

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-145

PHARMACOLOGY REVIEW



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-145
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PRODUCT: ISENTRESS™
INTENDED CLINICAL POPULATION: HIV-infected patients
SPONSOR: Merck & Co., Inc
DOCUMENTS REVIEWED: Electronic
REVIEW DIVISION: Division of Antiviral Products (HFD-530)
PHARM/TOX REVIEWER: Ita Yuen, PhD
PHARM/TOX SUPERVISOR: Hanan Ghantous, PhD, DABT
DIVISION DIRECTOR: Debra Birnkrant, MD
PROJECT MANAGER: Monica Zeballos, PharmD

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Yes

B. Recommendation for nonclinical studies

Dosing in the two-year carcinogenicity studies in rats and mice are ongoing and will continue until 4th quarter of 2007. The final study reports are anticipated to become available by the 3rd quarter of 2008. Since the present NDA submission is for the accelerated approval, the studies included in this NDA are adequate for nonclinical safety evaluation. The results of the carcinogenicity studies should be available by the time traditional approval is sought.

C. Recommendations on labeling

The language included in the sponsor's labeling for the "Carcinogenesis, Mutagenesis, Impairment of Fertility", "Pregnancy Category C", "Labor and Delivery", and "Nursing Mothers" sections has been changed and the label currently reads as follows:

8.1 Pregnancy





8.3 Nursing Mothers



8.4 Pediatric Use

Safety and effectiveness in pediatric patients less than 16 years of age have not been established.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term (2-year) carcinogenicity studies of raltegravir in rodents are ongoing.

No evidence of mutagenicity or genotoxicity was observed in *in vitro* microbial mutagenesis (Ames) tests, *in vitro* alkaline elution assays for DNA breakage, and *in vitro* and *in vivo* chromosomal aberration studies.

No effect on fertility was seen in male and female rats at doses up to 600 mg/kg/day which resulted in a 3-fold exposure above the exposure at the recommended human dose.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The safety profile of raltegravir has been extensively characterized in rats, mice, rabbits, and dogs. The absorption, distribution, metabolism, and excretion (ADME) profiles of raltegravir in these species are similar to that in humans which made them appropriate animal models for the nonclinical safety evaluation. The toxicological, genotoxic, allergenic, immunologic, and reproductive and developmental toxicological potentials as well as raltegravir's effects on cardiovascular, neurological, respiratory, gastrointestinal, renal, and other systems were evaluated. The assessment of carcinogenic potential for raltegravir is ongoing in rats and mice. The dosing phase is expected to end at the 4th quarter of 2007. All of the pivotal toxicology studies employed adequate range of doses that were administered via clinical route of administration (oral) and produced sufficient systemic exposures and safety margins over that at clinical dose of 400 mg BID. Raltegravir was found to readily cross blood-brain and blood-placental barriers and was secreted in milk. Doses used in a myriad of general toxicology studies ranged from 5 to 1000 mg/kg/day. The highest doses investigated following chronic oral administration of raltegravir were 360 mg/kg/day in dogs (12 month administration) and 600 mg/kg/day in rats (26 weeks administration). The exposures at these doses were 5- and 3-fold over that at the clinical dose of 400 mg BID, respectively. At these doses, raltegravir was found to be well tolerated and produced few or no adverse effects at doses studied. One notable exception was the irritation to the mucosal surfaces raltegravir came in contact with.

Rodents seemed to be more sensitive to the irritation to mucosal surfaces by raltegravir than dogs and rabbits. This irritation was dose- and duration-related but was independent of age. Raltegravir at doses ≥ 120 mg/kg/day caused dose-related increases in salivation (probably because of poor palatability), the incidence of glandular mucosal degeneration and erosion in stomach, and the incidence and severity of inflammation in nose and nasopharynx (presumably due to aspiration of drug formulation during drug administration) in adult rats. In addition, decreased food consumption, decreased body weight, and mortality were also associated with raltegravir at a dose of 600 mg/kg/day. Similar irritation to mucosal surfaces was also observed in young rats (5-56 days old) that were administered similar doses of raltegravir. The only difference in the manifestation of stomach irritation was that nonglandular mucosa instead of glandular mucosa was affected in the young rats. No additional toxicities were noted in juvenile rats indicating that juvenile rats were not more sensitive to drug effects than adult rats. The mucosal irritation was evident in mice. Deaths were observed at doses ≥ 500 mg/kg/day. They were related to the increases in the incidences of gastrointestinal bloating glandular mucosal multifocal erosion in stomach.

