterol. At the end of 30-day recovery study, no difference was observed between treated and control groups. However, much higher control values were observed in this stage, such as cholesterol (male 48% and female 14% higher than the control values at the end of treatment), and triglyceride (male 25% and female 20% higher than the control values at the end of treatment).

Group	C	LD	MD	HD	C	LD	MD	HD	C	HD	C	HD
# of Animal	8	8	8	8	8	8	8	8	6	6	6	6
Cholesterol (mg/g)	3.26	3.05 (94)	2.79 (86*)	2.59 (79**)	2.40	2.46 (103)	2.71 (113**)	2.87 (120**)	4.81	4.25	2.73	2.69
Triglyceride (mg/g)	21.4	15.1 (71*)	12.6 (59**)	8.9 (42**)	9.1	8.9 (98)	7.0 (77)	8.5 (93)	26.7	22.7	10.9	9.7

( ): % of control; *,**: Statistically significant at $p<0.05$ and $p<0.01$.

ORGAN WEIGHT (at necropsy):

–Liver: slight ↑ in relative weight in MDf (6%) and HDf (8%) at the end of treatment.

GROSS PATHOLOGY AND HISTOPATHOLOGY (at necropsy):

MD rats exhibited slight to moderate histopathology changes only in liver. HD exhibited more severe histopathology change in liver, as well as other organs. Males exhibited more severe and higher frequent changes than females. At the end of 30-day recovery, all changes observed during treatment disappeared.

Findings at the End of Dosage	Sex	♂	♀	♂	♀
	No. of Rats	10	10	10	10
Stomach					
Mucosal thickening in forestomach		0	0	1	0
Mucosal erosion in glandular stomach		0	0	2	0
Liver					
Cytoplasmic eosinophilic change mainly in periportal hepatocyte	+	3	2	0	3
	++	2	2	2	7
	+++	0	0	8	0
Cytoplasmic inclusion body mainly in periportal hepatocyte	±	1	0	4	1
	+	0	0	2	0
Single cell necrosis mainly in periportal hepatocyte	±	1	0	4	3
	+	1	0	2	0
Irregular arrangement of hepatic cell cord	±	1	2	3	0
	+	1	1	5	6
	++	0	0	2	1
Adrenal					
Thickening of zona glomerulosa	+	0	0	1	2
Stomach					
Hyperkeratosis in forestomach	±	0	0	3	1
Thyroid					
Thickening of follicular epithelium	±	0	0	2	2
Pancreas					
Single cell necrosis in acinar cell	±	0	0	0	1

±: very slight, +: slight, ++: moderate, +++: severe

SUMMARY

Title	ONE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN RATS (B-017-L)							
Animal	SD rats, 7 weeks old.							
Route	Oral gavage							
Dose (mg/kg/day)	0	15	50		150		150 recovery	
Sex and # of animal	♂/♀	♂/♀	♂	♀	♂	♀	♂	♀
	10	10	10	10	10	10	6	6
Mortality	None							
Clinical sign	-							
Body weight	-							
Food consumption	-				↓ 5% 1st 2 wks	-	-	
Ophthalmology	-							
Auditory test	-							
ECG	-							
Urinalysis	-							
Hematology	-	-	-	PT↓	PT↓	PT, APTT↓	-	
Blood chemistry	-	-	-	TG↓, LAP↑			-	
Liver chemistry: TG	-	♂ ↓29%	↓41%	-	↓58%	-	-	
Cholesterol	-	-	↓14%	↑13%	↓21%	↑20%	-	
Organ weight: liver	-	-	-	↑6%	-	↑ 8%	-	
Autopsy	-		-	-	Stomach change	-	-	
Histopathology	-		Slight to moderate liver change		More severe liver, stomach, adrenal, thyroid, and pancreas change		-	
Reversibility	Good							
Conclusion	NOAEL is 15 mg/kg							

-: No remarkable findings. Empty cell: no data.

Study Title: Three-month Repeated Oral Dose Toxicity Of S-4522 In Rats
Study No.: F-009-L
Amendment #, Vol #, and Page #: SN000 Vol 5 Page 1 and SN005 Vol 2 Page 1

NOTE: Performed by ——— Study period: 5/92-6/93. Final study report dated June 3, 1993. Lot No. 54. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: SD rats were obtained from ——— Dosing started at 6 weeks of age. 12 rats/sex/group were administered S-4522 orally by gavage in 5% aqueous solution of gum arabic at doses 0, 10, 30, and 100 mg/kg/day for three month. 6 rats/sex/group (control and HD only) were continued for another 4-week recovery study. Dose volume was 5 ml/kg for treated groups. MM-6 diet and tap water were provided *ad libitum*.

Control	0	18	18
LD	10	12	12
MD	30	12	12
HD	100	18	18

RESULTS:

CLINICAL SIGNS (daily observation):

HD animals exhibited hypoactivity, piloerection, bradypnea, hypothermia, loss of hair, lacrimation. Some became weak to death or the moribund state for sacrifice.

MORTALITY:

Control	0	0/18	0/12
LD	10	1/12*	0/12
MD	30	0/12	0/12
HD	100	15/18* on day 11-19 of dosing	3/18 on day 13, 53 and 57 of dosing

*: 1 LD male and 1 HD male death appeared to be due to mal-intubation.

BODY WEIGHT (twice a week in the first 4 weeks and once a week after that):

8	86%**	96%
12	72%**	92%**
19	87%**	93%*

Values in ( ) are % of control. *,**: Statistically significant at $p<0.05$ and $p<0.01$.

FOOD CONSUMPTION (once a week):

HDm deceased by 53% on the second week of administration.

AUDITORY TEST (before dosing, week 13, and week 4 post dosing):

No abnormal incidence observed.

OPHTHALMOLOGIC EXAMINATION (before dosing, week 13, and week 4 post dosing):

No abnormality observed.

URINALYSIS (week 4 and 13, and week 4 post dosing):

1 HDf during administration and 2 HDf during recovery had occult blood (+) and red blood cells were found in the sediments.

HEMATOLOGICAL EXAMINATION (at sacrifice):

HD had slight low hemoglobin (94% of control) in males and MCH (96% of control) in females.

The sacrificed moribunds had increased hematocrit, hemoglobin, RBC and decreased lymphocyte.

MYELOGRAM (at sacrifice):

HD males: ↑ promyelocytes (149% of control) and plasma cells (189% of control).

HD females: ↓ myelocytes (64% of control).

Sacrificed moribunds: ↑ M/E ratio and neutrophil, and ↓ polychromatic normoblast.

BLOOD CHEMISTRY EXAMINATION (at sacrifice):

LD		Na ↓ 1%
MD		total cholesterol ↑ 38% *, α_2-globulin ratio ↑ 12% **
HD	GOT ↑ 67% **	phospholipid ↑ 34% **, P ↑ 42% *, α_2-globulin ↑ 15% ** total cholesterol ↑ 66% **, triglycerides ↓ 73% *

*,**: Statistically significant at $p<0.05$ and $p<0.01$.

Sacrificed moribunds: GOT, GPT, ALP, LDH, CPK, A/G ratio, BUN, total bilirubin ↑; triglycerides ↓.

LIVER BIOCHEMISTRY EXAMINATION (at sacrifice):

HDm: phospholipid ↑ 11% *
HDf: triglycerides ↓ 42% **
ORGAN WEIGHT (at sacrifice):
HDm: thyroid ↑ (absolute weight 124% of control *)
HDf: liver ↑ (relative wt 115% control **) and spleen ↑ (relative wt 122% of control *)
Dead and moribunds: ↓ thymus and spleen
GROSS PATHOLOGY AND HISTOPATHOLOGY:
Animal died during the experimental period:
HD: 14/18 male and 3/18 female exhibited emaciation, ↓ abdominal adipose tissue, involution of thymus, atrophy of spleen, mucosal thickening forestomach, and enlargement of pancreas. Histopathological examination revealed hypertrophy of perilobular hepatocytes, diffuse lymphocytes depletion or fatty metamorphosis in the thymus, atrophy of white pulps of the spleen, mucosal hyperkeratosis of the forestomach.
1 LDm and 1 HDm death appeared to be due to mal-intubation.
Animal scheduled sacrificed:

Findings at the End of Dosage	♂	♀	♂	♀
	12	12	3	9
Necropsy Examination				
Forestomach				
Mucosal thickening			1	2
Microscopical Examination				
Liver				
Hypertrophy of perilobular hepatocytes	5	5	3	6
Polypoidy of perilobular hepatocytes	3		3	4
Perilobular fibrosis	2		3	2
Shrinkage in the size of lobules			3	
Bile duct proliferation			2	1
perilobular foci of altered hepatocytes			3	1
Stomach				
Hyperkeratosis in mucosa of forestomach			1	1
Heart				
Focal myocardial necrosis and Inflammatory cell infiltration in ventricle	a few		a few	
Lung and Bronchus				
Foamy cells in alveoli and Foreign body pneumonia	1			
Kidney and Urinary Bladder				
Dysplasia of kidney	1			
Genital Organs				
Seminiferous tubuli atrophy	1			
Lutein cyst		1		
Electronic Microscopical Examination				
Liver				
↑ smooth surfaced endoplasmic reticulum and free ribosomes in perilobular hepatocytes	12	12	3	9

SUMMARY

Title	THREE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN RATS (F-009-L)							
Animal	SD rats, 6 weeks old.							
Route	Oral gavage							
Dose (mg/kg/day)	0		10		30		100	
Sex and # of animal	♂	♀	♂	♀	♂	♀	♂	♀
	12	12	12	12	12	12	18	18
Mortality	-		1	-	-		15	3
Clinical sign	-						Hypoactivity, piloerection, loss of hair, bradypnea, emaciation, hypothermia, lacrimation, moribund to death	
Body weight	-						↓ up to 28%	↓ up to 8%
Food consumption	-						↓ 53% on 2nd week	-
Ophthalmology	-							
Auditory test	-							
ECG								
Urinalysis	-						-	1 occult blood (+)
Hematology	-						Hb↓ 6%	MCH↓ 4%
Myelogram	-						promyelocytes ↑49%, plasma cells ↑89%	myelocytes ↓36%
Blood chemistry	-		-		-	CHO↑ 38%	GOT ↑ 67%	PL↑34%, P↑42%, α_2-globulin↑15%, CHO↑66%, TG↓27%
Liver chemistry	-						PL ↑ 11%	TG↓ 42%
Organ weight	-						thyroid ↑ 24%	Liver ↑ 15%, spleen ↑ 22%
Autopsy	-						Stomach mucosal thickening, ♂ (1/3), ♀ (2/9)	
Histopathology	-				Slight liver change		severe liver change (dose related hypertrophy of perilobular hepatocytes)	
Reversibility	Fair							
Conclusion	NOAEL is 10 mg/kg							

-: No remarkable findings. Empty cell: no data.

Study Title: Six-month Repeated Oral Dose Toxicity Of S-4522 In Rats
Study No.: F13-L
Amendment #, Vol #, and Page #: SN000 Vol 7 Page 1 and SN009 Vol. 1 Page 1

NOTE: Performed by [redacted] Study period: 11/93-3/95. Final study report dated March 15, 1995. Lot No. R39001. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: SD rats were obtained from [redacted] Dosing started at 6 weeks of age. 20 rats/sex/group were administered S-4522 orally by gavage in 5% aqueous solution of gum arabic at doses 0, 2, 6, and 20 mg/kg/day for six month. 10 rats/sex/group (control and HD only) were continued for another two-month recovery study. Additional 24 rat/sex/group were used for plasma drug concentration analysis. Dose volume was 5 ml/kg/day for S-4522 treated groups. MM-6 pellet diet and tap water were provided *ad libitum*.