Males were more sensitive to the irritation than female mice. Irritation to mucosal surfaces was dose-limiting (mortality in rats and mice and >10% reduction in body weight gain in rats). It was independent of formulation. While the vehicle used in dogs, rabbits, and mice was 0.5% methylcellulose and 80% PEG 400 in rats, this type of toxicity was seen in rats and mice only. In addition, the toxicity was likely related to the local (stomach, nose, or nasopharynx) concentrations of raltegravir rather than the systemic exposures. Dogs had highest and longest systemic exposure to raltegravir, however, no adverse effect was observed.

The no-adverse-effect level (NOAEL) is 50 mg/kg/day (0.25-fold of the exposure in humans at 800 mg/day) in mice, 30 mg/kg/day (0.9-fold of the exposure in humans at 800 mg/day) in rats, >360 mg/kg/day (5-fold of the exposure in humans at 800 mg/kg/day) in dogs, and >1000 mg/kg/day (3.7-fold of the human exposure at 800 mg/kg/day) in pregnant rabbits.

The genotoxic potential of raltegravir was investigated in three *in vitro* and one *in vivo* genotoxicity assays. It was found not to be mutagenic or clastogenic with or without metabolic activation. The carcinogenic potential of raltegravir is being evaluated in two-year carcinogenicity studies in rats and mice. Dosing is still ongoing in the two studies and will be completed during 4th quarter of 2007.

Male and female fertility were assessed either by direct oral dosing to young (5-56 days old) and adult rats or by exposure *in utero* and via breast milk. The results indicated that fertility was not affected at doses as high as 600 mg/kg (about 3-times human exposure) in rats. *In utero* exposure to raltegravir did not adversely affect embryo and fetal survival, weight, and external, skeletal, and visceral development in rabbits at doses up to 1000 mg/kg/day (4-fold human exposure at 800 mg/day). Fetal plasma drug concentrations were about 2% of those in maternal plasma at 1 and 24 hours postdose, respectively. However, an increased incidence of supernumerary ribs in rat fetuses exposed *in utero* to 600 mg/kg/day (3-fold human exposure at 800 mg/day) raltegravir was observed. Mean drug concentrations in rat fetal plasma were approximately 1.5- to 2.5-fold greater than those in maternal plasma at 1 and 24 hours postdose, respectively. Based on the skeletal finding in rats, raltegravir will be classified under "Pregnancy Category C" and is not recommended for use during pregnancy unless necessary. It was also secreted into rat milk. Mean drug concentration in milk at 2 hours postdose was approximately 3-fold that in maternal plasma. Exposure to this drug *in utero* or in milk did not affect pup delivery or neonatal development in rats. The second generations exhibited normal behavior and postnatal development, growth, sexual maturity, and fertility. Young rats were similarly sensitive to raltegravir as adult rats. The same type of mucosal surface irritation was observed in 5-56 day-old rats administered the same dose range as adults. The No-adverse-effect level (NOAEL) for

reproductive toxicity is 1000 mg/kg/day for rabbits (3.7-fold human exposure at 800 mg/day) and 300 mg/kg/day for rats (2.2-fold human exposure at 800 mg/day).

The toxicity of intravenous administration of raltegravir was investigated in dogs and female rats. MK-0518, the monopotassium salt, was used. Mortality was associated with single intravenous doses ≥ 200 mg/kg/day in female rats. Treatment-related clinical signs including recumbency, gasping, labored breathing, and decreased activity usually preceded deaths which occurred within 8 minutes postdose. Mortality was also observed in a male dog that was intended to have received 400 mg/kg MK-0518 intravenously. Treatment-related mortality occurred after 358 mg/kg was administered and was believed to be associated with cardiac arrhythmia caused by the high amount of potassium in MK-0518 rather than the direct effect of the MK-0518 molecule. Increases in aspartate aminotransferase (both sexes), alanine aminotransferase (male only), and alkaline phosphatase (male only) levels without the corresponding histopathological findings in liver were associated with 100 mg/kg dose. At the same dose, treatment-related interstitial inflammation of kidney cortex was seen in the female dog. These effects likely reflected the systemic toxicity of raltegravir. Since raltegravir will be administered orally in humans and no plan for the development for any intravenous formulation is proposed by the sponsor, the descriptions and results of intravenous toxicity study is not included in the label.