Control	0	30	30	24	24
LD	2	20	20	24	24
MD	6	20	20	24	24
HD	20	30	30	24	24

RESULTS:

CLINICAL SIGNS (daily observation):

No treatment-related changes.

MORTALITY:

1 HDm death appeared to be due to dosing error.

BODY WEIGHT (twice weekly in the first 4 weeks and once weekly after that):

No treatment-related changes.

FOOD CONSUMPTION (once weekly):

HDm ↓ slightly (5%) on the first week of administration.

AUDITORY TEST (weeks 13 and 26 of dosing and week 9 post-dosing):

No abnormal incidence observed.

OPHTHALMOLOGIC EXAMINATION (weeks 13 and 26 of dosing and week 9 post-dosing):

No treatment-related changes.

URINALYSIS (weeks 4, 13, and 26 of dosing, week 9 post-dosing):

1 HDm had occult blood (+) (red blood cells were found in the sediments), but repeated test with fresh urine was (-).

HEMATOLOGICAL EXAMINATION (at sacrifice):

HDm: MCH ↓ (4%)

HDf: MCHC (3%), eosinophil (45%), hemoglobin (3%) ↓

MDf: MCHC (2%), eosinophil (53%) ↓.

LDf: MCHC (2%) ↓.

MYELOGRAM (at sacrifice):

No data.

BLOOD CHEMISTRY EXAMINATION (at sacrifice):

HDf: glucose ↑9%, total cholesterol ↑55% and phospholipids ↑ 35%

At the end of recovery: HDm: GOT ↑41%, total bilirubin ↑, glucose ↓ 14%

HDf: urea nitrogen ↑ 28% and creatinine ↑27%

LIVER BIOCHEMISTRY:

No data.

TOXICOKINETICS:

Peak (T_{max}) at 30 min after dosing

AUC_{0-24} increased dose-dependently

C_{max} and AUC_{0-24} values on day 91 and 182 were higher than those on day 1, but no overt sign of drug accumulation was detected

No sex difference

2	1	12.5	0.5	79.1
	91	15.6	0.5	96.7
	182	33.6	0.5	149.0
6	1	60.6	0.5	239.0
	91	66.1	0.5	254.0
	182	130.0	0.5	424.0
20	1	475.0	0.5	1310.0
	91	708.0	0.5	1950.0
	182	885.0	0.5	2410.0

ORGAN WEIGHT:

HDf: liver ↑ 19% and heart ↑ 11%

MDm: testis ↓ 10%

GROSS PATHOLOGY:

No treatment-related changes.

HISTOPATHOLOGY:

1 HDm died on day 35, histopathological examination revealed congestion and edema of lung and bronchi an congestion of the liver, indicating the death was due to dosing error.

		♂	♀	♂	♀	♂	♀	♂	♀
		20	20	20	20	20	20	19	20
Liver									
Hypertrophy of perilobular hepatocytes	±					3	2	4	3
	+						1	5	4
Perilobular fibrosis	+					1		1	
Vacuolar degeneration of perilobular hepatocytes	±	2		5	1	1	2	2	
	+	4		9		1	1	8	2
	++					4		5	
Foci of cellular alteration (basophilic cells)	p							2	1
Bile duct proliferation	±	2	4	4	4	3	2	4	2
	+								2
Vacuolar degeneration of centrilobular hepatocytes	±	3		1		2			
	+	4		2		1			
Heart									
Focal myocardial necrosis and Inflammatory cell infiltration	±	8		5		2		2	
	+			1		9		2	
Focal myocardial fibrosis	±			2				2	
	+	1		2				2	
Kidneys and Urinary Bladder									
Glomerulonephrosis	+			1					
Bladder Cystitis	+				1				
Lung and Bronchus									
Foreign body granuloma	+					1			
	++	1							
Stomach									

Epidermal cyst in submucosa of glandular stomach	p			1					
Spleen									
Abscess formation	+			1		1			

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	SIX-MONTH REPEATED ORAL TOXICITY OF S-4522 IN RATS (F-013-L)							
Animal	SD rats, 6 weeks old							
Route	Oral gavage							
Dose (mg/kg/day)	Control		2		6		20	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
Main study	30	30	20	20	20	20	30	30
TK study	24	24	24	24	24	24	24	
Mortality	0	0	0	0	0	0	1*	0
Clinical sign	-							
Body weight	-							
Food consumption	-						↓5%	-
Ophthalmology	-							
Auditory test	-							
Urinalysis	-							
Hematology	-							
Blood chemistry	-							
Toxicokinetics	-		T_{max} = 0.5 hr, no drug accumulation					
Organ weight	-				Testis ↓ 10%	-	-	Liver ↑ 19% Heart ↑ 11%
Autopsy	-							
Histopathology (liver)	-				Hypertrophy of perilobular hepatocytes Perilobular fibrosis Vacuolar degeneration of perilobular hepatocytes Foci of cellular alteration (basophilic cells) Bile duct proliferation			
Reversibility	Good							
Conclusion	NOAEL is 2 mg/kg							

*: Death due to a dosing error. -: No remarkable findings.

Study Title: One-Month Repeated Oral Toxicity Study Of S-4522 In Dogs
Study No.: B-020-L
Amendment #, Vol #, and Page #: SN000 Vol 10 Page 1 and SN014 Vol. 1 Page 1

NOTE: Performed by Shionogi & Co., Ltd., Japan. Study period: 5/92-3/93. Final study report dated March 26, 1993. Lot No. 54. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Beagle dogs were obtained from [redacted]. Dosing started at 6 months of age. 3 dogs/sex/group were administered S-4522 orally by gavage in 5-fold triturate with lactose at doses 0, 10, 30, and 90 mg/kg/day for one month. 2 dogs/sex/group (control and HD only) were continued for another one-month recovery study. Control group received 360 mg/kg lactose.

Control	0	5	5
LD	10	3	3
MD	30	3	3
HD	90	5	5

RESULTS:

CLINICAL SIGNS (daily observation):

HD showed high incidence of soft feces.

MORTALITY:

1 HDf showed extremely deteriorated condition and was sacrificed in extremis on day 24.

BODY WEIGHT (every 5 days pre- and during dosing, every 10 day post dosing):

No treatment-related changes.

FOOD CONSUMPTION (daily):

No treatment-related changes.

AUDITORY TEST (day –5, 24 and 28R):

No abnormal incidence observed.

OPHTHALMOLOGIC EXAMINATION (day –3 or –4, 22 or 23, and 26R):

No treatment-related changes.

ELECTROCARDIOGRAPHIC EXAMINATION (day –11, 25, and 27R):

No treatment-related changes.

URINALYSIS (days –11 and –4, 15 and 25, and 8R and 29R):

No treatment-related changes.

HEMATOLOGICAL EXAMINATION (days –10 and -3, 14 and 28, 7R and 28R): Day 28

Sex	♂	♀	♂	♀	♂	♀	♂	♀
Control	6.34	6.35	14.8	14.7	45.3	45.2	12.26	10.53
LD	6.48	6.33	14.7	15.0	45.2	46.6	11.19	10.62
MD	5.92 (7%)	5.66 (11%)	13.8 (7%)	13.1* (11%)	42.1	40.4* (10%)	12.83	11.21
HD	5.59* (12%)	5.83 (8%)	13.0** (12%)	13.7 (7%)	39.9** (12%)	42.4	13.72 (12%)	13.10 (24%)

( ): % of change comparing with control group.

The animal sacrificed in extremis exhibited ↑ RBC, HBG, HCT, WBC.

MYELOGRAM (at sacrifice):

The animal sacrificed in extremis had only half nucleated bone marrow cells as the control.

BLOOD CHEMISTRY EXAMINATION (days –10 and -3, 14 and 28, 7R and 28R):

Day 14

Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control	25	26	27	29	113	134	155	150	30	26
LD	24	27	37	39	133	117	134	103* (31%)	29	18 (31%)
MD	32* (28%)	31	58 (115%)	40 (38%)	146	140	98** (37%)	88** (41%)	24 -	9** (65%)
HD	38** (52%)	42** (62%)	85 (215%)	62 (114%)	188 (66%)	153	78** (50%)	76** (49%)	14 (53%)	14* (46%)

Day 14

Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control	27	28	29	33	100	49	156	145	29	24
LD	29	28	44 (52%)	43	124 (24%)	37* (24%)	125* (20%)	100** (31%)	28	17 (29%)
MD	38* (41%)	32	76** (163%)	62** (88%)	142* (42%)	35* (29%)	83** (53%)	76** (48%)	16 (45%)	8** (67%)
HD	39** (44%)	40** (43%)	56* (93%)	50 (52%)	136* (36%)	34* (31%)	70** (55%)	73** (50%)	15 (48%)	12* (50%)

( ): % of change comparing with control group. *,**: significant difference.

The animal sacrificed in extremis exhibited ↑ GOT, LDH, CPK activity, creatine, urea nitrogen, total bilirubin; ↓ total cholesterol, glucose, total protein, albumin and electrolytes.

LIVER BIOCHEMISTRY (at sacrifice):

No obvious changes in total protein, total cholesterol, phospholipids, triglyceride, and activity of P-450, coumar. Dmase, coumar. Dease, coumar. DPase.

TOXICOKINETICS (Non-GLP, days 0 and 29):

Peak (T_{max}) ranged from 1.3 hr for LD and MD to 3.2 hr for HD

C_{max} and AUC_{0-24} increased dose-dependently

No drug accumulation. No sex difference

10	0	1.3	1.3	0.29	0.20	1.52	1.11
10	29	2.0	1.7	0.14	0.14		
30	0	1.3	2.3	0.55	0.84	3.52	3.89
30	29	1.7	2.0	0.58	0.49		
100	0	3.2	3.0	2.63	4.77	19.91	23.94
100	29	3.0	3.5	8.99	2.68		

ORGAN WEIGHT:

HDf: lung ↓ 17%.

The animal sacrificed in extremis exhibited ↑ lungs, kidneys, spleen, pancreas and adrenals.

PATHOLOGICAL EXAMINATION:

Animal sacrificed in extremis:

Liver/biliary tracts: hematoma (3X3 cm), thrombus, atrophy of hepatocytes and hyperplasia of the bile duct, hemorrhage of serosa and mucosa of the gallbladder.

APPEARS THIS WAY
ON ORIGINAL

Animals necropsied on schedule:

		♂	♀	♂	♀	♂	♀	♂	♀
		3	3	3	3	3	3	3	3
Gallbladder									
Ectopic pancreas	p			1					
Lamina propria mucosae, edema	±							1	1
	+					2		1	2
Lamina propria mucosae, hemorrhage	+					1			1
	++					1		1	1
Lamina propria mucosae, inflammatory cell infiltration	±					1			2
	+							2	
Liver									
Hepatocyte inclusion body	p					1			
Testis									
Seminiferous tubule, giant cells increase	±							1	
Eye									
Retina, dysplasia	p		1	1	1			2	1
Retina, loss	p								1

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	ONE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN DOGS (B-020-L)							
Animal	Beagle dogs, 6 months old							
Route	Oral gavage							
Dose (mg/kg/day)	Control		10		30		90	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	5	5	3	3	3	3	5	5
Mortality	0	0	0	0	0	0	0	1*
Clinical sign	-						Soft feces	
Body weight	-							
Food consumption	-							
Ophthalmology	-							
Auditory test	-							
Urinalysis	-							
Hematology	-				↓ RBC, ↓ HGB, ↓ HCT			
Myelogram	-							
Blood chemistry	-				↑ GOT, ↑ GPT, ↑ ALP, ↓ LDH, ↓ CHO, ↓ TG			
Liver Biochemistry	-							
Toxicokinetics	-		T_{max} = 1.3 – 3.2 hr, C_{max} and AUC_{0-24} increased dose-dependently No drug accumulation, No sex difference					
Organ weight	-							↑ lung 17%
Autopsy	-							
Histopathology	-				Gallbladder: cholecycstitis Testis: giant cells in testicular seminiferous tubules			
Reversibility	Good							
Conclusion	NOAEL is 10 mg/kg							

*: sacrificed in extremis. -: No remarkable findings.

Study Title: Three-Month Repeated Oral Toxicity Study Of S-4522 In Dogs
Study No.: B-021-L
Amendment #, Vol #, and Page #: SN000 Vol 11 Page 1

NOTE: Performed by Shionogi & Co., Ltd., Japan. Study period: 8/92-5/94. Final study report dated May 13, 1994. Lot No. 55. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Beagle dogs were obtained from [redacted] Dosing started at 7 months of age. 3 dogs/sex/group were administered S-4522 orally by gavage in 1:10 dilution with lactose at doses 0, 7.5, 15, and 30 mg/kg/day for three month. 3 dogs/sex/group (control and HD only) were continued for another 30-day recovery study.

Group	Dose (mg/kg)	Male #	Female #
Control	0	6	6
LD	7.5	3	3
MD	15	3	3
HD	30	6	6

RESULTS:
CLINICAL SIGNS (daily):
1/6 HDf had hypoactivity on days 50-52.
MORTALITY:
None.
BODY WEIGHT (every 7 days):
1/6 HDf exhibited body weight decrease on days 49 and 56.
FOOD CONSUMPTION (daily):
1/6 HDf had decreased food consumption on days 49-52.
AUDITORY TEST (days -7, 49, 89 and 25R):
No abnormal incidence observed.
OPHTHALMOLOGIC EXAMINATION (days -13, 46, 83 and 26R for male, days -10, 47, 84 and 26R for female):
Slight opacity of lens was observed in HD.

	During Dosing	End of administration	Middle of recovery	End of recovery
HDm	0/6	1/6	2/3	-
HDf	0/6	2/6	1/3	1/3

URINALYSIS (days -16, -2, 29, 57, 89, 5R and 26R):
No treatment-related changes.
ELECTROCARDIOGRAPHICAL EXAMINATION (days -6, 48, 85 and 25R):
No treatment-related changes.
HEMATOLOGICAL EXAMINATION (days -15, -3, 27, 55, 88, 5R, and 27R):
No treatment-related changes.
MYELOGRAM (at sacrifice):
No treatment-related changes.
BLOOD CHEMISTRY EXAMINATION (days -15, -3, 27, 55, 88, 5R and 27R):
HD: ↓ Cholesterol (40-50%), ↓ triglyceride (48-66%); 1 HDm ↑ GPT (from 23-26 pre-dosing to 158-228 during dosing).

MD: ↓ Cholesterol (25-35%), ↓ triglyceride (30-77%).
LD: ↓ Cholesterol (25-47%), ↓ triglyceride (41-52%).

LIVER BIOCHEMISTRY (at sacrifice):

No obvious changes in total protein, total cholesterol, phospholipids, triglyceride, and activity of P-450, coumar. Dmase, coumar. Dease, coumar. DPase.

TOXICOKINETICS (Non-GLP, days 0, 41, and 82):

Peak (T_{max}) ranged from 1.3 hr to 2.7 hr
C_{max} increased dose-dependently.
No drug accumulation. No sex difference

7.5	0	1.3	1.3	0.26	0.25
7.5	41	1.7	1.3	0.32	0.21
7.5	82	1.3	3.0	0.35	0.15
15	0	2.0	1.3	0.34	0.75
15	41	2.0	2.3	0.27	0.75
15	82	1.7	2.0	0.41	0.44
30	0	2.5	1.5	1.30	1.71
30	41	2.0	2.3	2.60	1.83
30	82	2.7	1.8	1.42	1.28

ORGAN WEIGHT:

No treatment-related changes.

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		♂	♀	♂	♀	♂	♀	♂	♀
		3	3	3	3	3	3	3	3
Gallbladder									
Mucosa, red speck or point	p				1	1	1	2	3
Lamina propria mucosae, hemorrhage	±					2	3		1
	+			1		1		1	1
	++				1			2	1
Lamina propria mucosae, inflammatory cell infiltration	±			1	1	1		1	1
	+								

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	THREE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN DOGS (B-021-L)							
Animal	Beagle dogs, 7 months old							
Route	Oral gavage							
Dose (mg/kg/day)	Control		7.5		15		30	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	6	6	3	3	3	3	6	6
Mortality	None							
Clinical sign	-							1 hypoactive
Body weight	-							1 ↓
Food consumption	-							– 1 ↓

Ophthalmology	-		1 opacity	2 opacity
Auditory test	-			
Urinalysis	-			
Hematology	-			
Myelogram	-			
Blood chemistry	-	↓CHO, ↓TG, ↑GPT in 1 HDm		
Liver Biochemistry	-			
Toxicokinetics	-	T_{max} = 1.3 – 2.7 hr, C_{max} increased dose-dependently No drug accumulation, No sex difference		
Organ weight	-			
Autopsy		Red areas on the surface of mucosa of gallbladder		
Histopathology	-	Gallbladder: hemorrhage and inflammatory cell infiltration in lamina propria mucosae		
Reversibility	Good			
Conclusion	No NOAEL could be estimated in this study			

*: sacrificed in extremis. -: No remarkable findings.

Study Title: Investigative 6-Month Repeated Oral Toxicity Study Of S-4522 In Dogs
Study No.: B-037-L
Amendment #, Vol #, and Page #: SN000 Vol 12 Page 1

NOTE: Performed by Shionogi & Co., Ltd., Japan. Study period: 2/93-12/94. Final study report dated December 9, 1994. Lot No. 55. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Beagle dogs were obtained from ——— Dosing started at 6-7 months of age. 3 dogs/sex/group were administered S-4522 orally by gavage in 10-fold triturate with lactose at doses 1 and 4 mg/kg/day for six months.

LD	1	3	3
HD	4	3	3

RESULTS:
CLINICAL SIGNS (daily):
Abnormal feces, vomiting were observed in all animals.
MORTALITY:
None.
BODY WEIGHT (every 14 days):
No dose-related changes.
FOOD CONSUMPTION (daily):
1 HDf and 1 LDf had decreased food consumption.
OPHTHALMOLOGIC EXAMINATION (days –3, 92 and 180):
No dose-related changes.
HEMATOLOGICAL EXAMINATION (days –13, -2, 41, 82, 125 and 181):
No dose-related changes.

BLOOD CHEMISTRY EXAMINATION (days –13, -2, 41, 82, 125, 139, 160 and 181):
↓ ALP (50%), ↓ CPK (50%), ↓ CHO (40%).

LIVER BIOCHEMISTRY (at sacrifice):
No obvious changes in total protein, total cholesterol, phospholipids, triglyceride, and activity of P-450, coumar. Dmase, coumar. Dease, coumar. DPase.

TOXICOKINETICS (Non-GLP, days 0, 89 and 179):
Peak (T_{max}) ranged from 1.5 hr to 3.2 hr
C_{max} and AUC_{0-24} increased dose-dependently
No drug accumulation.

Dose (mg/kg)	Days of Treatment	T_{max} (Hr)	C_{max} (ng/ml)	AUC_{0-24} (ng.hr/ml)
1	0	1.5	16.7	95.6
1	89	1.7	13.1	76.4
1	179	3.2	18.0	122
4	0	1.8	120	639
4	89	2.3	61.2	384
4	179	2.0	111	661

ORGAN WEIGHT:
No dose-related changes.

GROSS PATHOLOGY AND HISTOPATHOLOGY:
No dose-related changes.

SUMMARY

Title	Investigative 6-month repeated oral toxicity of S-4522 in dogs (B-037-I)			
Animal	Beagle dogs, 6-7 months old			
Route	Oral gavage			
Dose (mg/kg/day)	1		4	
# of animal	♂	♀	♂	♀
	3	3	3	3
Mortality	None			
Clinical sign	Abnormal feces, vomiting			
Body weight	-			
Food consumption	-			
Ophthalmology	-			
Auditory test				
Urinalysis				
Hematology	-			
Myelogram				
Blood chemistry	↓ ALP, ↓ CPK, ↓ CHO			
Liver Biochemistry	-			
Toxicokinetics	T_{max} = 1.5 – 3.2 hr, C_{max} and AUC_{0-24} increased dose-dependently No drug accumulation, No sex difference			
Organ weight	-			
Autopsy	-			
Histopathology	-			
Reversibility				
Conclusion	NOAEL is 4 mg/kg			

*: sacrificed in extremis. -: No remarkable findings.

Study Title: Twelve-Month Repeated Oral Toxicity Study Of S-4522 In Dogs
Study No.: B-046-L
Amendment #, Vol #, and Page #: SN000 Vol 13 Page 1 and SN020 Vol. 1 Page 1

NOTE: Performed by Shionogi & Co., Ltd., Japan. Study period: 12/93-1/96. Final study report dated January 25, 1996. Lot No. R39001. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Beagle dogs were obtained from [redacted]. Dosing started at 6 months of age. 4 dogs/sex/group were administered S-4522 orally by gavage in 10-fold triturate with lactose at doses 0, 1, 3, and 6 mg/kg/day for 12 months. 3 dogs/sex/group (control and HD only) were continued for another 1 month recovery study.

Control	0	7	7
LD	1	4	4
MD	3	4	4
HD	6	7	7

RESULTS:
CLINICAL SIGNS (daily):
No dose-related changes.
MORTALITY:
None.
BODY WEIGHT (every 7 days):
No dose-related changes.
FOOD CONSUMPTION (daily):
No dose-related changes.
AUDITORY TEST (days –7, 91, 175, 266, 360 and 27R):
No abnormal incidence observed.
OPHTHALMOLOGIC EXAMINATION (days –8, 92, 181, 273, 358 and 22R):
Opacity in 2 HDm and 1 LDm.
ELECTROCARDIOGRAPHICAL EXAMINATION (days -13, 84, 176, 265, 356 and 23R):
No dose-related changes.
URINALYSIS (days –20, -5, 87, 178, 269, 358, 7R and 27R):
No dose-related changes.
HEMATOLOGICAL EXAMINATION (days –19, -7, 85, 183, 268, 360, 8R and 26R):
No dose-related changes.
MYELOGRAM (at sacrifice):
No dose-related changes.

BLOOD CHEMISTRY EXAMINATION (days -19, -7, 85, 183, 268, 360, 8R and 26R):

	C	LD	MD	HD	C	LD	MD	HD	C	HD	C	HD
	7	7	7	7	7	7	7	7	3	3	3	3
Cholesterol (mg/dl)	155	144 (93%)	97* (63%)	97* (63%)	185	148 (80%)	139 (75%)	114** (62%)	175	151	158	193
Triglyceride (mg/dl)	31	18* (58%)	17* (55%)	15** (48%)	41	17 (41%)	29 (71%)	15* (37%)	30	30	47	36

( ): %of control; *,**: Statistically significant at $p<0.05$ and $p<0.01$.

LIVER BIOCHEMISTRY (at sacrifice):

No obvious changes in total protein, total cholesterol, phospholipids, triglyceride, and activity of P-450, coumar. Dmase, coumar. Dease, coumar. DPase.

TOXICOKINETICS (Non-GLP, days 0, 184 and 363):

T_{max} ranged from 1.4 hr to 2.4 hr

C_{max} and AUC_{0-24} increased dose-dependently and tended to increase with repeated dosing.

No gender difference.

1	0	1.9	16.6	85.8
1	184	2.4	25.8	154
1	363	2.5	27.1	237
3	0	1.8	61.5	303
3	184	2.0	63.3	446
3	363	2.0	88.3	703
6	0	1.4	267	1230
6	184	1.8	658	2130
6	363	1.7	816	3120

ORGAN WEIGHT:

No treatment-related changes.

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		♂	♀	♂	♀	♂	♀	♂	♀
		4	4	4	4	4	4	4	4
Gallbladder									
Fundic region mucosa: red point	p							1	1
Lamina propria mucosae, hemorrhage	±								1
	+							1	
Liver									
Hepatocytes: atrophy	++							1	

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	TWELVE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN DOGS (B-046-L)							
Animal	Beagle dogs, 6 months old							
Route	Oral gavage							
Dose (mg/kg/day)	0		1		3		6	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	7	7	4	4	4	4	7	7
Mortality	None							
Clinical sign	-							
Body weight	-							
Food consumption	-							
Ophthalmology	-	-	1 opacity	-	-	-	2 opacity	-
Auditory test	-							
Urinalysis	-							
Hematology	-							
Myelogram	-							
Blood chemistry	↓ CHO, ↓ TG							
Liver Biochemistry	-							
Toxicokinetics	T_{max} = 1.4 – 2.4 hr, C_{max} and AUC_{0-24} increased dose-dependently No drug accumulation, No sex difference							
Organ weight	-							
Autopsy	-						Gallbladder: red point in fundic region mucosa	
Histopathology	-						Gallbladder: hemorrhage in lamina propria mucosa Liver: hepatocytes: atrophy	
Reversibility	Good							
Conclusion	NOAEL is 3 mg/kg							

*: sacrificed in extremis. -: No remarkable findings.

Study Title: Six-Month Repeated Oral Toxicity Study Of S-4522 In Monkey
Study No.: F-014-L
Amendment #, Vol #, and Page #: SN000 Vol 14 Page 1 and SN005 Vol 4 Page 1

NOTE: Performed by ——— Study period: 11/93-10/94. Final study report dated October 11, 1994. Lot No. R39001. GLP statements (Japan) provided.

EXPERIMENTAL DESIGN: Cynomolgus monkeys were obtained from ——— Dosing started at 3-6 years of age. 3 monkeys/sex/group were administered S-4522 orally by gavage in 5% gum arabic solution at doses 0, 10, and 30 mg/kg/day for 6 months.

Control	0	3	3
LD	10	3	3
HD	30	3	3

RESULTS:

CLINICAL SIGNS (twice daily):
No dose-related changes.

MORTALITY:
None.

BODY WEIGHT (weekly):
No dose-related changes.

FOOD CONSUMPTION (daily):
No dose-related changes.

OPHTHALMOLOGIC EXAMINATION (weeks -2, 13 and 26):
No dose-related change in visual and slit lamp examination and ocular fundus examination.

URINALYSIS (weeks -3, 1, 4, 13 and 26):
No dose-related changes.

HEMATOLOGICAL EXAMINATION (weeks -3, 1, 4, 13 and 26):
No dose-related changes.

MYELOGRAM (at sacrifice):
No dose-related changes.

BLOOD CHEMISTRY EXAMINATION (weeks -3, 1, 4, 13 and 26):

	C	LD	HD	C	LD	HD
	3	3	3	3	3	3
Cholesterol (mg/dl)	127.4	82.7 (65)	69.7 (55)	128.3	76.0 (59**)	80.3 (63**)
Triglyceride (mg/dl)	33.0	29.7 (90)	19.0 (58)	35.0	27.0 (77)	19.3 (55)

( ): % of control; *,**: Statistically significant at $p<0.05$ and $p<0.01$.

Plasma total cholesterol concentration was decreased in both males and females from week 1 through the end of dosing period.

LIVER BIOCHEMISTRY:
No data.

TOXICOKINETICS (No-GLP Study, days 0, 90 and 181):

10	2.7-6.0	24.8-32.3	313-352	2.3-6.0	21.1-58.7	246-818
30		92.5-336	1300-4180		49.9-77.4	733-1090

ORGAN WEIGHT:

HD: Submandibular gland ↓ 47% in males
Liver ↓ 32% in males
Brain ↑ 16% in females
Spleen ↑ 49% in females

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		♂	♀	♂	♀	♂	♀
		3	3	3	3	3	3
Testis							
Softness, milky white epididymis	+					1	
Decrease in spermatogenic epithelium	+					1	
Giant cell in seminiferous tubule	+					1	
Vacuolation in seminiferous tubular epithelium	+					1	
Pancreas							
Granuloma	+						1
Vacuolation of acinar cell	±						1
	+				2	1	
Liver							
Mononuclear cell infiltration in Glisson's sheath	±						1
	+			1		1	
Adrenal							
Necrosis of parenchyma	+						1
Mononuclear cell infiltration	+						1
Thyroid							
Ectopic thymus	+	1		1		1	1

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

APPEARS THIS WAY
ON ORIGINAL

SUMMARY

Title	SIX-MONTH REPEATED ORAL TOXICITY OF S-4522 IN MONKEYS (F-014-L)					
Animal	Cynomolgus monkeys, 3-6 years old					
Route	Oral gavage					
Dose (mg/kg/day)	0		10		30	
# of animal	♂	♀	♂	♀	♂	♀
	3	3	3	3	3	3
Mortality	None					
Clinical sign	-					
Body weight	-					
Food consumption	-					
Ophthalmology	-					
Auditory test	-					
Urinalysis	-					
Hematology	-					
Myelogram	-					
Blood chemistry	↓ CHO, ↓ TG					
Liver Biochemistry	-					
Toxicokinetics	T_{max} = 2.3-6.0 hr, C_{max} and AUC_{0-24} increased dose-dependently No drug accumulation, No sex difference					
Organ weight	-				↓ liver, ↓ Submandibular gland, ↑ brain, ↑ spleen	
Autopsy	-				Testis: softness, milk white epididymis	
Histopathology	-				Testis: ↓ spermatogenic epithelium, giant cell in seminiferous tubulae, vacuolation in seminiferous tubular epithelium. Adrenal: necrosis of parenchyma, mononuclear cell infiltration	
			Pancreas: granuloma, vacuolation of acinar cell. Liver: mononuclear cell infiltration in Glisson's sheath Thyroid: ectopic thymus			
Reversibility	-					
Conclusion	NOAEL is below 10 mg/kg					

*: sacrificed in extremis. -: No remarkable findings.

APPEARS THIS WAY
ON ORIGINAL

Overall Toxicology Summary Overall Toxicology Summary

Animal	Rat (Jcl:SD)	Dog (Beagle)	Rat (Jcl:SD)	Rat (Jcl:SD)	Rat (Jcl:SD)
Animal #	6/sex/group	1/sex/group	10/sex/group	12/sex/group	20/sex/group
Dose (mg/kg)	1000, 2000	1000, 2000	0,15,50,150	0,10, 30, 100	0,2,6,20
Duration	Single	Single	1 month	3 months	6 months
Route	Oral gavage	Oral gavage	Oral gavage	Oral gavage	Oral gavage
Findings	No changes	Vomiting GOT,GPT, CPK↑ No hist. changes	Liver toxicity in 50, 150 mkd	Death in 100 mkd, liver toxicity in 30 mkd	Liver toxicity in 6,20 mkd
Target organ			Liver	Liver, Forestamach	Liver
Reversible			Yes	Yes	Yes
Conclusion	Lethal single dose > 2000	Lethal single dose > 2000	NOAEL is 15 mkd	NOAEL is 10 mkd	NOAEL is 2 mkd

Animal	Dog (Beagle)	Dog (Beagle)	Dog (Beagle)	Dog (Beagle)	Monkey (Cynomolgus)
Animal #	3/sex/group	3/sex/group	3/sex/group	4/sex/group	3/sex/group
Dose (mg/kg)	0,10,30,90	0,7.5,15,30	1,4	0,1,3,6	0,10,30
Duration	1 month	3 months	6 months	12 months	6 months
Route	Oral gavage	Oral gavage	Oral gavage	Oral gavage	Oral gavage
Findings	Death in 90 mkd, GOT,GPT, ALP↑ in 30 mkd	GOT,GPT, ALP↑ in 30 mkd, gallbladder change in all doses	No changes	Gallbladder changes in 6 mkd	Testis changes in 30 mkd
Target organ	Gallbladder	Gallbladder		Gallbladder	Testis
Reversible	Yes	Yes		Yes	
Conclusion	NOAEL is 10 mkd	No NOAEL established	NOAEL is 4 mkd	NOAEL is 3 mkd	NOAEL is 10 mkd

Histopathology Inventory for IND 56,385

Study	B-17-L	F-09-L	F-13-L	B-20-L	B-21-L	B-37-L	B-46-L	F-14-L
Species	Rat	Rat	Rat	Dog	Dog	Dog	Dog	Monkey
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*
Aorta				X				X
Bone Marrow smear			X	X	X		X	X
Bone (femur)			X					
Brain	X*	X*	X*	X*	X*	X*	X*	X*
Cecum				X	X	X	X	X
Cervix								
Colon				X	X	X	X	X
Duodenum				X	X	X	X	X
Epididymis		X*	X*	X	X	X	X	X*
Esophagus		X		X	X	X	X	X
Eye		X		X	X	X	X	X
Fallopian tube								
Gall bladder				X				X
Gross lesions								
Harderian gland								
Heart	X*	X*	X*		X*	X*	X*	X*
Hypophysis								
Ileum				X	X	X	X	X
Injection site								
Jejunum				X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland								
Larynx								
Liver	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical					X	X	X	X
Lymph nodes mandibular		X	X					X*
Lymph nodes, mesenteric		X	X	X	X	X	X	X
Mammary Gland					X	X	X	X
Nasal cavity								
Optic nerves				X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X		X*	X*	X*	X*
Parathyroid		X	X	X*				
Peripheral nerve								
Pharynx								
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*	X*	X*	X*
Rectum				X				X
Salivary gland	X*	X*	X*	X				
Sciatic nerve				X	X	X	X	X
Seminal vesicles								X*
Skeletal muscle					X	X	X	
Skin				X	X	X	X	X
Spinal cord				X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*	X*
Sternum		X		X	X	X	X	X
Stomach	X	X	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*	X*	X*	X*
Thyroid	X	X	X	X*	X*	X*	X*	X*
Tongue				X	X	X	X	X
Trachea				X	X	X	X	X
Urinary bladder		X	X	X	X	X	X	X*
Uterus	X*	X*	X*	X*	X*	X*	X*	X*
Vagina								X
Zymbal gland								

* organ weight obtained

Carcinogenicity

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals

Application number: IND 56,385
Division: Division of Metabolic & Endocrine Drug Products, HFD-510
CAS#:
Drug name: ZD4522
Pharmacological Classification: HMG CoA reductase inhibitor
Sponsor/Applicant: ZENECA Pharmaceuticals Inc., Wilmington, DE 19850.
Sponsor/Applicant contact name: Linda G. Knapp
Sponsor/Applicant telephone and fax number: (302) 886-2289, (302) 886-2822(fax)
Date submitted: November 30, 1998
45-day date (from submission stamp date): January 14, 1999
P/T Reviewer(s): John Zhaolong Gong
Date Review Completed: January 4, 1999
Date of CAC review: January 12, 1999
CAC members: Joe DeGeorge, Joe Contrera and Al DeFelice

Summary of Proposal for Review

Species/strain:	Mice (B6C3F1)	
Number/sex/dose:	51	
Route:	Orally by gavage	
	Male	**Female**
Doses proposed (mg/kg/day): (Ongoing study 5 months)	0, 0, 10, 60, 200, 400	0, 0, 10, 60, 200, 400

Basis of dose selection:

MTD: 200 mkd causes increase in liver weight (24% in males and 16% in females) and minimal histological changes in liver without significant body weight change.

AUC: In the dose selection document (SN006, page 1), Sponsor claims that 400 mkd is expected to give a *30-fold* margin over human exposure (assuming a mean AUC of 8690 ng.hr/ml in the mouse for the sexes combined at a dose of 400 mg/kg; 290 ng.hr/ml in man at 40 mg based on AUC's of 38, 77, and 135 ng.hr/ml at a single dose of 5, 10 and 20 mg).

In the current status report of the ongoing carcinogenicity study (SN006, page 60), Sponsor claims the dose of 200 mkd provides a *6-fold* margin over the proposed maximum human exposure (assuming a mean AUC of 4350 ng.hr/ml in the mouse for the sexes combined at a dose of 200 mkd and a maximum mean AUC of 700 ng.hr/ml in man).

Kinetics Submitted:	**Mice**	**Human**
Pharmacokinetics	13-weeks	Single dose

Metabolism:	No data	In vitro
Protein binding:	No data	91.1-93.3%

Notable design features: Proposed route is orally by gavage for 80 weeks

Summary of Recommendations to CAC:

	Male	**Female**
Doses in ongoing study:	0, 0, 10, 60, 200	0, 0, 10, 60, 200

Basis for recommendation:

200 mkd appears to be appropriate as the high dose of the carcinogenicity study:

1. The high dose (200 mkd) in the 13-week study produced minimal histological changes in the liver (liver weight ↑ 24% in males and 16% in females, minimal centrilobular hepatocyte hypertrophy) without body weight change.
2. In the carcinogenicity study that Sponsor started 5 months ago on June 10, 1998. No adverse effect was observed in 10, 60 and 200 mkd group. Six animals in the female high dose group (400 mkd) were sacrificed between week 1 and 2. In 5/6 animals, the poor condition was assessed to be due to the drug. Histopathological examination of the sacrificed animals reveals damages consisting of hepatocyte vacuolation with single cell necrosis in liver and squamous cell hyperplasia with or without hyperkeratosis and gastritis in the stomach. In 2 animals, tubular degeneration is also evident in the kidney. Although this toxicity dose not appear to be very severe (the animals were sacrificed), it is occurred so early in the study would suggest that the 400 mkd group would not survive the study. Therefore, 200 mkd appears to be a reasonable high dose.

CAC concurrence and Recommendations:

The committee concurs with 200 mg/kg as the MTD (new high dose) provided that the pathology report can rule out gavage accidents or bleeding accidents as the cause of the deaths at 400 mg/kg.

The committee noted that the standard acceptable duration for a mouse carcinogenicity study is 104 weeks. If excessive deaths occur, it is recommended that the division and CAC be consulted prior early termination of the study.

Comments:

The duration of carcinogenicity study in mice should be 104 weeks. Eighty weeks in this ongoing study is insufficient. We cannot prospectively recommend an 80-week study duration in mice.

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals

Application number: IND 56,385
Division: Division of Metabolic & Endocrine Drug Products, HFD-510
Drug name: ZD4522
Pharmacological Classification: HMG CoA reductase inhibitor
Sponsor/Applicant: ZENECA Pharmaceuticals Inc., Wilmington, DE 19850.
Sponsor/Applicant contact name: Linda G. Knapp
Sponsor/Applicant telephone and fax number: (302) 886-2289, (302) 886-2822(fax)
Date submitted: November 30, 1998
45-day date (from submission stamp date): January 14, 1999
P/T Reviewer(s): John Zhaolong Gong
Date Review Completed: January 4, 1999
Date of CAC review: January 12, 1999
CAC members: Joe DeGeorge, Joe Contrera and Al DeFelice

Summary of proposal for review

Species/strain:	Rats (Sprague-Dawley Crl:SDi	
Number/sex/dose:	50	
Route:	Orally by gavage	
	Male	**Female**
Doses proposed (mg/kg/day): (ongoing study 6 months)	0, 0, 2, 20, 60, 80	0, 0, 2, 20, 60, 80

Basis of dose selection:

MTD: 60 mkd causes minimal histological changes in liver without significant body weight changes and adverse clinical signs.

AUC: In the dose selection document (SN006, page 34), Sponsor claims that 80 mkd is expected to provides a *30-fold* margin over human exposure (assuming AUC of 8960 ng.hr/ml in the rat at a dose of 80 mg/kg; 290 ng.hr/ml in man at 40 mg based on AUC's of 38, 77, and 135 ng.hr/ml at a single dose of 5, 10 and 20 mg).
In the current status report of the ongoing carcinogenicity study (SN006, page 60), Sponsor claims that the dose of 80 mkd provides a *13-fold* margin over the proposed maximum human exposure (assuming a mean AUC of 8960 ng.hr/ml in the rat for the sexes combined at a dose of 80 mkd and a maximum mean AUC of 700 ng.hr/ml in man).

Kinetics Submitted:	**Rats**	**Human**
Pharmacokinetics	3-months	single dose
Metabolism:	In vivo	In vitro
Protein binding:	95.5-96.5%	91.1-93.3%

Notable design features: Proposed route is orally by gavage

Summary of Recommendations to CAC:

	Male	Female
Doses in ongoing study:	0, 0, 2, 20, 60, 80	0, 0, 2, 20, 60, 80

Basis for recommendation:

The 80 mkd might be too low as the high dose of carcinogenicity study.

1. The high dose of the on-going carcinogenicity study failed to induce reasonable toxicity and appears to be below MTD. Sponsor started the carcinogenicity study 6 months ago on May 5, 1998. No adverse effect was observed in 2, 20 and 60 mkd groups in both males and females, and 80 mkd in female. High dose of 80 mkd only causes a 6% reduction of body weight gain in females.
2. The use of 80 mkd as high dose in carcinogenicity study is based on the 3-month study in SD rats. In that study, significant number of animal death occurred within first 3 weeks after dosing, which is not consistent with other studies, raising the problem of credibility of the 3-month study in SD rats.
3. In the 1-month study in SD rats, the high dose of 150 mkd has no effect on body weight, and only produces minimal to moderate liver damage.
4. In the 13-week sighting study in F344 rats, the high dose of 60 mkd has no effect on body weight, and only induces minimal liver damage. The sponsor indicates that PK appears to be similar in the F344 rats and SD rats. However, the dose ranging should have been performed in the appropriate test species.
5. In the experiment design, dose increase at a common ratio of 3 may be more appropriate.

CAC Concurrence and Recommendations:

The committee could not provide concurrence with the doses being evaluated based on the data provided.

The committee recommended that the sponsor conduct a short study at sufficiently high doses to determine the MTD and the proximity of the current doses to the MTD, using the same animal strain and the same diet and mode of administration as the study currently being conducted. This could provide information about the utility of the doses being evaluated in the ongoing study in the event there are no clear findings of toxicity in the carcinogenicity study upon completion. Alternatively, the carcinogenicity study could be determined as adequate if sufficient evidence of toxicity or carcinogenic potential were observed in the completed 2 year study.

Comments:

Regarding the effects of calcium on the results of ZD4522 toxicity, the Sponsor claims that the calcium concentration in the diet has a significant influence on ZD4522 toxicity. The reviewer has a different view on this matter.

Sponsor carried out the following relevant studies.

(A) A 13-week sighting study in F344 rats (6, 20, 60 mkd). In this study, the high dose of 60 mkd has no effect on body weight, and only induces minimal liver damage (bile duct hyperplasia, peribiliary fibrosis, etc).

(B) A 3-month study in SD rats (10, 30, 100 mkd). In this study, the high dose of 100 mkd causes death of 15/18 males and 3/18 female within 3 weeks of dosing. 30 mkd only causes minimal liver damage.

(C) A 1-month study in SD rats (15, 50, 150 mkd). In this study, the high dose of 150 mkd produces minimal to moderate liver damage, but has no effect on body weight. 50 mkd only induces minimal liver damage.

(D) A 8-day study in SD rats (150 mkd) to compare the effects of diets on S-4522 toxicity: Four diets were compared and significant different body weight decrease and histopathological change were observed among different diet treated groups.

Sponsor claims that the different results between studies (B) and (C) are due to the difference in calcium concentration in the diet. High calcium diet (1.80%, CA-1 diet) was used in study (C) and low calcium diet (1.02%, MM-6 diet) was used in study (B). Sponsor proposes that lower calcium increases ZD4522 absorption (Vol. 1, Page 39) and thus increases its toxicity. This hypothesis is not supported by the ongoing carcinogenicity study, which Sponsor started 6 month ago (May 5, 1998). In that study, an even lower calcium diet (0.66%, SQC Rat and Mouse Maintenance Diet No 1, expanded) is being used. No adverse effect has been observed, except a 6% reduction of body weight gain in the females high dose group (80 mkd) after 20 week dosing. Therefore, calcium concentration in diet can not completely explain the high mortality in the 3-month study in SD rats, other factors could also account for the difference observed between (B) and (C). Study (D) compares four diets regarding their influence on S-4522 toxicity. Animals exhibit difference in body weight and organ damage among different diet treated groups. The concentration of Vitamin A, nicotinamide, Ca, P were found different among the diets.

APPEARS THIS WAY
ON ORIGINAL

Pharmacologist's Review of IND 56,385
(SN000 submitted on July 10, 1998, SN005 submitted on October, 12 1998, and SN006 submitted on November 25, 1998)

Drug: ZD4522 (S-4522)

Sponsor: ZENECA Pharmaceuticals Inc., Wilmington, DE 19850.

Reviewer: John Zhaolong Gong, Ph.D., HFD-510

Relevant NDA: Lovastatin (NDA 19,643), Simvastatin (NDA 19,766), Pravastatin (NDA 19,898), Fluvastatin (NDA 20,261), Atorvastatin (NDA 20,702), Cerivastatin (NDA 20, 740)

Drug Class: HMG CoA reductase inhibitor

Indication: primary hypercholesterolemia and mixed dyslipidemias

Clinical Status: Phase II

Summary of Pharmacology:

ZD4522, a novel member of the statin class of lipid lowering agents, is a synthetic 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor. Since HMG CoA reductase is the major rate-controlling step in the pathway for cholesterol synthesis, statins have proven to be clinically effective in the reduction of plasma levels of LDL and VLDL and are marketed world-wide for the lowering of total cholesterol/LDL-cholesterol levels. First generation statins (lovastatin, pravastatin and simvastatin) are prodrug derivatives of fungal metabolites, whereas ZD4522 is structurally similar to the synthetic second generation statins (super statins, such as, atorvastatin, fluvastatin and cerivastatin).

Reviews Contents:

1. One-month repeated oral toxicity of S-4522 in rats
2. Three-month repeated oral dose toxicity of S-4522 in rats
3. Six-month repeated oral dose toxicity of S-4522 in rats
4. Toxicity study in mice by repeated oral administration for 2 weeks
5. Preliminary carcinogenicity study:
   Toxicity to mice by repeated oral administration for 13 weeks
6. Preliminary carcinogenicity study:
   Toxicity to rats by repeated oral administration for 13 weeks
7. Supplement toxicity studies of S-4522 in rats:
   Toxicological characterization of effective compounds
8. Ongoing two year oncogenicity study in mice
9. Ongoing two year oncogenicity study in rats
10. Communication to Sponsor

REVIEW OF STUDY B-017-L (SN000 Vol 4 Page 1):
ONE-MONTH REPEATED ORAL TOXICITY OF S-4522 IN RATS
Note: This study was reviewed under the Toxicology section of this review.

REVIEW OF STUDY F-009-L (SN000 Vol 5 Page 1 and SN005 Vol 2 Page 1):
THREE-MONTH REPEATED ORAL DOSE TOXICITY OF S-4522 IN RATS
Note: This study was reviewed under the Toxicology section of this review.

REVIEW OF STUDY F13-L (SN000 Vol 7 Page 1):
SIX-MONTH REPEATED ORAL DOSE TOXICITY OF S-4522 IN RATS
Note: This study was reviewed under the Toxicology section of this review.

REVIEW OF STUDY SGI143943218 (SN000 Vol 15 Page 1 and SN005 Vol 5 Page 1):
TOXICITY STUDY IN MICE BY REPEATED ORAL ADMINISTRATION FOR 2 WEEKS

NOTE: Performed by [redacted] Study period: 9/94-12/94. Final study report dated April 11, 1995. Lot No. R39001. GLP statements provided.

EXPERIMENTAL DESIGN: Mice ($B_6C_3F_1$) were obtained from [redacted]
Dosing started at 39-41 days of age. 6 mice/sex/group were administered S-4522 orally by gavage in 5% gum arabic solution at doses 0, 20, 60 and 200 mg/kg/day for 17 days.

Group	Dose (mg/kg)	♂	♀
Control	0	6	6
LD	20	6	6
MD	60	6	6
HD	200	6	6

RESULTS:

CLINICAL SIGNS (daily):
No dose-related changes.

MORTALITY:
3 male death due to overexposure to ether anaesthetic.

BODY WEIGHT (twice a week):
No dose-related changes.

FOOD CONSUMPTION (weekly):
No dose-related changes.

HEMATOLOGICAL EXAMINATION (at sacrifice):
No dose-related changes.

ORGAN WEIGHT:

HD: Liver ↑ 18% in males, ↑ 11% in females.

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		0		LD		MD		HD	
		♂	♀	♂	♀	♂	♀	♂	♀
		6	6	6	6	6	6	6	6
Liver									
Hepatocyte hypertrophy - centrilobular	±							5	3

–Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	TOXICITY STUDY IN MICE BY REPEATED ORAL ADMINISTRATION FOR 2 WEEKS (SGI143943218)							
Animal	Mice ($B_6C_3F_1$), 39-41 days old							
Route	Oral gavage							
Dose (mg/kg/day)	0		20		60		200	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	6	6	6	6	6	6	6	6
Mortality	3 male death due to overexposure to ether anaesthetic							
Clinical sign	-							
Body weight	-							
Food consumption	-							
Ophthalmology								
Auditory test								
Urinalysis								
Hematology	-							
Myelogram								
Blood chemistry								
Liver Biochemistry								
Toxicokinetics								
Organ weight	-						liver ↑ 18% in males, ↑ 11% in females	
Autopsy	-							
Histopathology	-						Liver: Hepatocyte hypertrophy – centrilobular	
Reversibility								
Conclusion	NOAEL is 60 mg/kg							

*: sacrificed in extremis. -: No remarkable findings.

REVIEW OF STUDY SGI144952323 (F-015-L) (SN000 Vol 15 Pg 128 and SN016 Vol 1 Pg 1):
PRELIMINARY CARCINOGENICITY STUDY: TOXICITY TO MICE BY REPEATED ORAL ADMINISTRATION FOR 13 WEEKS

NOTE: Performed by [redacted]. Study period: 3/95-11/95. Final study report dated November 1, 1995. Lot No. R39001. GLP statements provided.

EXPERIMENTAL DESIGN: Mice ($B_6C_3F_1$) were obtained from ———. Dosing started at 41-43 days of age. 10 mice/sex/group were administered S-4522 orally by gavage in 5% gum arabic solution at doses 0, 20, 60 and 200 mg/kg/day for 13 weeks. Another 36 mice/group/sex were used for toxicokinetic study on day 1 and week 13.

Group	Dose (mg/kg)	Main		Satellite	
		♂	♀	♂	♀
Control	0	10	10		
LD	20	10	10	36	36
MD	60	10	10	36	36
HD	200	10	10	36	36

RESULTS:

CLINICAL SIGNS (daily):
No dose-related changes.

MORTALITY:
2 death not related to treatment.

BODY WEIGHT (weekly):
No dose-related changes.

FOOD CONSUMPTION (weekly):
No dose-related changes.

HEMATOLOGICAL EXAMINATION (at sacrifice):
No dose-related changes.

TOXICOKINETICS (day 1 and week 13, from SN006, Page 2):

Dose (mg/kg)	Sex	Day 1			Day 89		
		T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-12} (ng.hr/ml)	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-12} (ng.hr/ml)
20	♂	8	25.7	213	0.5	57.1	145
	♀	0.5	195	550	0.5	114	322
60	♂	0.5	1230	1480	0.5	494	722
	♀	0.5	1930	2870	0.5	393	969
200	♂	0.5	13400	19000	0.5	2520	3080
	♀	0.5	7060	19700	0.5	2860	5610

ORGAN WEIGHT:
HD: Liver ↑ 22% in males, ↑ 15% in females.
MD: Liver ↑ 10% in females

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		0		LD		MD		HD	
		♂	♀	♂	♀	♂	♀	♂	♀
		6	6	6	6	6	6	6	6
Liver									
Hepatocyte enlargement - centrilobular	±	0		1		6		10	7

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	PRELIMINARY CARCINOGENICITY STUDY: TOXICITY TO MICE BY REPEATED ORAL ADMINISTRATION FOR 13 WEEKS (SGI144952323, F-0150L)							
Animal	Mice ($B_6C_3F_1$), 41-43 days old							
Route	Oral gavage							
Dose (mg/kg/day)	0		20		60		200	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	10	10	10	10	10	10	10	10
Mortality	2 death not related to treatment							
Clinical sign	-							
Body weight	-							
Food consumption	-							
Ophthalmology								
Auditory test								
Urinalysis								
Hematology	-							
Myelogram								
Blood chemistry								
Liver Biochemistry								
Toxicokinetics								
Organ weight - Liver	-				↑ 22% in ♂		↑ 22% in ♂, ↑ 15% in ♀	
Autopsy	-							
Histopathology	-				Liver: Hepatocyte hypertrophy – centrilobular			
Reversibility								
Conclusion	NOAEL is 20 mg/kg							

*: sacrificed in extremis. -: No remarkable findings.

REVIEW OF STUDY SGI145951544 (F-016-L) (SN000 Vol 16 Pg 1 and SN016 Vol 2 Pg 1):
PRELIMINARY CARCINOGENICITY STUDY: TOXICITY TO RATS BY REPEATED ORAL ADMINISTRATION FOR 13 WEEKS

NOTE: Performed by ——— Study period: 9/94-8/95. Final study report dated August 30, 1995. Lot No. R39001. GLP statements provided.

EXPERIMENTAL DESIGN: Rats (F-344, DF Crl BR) were obtained from ——— ——— Dosing started at 42-44 days of age. 10 rats/sex/group were administered S-4522 orally by gavage in 5% gum arabic solution at doses 0, 6, 20, and 60 mg/kg/day for 13 weeks.

Another 12 rats/group/sex were used for toxicokinetic study on day 1 and week 13. SDS Rat and Mouse No. 1 maintenance diet and tap water were provided *ad libitum*.

Group	Dose (mg/kg)	Main		Satellite	
		♂	♀	♂	♀
Control	0	10	10		
LD	6	10	10	12	12
MD	20	10	10	12	12
HD	60	10	10	12	12

RESULTS:

CLINICAL SIGNS (daily):

No dose-related changes.

MORTALITY:

2 death due to excess of anaesthetic.

BODY WEIGHT (weekly):

No dose-related changes.

FOOD CONSUMPTION (weekly):

No dose-related changes.

OPHTHALMOLOGIC EXAMINATION (pre-dosing and week 13):

No treatment related changes.

HEMATOLOGICAL EXAMINATION (at sacrifice):

Platelet: HDm ↓ 12%, HDf ↓ 10%, MDf ↓ 14%.

BLOOD CHEMISTRY EXAMINATION (at sacrifice):

Group	GPT		GOT		ALP		CPK		Cholesterol	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control										
LD	117**		135*				124		117	
MD	146**		153**		113**	117	117	118		
HD	151**	144**	180**	144**	131**	135**	130	134	116**	

Values are % of control; *,**: Statistically significant at $p<0.05$ and $p<0.01$.

TOXICOKINETICS (day 1 and week 13, from SN006, Page 34):

Dose (mg/kg)	Day 1			Day 90		
	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-12} (ng.hr/ml)	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-12} (ng.hr/ml)
6	0.5	19	122	0.5	30.4	240
20	0.5	82.2	340	0.5	389	1130
60	0.5	381	1350	0.5	3490	6720

ORGAN WEIGHT:

Group	Liver		Spleen	
Sex	♂	♀	♂	♀
Control				
LD	90*		110*	113*
MD	95*		107*	115**
HD	91**		117**	115**

Values are % of control; *,**: Statistically significant at $p<0.05$ and $p<0.01$.

GROSS PATHOLOGY AND HISTOPATHOLOGY:

		0		LD		MD		HD	
		♂	♀	♂	♀	♂	♀	♂	♀
		10	10	10	10	10	10	10	10
Liver									
Bile duct hyperplasia	±					8	5	5	
	+							5	9
Peribiliary fibrosis	±							4	
	+							5	9
Basophilic hepatocytes - focal	±				3	4	7	2	5
	+							3	4
	++							1	
Hepatocyte hypertrophy – periportal	±							7	4
Hepatocyte hypertrophy – generalized	±					5	6		
	+							1	3
Hepatocyte mitoses	±					5	3	4	5
	+							1	
Hepatocyte necrosis - centrilobular	±							4	2
Granulomatous inflammation	±				3		4	2	1
	+						1	2	4
	++							1	1
Pericholangitis	±								1
	+							1	1

Empty cell: no change; ±: very slight; +: slight; ++:moderate; p: present

SUMMARY

Title	PRELIMINARY CARCINOGENICITY STUDY: TOXICITY TO RATS BY REPEATED ORAL ADMINISTRATION FOR 13 WEEKS (SGI145951544, F-016-L)							
Animal	Rats (F344), 42-43 days old							
Route	Oral gavage							
Dose (mg/kg/day)	0		6		20		60	
# of animal	♂	♀	♂	♀	♂	♀	♂	♀
	10	10	10	10	10	10	10	10
Mortality	2 death not related to treatment							
Clinical sign	-							
Body weight	-							
Food consumption	-							
Ophthalmology	-							
Auditory test								
Urinalysis								
Hematology: Platelet					↓ 14%		↓ 12%	↓ 10%
Myelogram								
Blood chemistry			GPT ↑ 17-51%, GOT ↑ 35-80%, ALP ↑113-135%, CPK ↑ 17-34%					
Liver Biochemistry								
Toxicokinetics								
Organ weight			Liver ↓ 5-10%, Spleen ↑ 7-17%					
Autopsy	-							
Histopathology	-				Liver/bile duct: hypertrophy			
Reversibility								
Conclusion	No NOAEL detected							

*: sacrificed in extremis. -: No remarkable findings.

RÉVIEW OF STUDY A1-01-01 (sub-report, SN005 Vol 5 Page 146):
SUPPLEMENT TOXICITY STUDIES OF S-4522 IN RATS:
TOXICOLOGICAL CHARACTERIZATION OF EFFECTIVE COMPOUNDS

NOTE: This study was reviewed under the Special Toxicology section of this review

REVIEW OF ONGOING STUDY TCM/1088 (SN006 Page 1):
ZD4522: TWO YEAR ONCOGENICITY STUDY IN MICE

NOTE: Performed by [redacted]. Study started on June 3, 1998. Report submitted on November 30, 1998. GLP statements provided.

EXPERIMENTAL DESIGN: Mice (B6C3F1 strain) were obtained from [redacted] Dosing started at 7 weeks of age. S-4522 was administered orally by gavage in 5% aqueous solution of gum arabic for two years. Satellite mice were used for toxicokinetic study. SQC Rat and Mouse Maintenance Diet No. 1 and tap water were provided *ad libitum*.

Group	Dose (mg/kg)	Main		Satellite	
		♂	♀	♂	♀
Control	0	51	51	9	9
LD	10	51	51	24	24
LMD	60	51	51	24	24
MD	200	51	51	24	24
HD	400*	51	51	24	24
Control	0	51	51	0	0

*: 400 mkd group was terminated on week 2 due to "extreme general toxicity".

RESULTS:

CLINICAL SIGNS (daily):
At week 16, LD, LMD, and MD group show no adverse clinical signs. HD male group showed signs of "extreme general toxicity" during the first two weeks.

MORTALITY:
HD males: 12/51 animals died or were sacrificed in extremis.

BODY WEIGHT (day 0, once a week for 16 weeks, once every 4 week thereafter):
No treatment-related changes.

FOOD CONSUMPTION (weekly for 16 weeks and once every 4 week thereafter):
No treatment-related changes.

WATER CONSUMPTION (1 week during 12, 15 and 18 months):
No data.

OPHTHALMOLOGIC EXAMINATION (day 0 and 6, 12 and 18 months):
No data.

TOXICOKINETICS (week 52):
No data.

HEMATOLOGICAL EXAMINATION (at necropsy):
No data.

ORGAN WEIGHT (at necropsy):

No data.

GROSS PATHOLOGY AND HISTOPATHOLOGY (at necropsy):

HD males: histopathological examination of 6 decedents revealed hepatocyte vacuolation with single cell necrosis in the liver and squamous cell hyperplasia with/without hyperkeratosis and gastritis in the stomach. In two of the animals tubular degeneration was also evident in the kidney.

SUMMARY

Title	ZD4522: TWO YEAR ONCOGENICITY STUDY IN MICE (TCM/1088)											
Animal	Mice (B6C3F1 strain), 7 weeks old.											
Route	Oral gavage											
Dose	0		0		10		60		200		400	
Sex and # of animal	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	51	51	51	51	51	51	51	51	51	51	51	51
Mortality	-										12	-
Clinical sign	-											
Body weight	-											
Food	-											
Organ weight												
Autopsy												
Histopathology											Liver, Stomach Damage	
Conclusion												

-: No remarkable findings. Empty cell: no data.

REVIEW OF ONGOING STUDY TCR/2852 (SN006 Page 33):
ZD4522: TWO YEAR ONCOGENICITY STUDY IN RATS

NOTE: Performed by _____ Study started on May 5, 1998. Report submitted on November 30, 1998. GLP statements provided.

EXPERIMENTAL DESIGN: Rats (SD, Crl:SDBR strain) were obtained from _____ Dosing started at 6.5 weeks of age. S-4522 was administered orally by gavage in 5% aqueous solution of gum arabic for two years. SQC Rat and Mouse Maintenance Diet No. 1 and tap water were provided *ad libitum*.

Group	Dose (mg/kg)	♂	♀
Control	0	50	50
LD	2	50	50
LMD	20	50	50
MD	60	50	50
HD	80	50	50
Control	0	50	50

RESULTS:

CLINICAL SIGNS (daily):
No treatment-related changes.

MORTALITY:
None.

BODY WEIGHT (day 0, once a week for 16 weeks, once every 4 week thereafter):
HD female group has a 6% reduction in body weight gain after 6 months of dosing.

FOOD CONSUMPTION (weekly for 16 weeks and once every 4 week thereafter):
No treatment-related changes.

WATER CONSUMPTION (1 week during 12, 15 and 18 months):
No data.

OPHTHALMOLOGIC EXAMINATION (day 0 and 6, 12, 18 and 24 months):
No data.

TOXICOKINETICS (week 1 and 12):
No data.

HEMATOLOGICAL EXAMINATION (at necropsy):
No data.

ORGAN WEIGHT (at necropsy):
No data.

GROSS PATHOLOGY AND HISTOPATHOLOGY (at necropsy):
No data.

SUMMARY

Title	ZD4522: TWO YEAR ONCOGENICITY STUDY IN RATS (TCR/2852)											
Animal	SD Rats (Crl:SD: train), 6.5 weeks old.											
Route	Oral gavage											
Dose (mg/kg/day)	0		0		2		20		60		80	
Sex and # of animal	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	50	50	50	50	50	50	50	50	50	50	50	50
Mortality												
Clinical sign	-											
Body weight gain	-										-	↓6%
Food consumption	-											
Organ weight												
Autopsy												
Histopathology												
Conclusion												

-: No remarkable findings. Empty cell: no data.

COMMUNICATION TO SPONSOR

Executive CAC meeting was held on January 26, 1999. The minutes of this meeting are attached.

The division agrees to the recommendations of the Executive CAC.

REPRODUCTIVE TOXICOLOGY

Study title: Study on oral administration of S-4522 prior to and in the early stages of pregnancy in rats
Submission: SN000 Vol 18 Page 1 and SN005 Vol 6 Page 1
Study No: S-4522-F-003-L (Contract No.
Study period: April 21, 1992 to May 20, 1993
Site and testing facility:
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: Lot No. 54
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Sprague-Dawley rats (Jcl:SD, SPF)
- Doses employed: 0, 5, 15 and 50 mg/kg/day
- Route of Administration: oral gavage
- Study Design: Males were treated for 9 weeks from the age of 6 weeks and further treated through the mating period until one day before autopsy. Females were treated for 2 weeks from the age of 9 weeks and further treated through the mating period until 7 days after the establishment of successful copulation. Males were sacrificed at age of 16-17 weeks. The dams were cesarean-sectioned on day 20 of pregnancy.
- Number of animals/sex/dosing group: 24
- Parameters and endpoints evaluated: Clinical signs of toxicity, survival and death, body weight, food consumption, estrus cycle, organ weight, histopathologic examination, fetus viability, sex ratio, body weight, external and visceral abnormality, skeletal abnormality and ossification.
- Statistical evaluations: Treated and control groups were statistically compared at the 5% and 1% levels of significance. For the fetal data, the litter was used as a unit in the analysis.

Results:

- Clinical signs: No treatment-related changes
- Mortality: None
- Body weight:
  Male: No treatment-related changes
  Female: Body weight gain was suppressed in the 50 mkd group during premating and pregnancy periods (↓ 4%).
- Food consumption:
  Male: No treatment-related changes
  Female: No consistent changes.
- Fertility in Males
  - In-life observations:
  - Terminal and Necroscopic evaluations:

- Spleen: 1 animal in 50mkd group exhibited hypertrophy and leukodermia, multiple necrosis with inflammatory cell infiltration.
  Kidney: weights decreased in all treated groups, with no histopathologic changes observed.

- Fertility and Early Embryonic Development in Females
  - In-life observations:
    Estrus cycles:

# of animals	24	24	24	24
# of estrus stage for 15 days	3.6	3.7	3.4	3.3
Period of estrus cycle (days)	4.4	4.1	4.1	4.3

Mating findings:

# of mated animals	24	24	24	24
# of copulated animals	24	24	24	23
# of pregnant animals	24	21	24	22

  - Terminal and Necroscopic evaluations:

# of animals	24	21	24	23
Autopsy	No abnormal changes			
Organ weight	Liver, ovary relative wt ↑ in 50 mkd group			
# of corpora lutea/dam	16.5	16.8	17.5	17.4
# of implantations/dam	15.9	15.9	15.5	16.8
Implantation ratio (%)	96.2	94.6	88.6*	96.3
# of resorptions/dam	0.75	1.05	1.21	0.78
Placental weight(g)	0.46	0.45	0.46	0.44

* significantly different from control $p<0.05$.

- Embryo-fetal Development
  - In-life observations:
  - Terminal and Necroscopic evaluations:
    - Dams:
    - Offspring:

# of live fetus/dam	15.2	14.9	14.3	16.0
Viability (%)	95.3	93.4	92.2	95.3
Sex ratio (♂/♀)	0.9	0.8	1.2	0.8
Body weight (g): ♂	3.62	3.55	3.51	3.50
Body weight (g): ♀	3.41	3.35	3.38	3.28*
External abnormality (%)	1/364 (0.3)	1/312 (0.3)	0	0
Short body, anury	1			
Short body, vestigial tail		1		
Visceral abnormality (%)	14/119 (11.8)	8/104 (7.7)	14/115 (12.2)	17/123 (13.8)

Tortuosity of ureter	5	3	11	9
Thymic remnant in neck	3	2	1	1
Supernumerary coronary orifice	1	1	0	3
Ventricular septal defect	5	0	0	0
Left umbilical artery	0	0	2	2
Skeletal abnormality (%)	1/244 (0.4)	0	1/229 (0.4)	0
Defect of thoracic vertebral arch	1			
Hypoplasia of thoracic vertebral body			1	
Skeletal variation (%)	51 (20.9)	51 (24.6)	52 (22.7)	47 (19.2)
Lumbar rib	40	39	37	38
Splitting of thoracic vertebral body	3	3	5	2
Asymmetry of sternebra	3	2	1	1
7 lumbar vertebrae	1	1	0	0
Lumbarization	1	0	1	1
Asymmetry of rib	1	0	0	0
14 ribs	0	1	1	0
Splitting of sternebra	0	0	2	1
Cervical rib	0	0	0	1
Lumbarization, lumbar rib	1	1	3	2
Splitting of thoracic vertebral body, lumbar rib	1	1	1	0
7 lumbar vertebrae, lumbar rib	0	1	1	0
Lumbar rib, asymmetry of sternebra	0	2	0	0
Splitting of thoracic vertebral body, asymmetry of rib	0	0	0	1
Ossification (# of ossification, mean)				
Phalanx of forelimb: Distal	10	10	9.58	10
Middle	0	0	0	0
Proximal	0.91	0.80	1.02	0.47
Metacarpus	7.27	6.99	7.03	7.07
Sternebra	5.56	5.38	5.12*	5.28*
Sacrococcygeal vertebra	7.83	7.63	7.49	7.60*

* significantly different from control $p<0.05$.

Summary and Evaluation:

S-4522 was repeatedly administered orally at doses of 5, 15 and 50 mkd to groups of 24 rats/sex prior to and in the early stages of pregnancy. Its effects on parental animals and fetuses were evaluated.

- General toxicity: No toxic symptoms nor death occurred. 50 mkd female group exhibited persistent suppression of body weight gain and sporadic decreases in the food consumption.
- Reproductive toxicity: No effect on estrus cycle, copulation, male/female fertility, ovulation, implantation and maintenance of pregnancy.
- Fetus development: No embryo-fetolethal and teratogenic effect. Slight suppression of fetal body weight and slightly retarded ossification was observed in 50 mkd group.
- Dose selection: Preliminary study was conducted at doses of 450, 150, 50 and 15 mkd for 14 days. Death occurred at 450 and 150 mkd groups. Body weight gain suppression and organ weight changes were observed in 50 mkd group, thus 50 mg/kg was set as the

high dose. In the present study, high dose (50mkd) induced minimal general toxicity in dams and slight suppression in fetal development. The AUC at similar dose in rats (on day 1 of 60 mkd) was 1350 ng.hr/ml, that is about 4 times the AUC at the maximum proposed human dose of 80 mg. Therefore, 50 mkd appears to be acceptable as the high dose.

- Conclusion: NOAEL for parental animals and for embryos/fetuses is 15 mg/kg/day.

Study title: Study on oral administration of S-4522 during the period of organogenesis in rats
Submission: SN000 Vol 19 Page 1 and SN005 Vol 7 Page 1
Study No: S-4522-F-011-L (Contract No [redacted])
Study period: January 6, 1994 to February 16, 1995
Site and testing facility:
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: Lot No. 56
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Sprague-Dawley rats (Jcl:SD, SPF)
- Doses employed: 0, 25, 50 and 100 mg/kg/day
- Route of Administration: oral gavage
- Study Design: Successfully copulated females were treated orally with S-4522 at 0, 25, 50 and 100 mkd for 11 days between pregnancy days 7 and 17. About 2/3 Dams were cesarean-sectioned on day 20 of pregnancy, and remaining 1/3 dams were allowed to go through spontaneous delivery. The pups were weaned on day 21 postpartum.
- Number of animals/dosing group: 36
- Parameters and endpoints evaluated:
  Dams (F_0): Clinical signs of toxicity, survival and death, body weight, food consumption, pregnancy length, gestation index (# females delivered live pups/# pregnant females), organ weight, number of corpora lutea and number of implantations, implantation index (# implantation/# corpora lutea), number of implantation sites, birth index (# live pups/# implantation sites).
  Fetuses: number of embryonic/fetal deaths, number of live fetuses, embryonic/fetal mortality index (# embryonic/fetal deaths/# implantations), sex, body weight, placental weight, external abnormality, internal abnormality, skeletal abnormality/variation, ossification.
  Preweaning observation of offspring (F_1): number of liveborn and stillbirth pups, sex, body weight, external appearance, stillbirth index (# stillbirth pups/# pups delivered), clinical signs of toxicity, survival/mortality, viability index (# lives on day 4 after birth/# liveborn pups), weaning index (# pups alive at weaning/# liveborn pups), physical development (appearance of pinna detachment, hair growth, eruption of the lower incisors, opening of the eyelids, functional development (righting reflex, pain response, traction strength, Preyer's reflex, hindleg withdrawal reflex, visual placing reflex).

Postweaning observation of offspring (F_1): at weaning (day 21 after birth), all F1 offspring excepting 2 pups/sex from each dam were sacrificed for histopathologic evaluation. The weanlings were observed for general toxicity and survival/mortality, sexual maturation (testicular descent or vaginal opening), body weight, behavioral and learning tests (open-field test, rotarod performance test, water multiple T-maze test), fertility (F_1 males and females were mated at the age of 11 weeks, copulation index, male fertility index, female fertility index, histopathologic examination, implantation index, embryonic/fetal mortality index, F_2 fetuses: sex, body weight, placental weight, external abnormality).

- Statistical evaluations: Treated and control groups were statistically compared at the 5% and 1% levels of significance. For the fetal data, the litter was used as a unit in the analysis.

Results:

- Clinical signs: No treatment-related changes
- Mortality: 1 death in 100 mkd group due to dosing error
- Body weight: No treatment-related changes
- Food consumption: No treatment-related changes
- Embryo-fetal Development
  - In-life observations:
  - Terminal and Necroscopic evaluations:
    - Dams:

# of animals	23	23	24	22
Autopsy	No abnormal changes			
Organ weight	Liver wt ↑ in 50 and 100 mkd groups			
# of corpora lutea/dam	16.6	16.8	17.7	17.6
# of implantations/dam	15.3	15.8	16.0	16.6
Implantation ratio (%)	90.3	94.3	90.4	94.1
# of resorptions/dam	1.2	1.2	1.6	1.3
Placental weight(g)	0.45	0.43	0.47	0.46

* significantly different from control $p<0.05$.

- Offspring:

# of live fetus/dam	14.1	14.6	14.3	15.3
Viability (%)	92.7	92.3	89.0	92.5
Sex ratio (♂/♀)	0.9	1.0	0.9	0.9
Body weight (g): ♂	3.52	3.55	3.60	3.51
♀	3.37	3.37	3.41	3.33
External abnormality (%)	2/324 (0.6)	0/335	0/344	0/337
Visceral abnormality (%)	11/108 (10.2)	20/112 (17.9)	21/119 (17.6)	18/115 (15.7)
Tortuosity of ureter	7	7	12	4
Thymic remnant in neck	1	4	3	8
Supernumerary coronary orifice	0	1	0	1

Ventricular septal defect	2	6	3	2
Left umbilical artery	0	1	1	2
Skeletal abnormality (%)	0	0	0	0
Skeletal hypoplasia	1	0	0	0
Skeletal variation (%)	78/214 (36.4)	78/223 (35.0)	68/225 (30.2)	67/222 (30.2)
Lumbar rib	65	64	61	49
Splitting of thoracic vertebral body	4	3	2	5
Asymmetry of sternebra	0	0	0	2
7 lumbar vertebrae				
Lumbarization	2	0	0	0
Asymmetry of rib	0	0	1	0
14 ribs	4	7	1	2
Splitting of sternebra	0	2	2	1
Cervical rib	0	0	0	1
Lumbarization, lumbar rib	0	1	0	2
Splitting of thoracic vertebral body, lumbar rib	1	1	0	3
Lumbar rib, splitting of sternebra	1	0	1	0
Lumbar rib, asymmetry of sternebra	0	0	0	2
Lumbarization, splitting of sternebra	1	0	0	0
Ossification (# of ossification, mean)				
Phalanx of forelimb: Distal	9.95	9.83	9.97	9.95
Middle	0	0	0	0
Proximal	0.44	0.33	0.58	0.60
Metacarpus	6.85	6.94	6.97	6.77
Sternebra	5.60	5.57	5.56	5.41
Sacrococcygeal vertebra	7.82	7.73	7.87	7.59

* significantly different from control $p<0.05$.

- Prenatal and postnatal development, including maternal function)
  - In-life observations:
    - Dams:

# of pregnant animals	12	12	12	12
Gestation period (days)	21.9	22.1	22.0	22.0
# of implantations/dam	17.0	15.3	15.8	15.7
# of live pups at birth/dam	15.1	13.8	14.1	13.8
Sex ratio	1.1	1.1	0.9	1.0
# of pups with external abnormality (%)	1 (0.6)	0	1 (0.6)	0

* Significantly different from control $p<0.05$.

    - Offspring:

# live pups at birth: ♂	86	86	80	82
♀	80	77	89	83
Viability at day 4 (%): ♂	100	98.9	100	97.6
♀	100	97.5	100	100

Viability at day 21 (%): ♂	97.7	100	100	100
♀	100	100	100	100
Body weight at birth (g): ♂	6.13	6.35	6.56	6.28
♀	5.71	6.00	6.18	5.99
Body weight at day 4 (g): ♂	9.35	10.55	10.41	10.34
♀	9.06	10.10	9.83	9.88
Body weight at day 21 (g): ♂	61.15	61.88	61.58	60.93
♀	59.04	60.14	59.15	59.01
Body weight at day 77 (g): ♂	437.2	438.3	451.0	454.1
♀	250.6	256.2	261.5	250.7
Separation of auricles (day 4) (%)	100	100	100	100
Emergence of abdominal hair (day 14) (%)	100	100	99	100
Eruption of lower incisors (day 14) (%)	100	100	100	100
Separation of eyelids (day 17) (%)	100	100	100	97.9
Descent of testes (days)	21.2	21.3	21.5	21.2
Opening of vagina (days)	32.0	31.9	31.7	32.0
# offspring examined	87	96	96	96
Right reflex	0	0	0	0
Pain response	0	0	0	0
Traction test	0	0	0	0
Preyer's reflex	0	0	0	0
Flexor response	0	0	0	0
Visual placing response	0	0	0	0
Open field test: latency time, ambulation, rearing, preening, grooming, defecation, urination	No significant difference observed			
Rotarod performance test: # falls	No significant difference observed			
Water multiple T-maze test: Time to reach the goal, # errors	No significant difference observed			
# copulated pairs	11/11	12/12	11/12	12/12
# pregnant pairs	10/11	11/12	11/11	10/12
# corpora lutea/dam	15.7	16.6	17.0	14.4
# implantations/dam	14.7	15.2	16.0	13.0
Implantation ratio (%)	93.6	91.3	94.1	90.3
Fetal death (%)	6.8	7.8	5.7	3.8
# live fetus/dam	13.7	14.0	15.1	12.5
Sex ratio	1.0	1.0	1.0	1.1
Body weight: ♂	3.5	3.3	3.6	3.5
♀	3.0	3.2	3.4	3.3
Placental weight (g)	0.43	0.45	0.46	0.48
External findings: abnormality	0	1	0	0

* Significantly different from control $p<0.05$.