In conclusion, except for the irritation to mucosal surfaces observed in rodents, raltegravir is considered safe in animals at multiples of exposure in humans.

B. Pharmacologic activity

Please see Dr. Sung Rhee's review.

C. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-145
Review number: 1
Sequence number/date/type of submission: 000/Jan. 18, 2007/Original
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Merck & Co., Inc.
126 E. Lincoln Ave.
P.O. Box 2000, RY 33-212
Rahway, NJ 07065-0900
732-594-4809

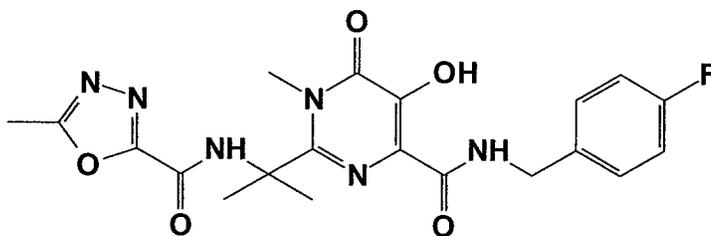
Manufacturer for drug substance: Merck & Co., Inc.
One Merck Drive
P.O. Box 100
Whitehouse Station, NJ 08889-0100

Reviewer name: Ita Yuen, Ph.D.
Division name: Division of Antiviral Products
HFD #: 530
Review completion date:

Drug:

Trade name: ISENTRESS™
Generic name: Raltegravir
Code name: MK-0518; L-000900612-003E
Chemical name: N-[(4-Fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-[1-methyl-1-[[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-4-pyrimidine-carboxamide monopotassium salt

CAS registry number: 871038-72-1
Molecular formula/molecular mass: C₂₀H₂₀FN₆O₅/482.51 g/mole
Structure:



Relevant INDs/NDAs/DMFs:	IND 69,928
Drug class:	HIV integrase inhibitor
Intended clinical population:	HIV-1 infected patients
Clinical formulation:	Film-coated tablets containing 400 mg (free phenol) or 434.4 mg (potassium salt) of MK-0518, lactose monohydrate, microcrystalline cellulose, anhydrous dibasic calcium phosphate, hypromellose 2208, poloxamer 407, sodium stearyl fumarate, and magnesium stearate; film coating containing _____ Pink _____ and purified water
Route of administration:	Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Ancillary pharmacology of L-000900612: and HIV integrase inhibitor (Report # PD003)
Cardiovascular telemetry assay in dogs (Report # TT #03-5642)
Cellular electrophysiological evaluation of a HIV integrase inhibitor on HERG (Report # TT #04-4721)
Functional observational battery assay (Study # TT #03-5720)
Rat respiratory assay (Report # TT #03-5635)
Pharmacokinetics of MK-0518 in Sprague-Dawley rats and beagle dogs after intravenous and oral administration (Study # PK-001)
Absorption kinetics of MK-0518 in rats and dogs (Study # PK004)
Tissue distribution of radioactivity after a single oral administration of [¹⁴C]-L-0612 to male rats (Study # PK-005)
Brain penetration of MK-0518 in CD-1 mice (Study # PK008)
Oral toxicokinetic study in rats with evaluation of placental and lactational transfer (Study TT #05-7170)
Oral toxicokinetic study in rabbits with evaluation of placental transfer (Study TT #05-7230)
In vivo metabolism and excretion of MK-0518 in Sprague-Dawley rats and beagle dogs (Study # PK002)
In vitro studies of MK-0518 (Study # PK003)
Metabolite profiles in urine, feces, and plasma of subjects administered [¹⁴C]MK-0518 (Study # PK007)
In vivo metabolism of MK-0518 in CD-1 mouse (Report # PK009)
Radioanalysis of biological samples following a single oral administration of [¹⁴C]L-000900612 (Merck study protocol no. 011) to healthy male subjects (Study # GIA00060)
Pharmacokinetic interaction between MK-0518 and atazanavir in rats (Study # PK010)
Exploratory acute oral-range finding study in mice (Study TT #03-2616)
Acute oral toxicity study in rats (Study TT #03-2619)
Exploratory single-rising dose oral tolerability study in dogs (Study TT #04-0080)
Exploratory single dose intravenous toxicity study in female rats (Study TT #06-2521)
Exploratory 7-day intravenous rising-dose tolerability study in dogs (TT #06-1036)
Eight-day oral toxicokinetic study in female rats (Study TT #05-6030)
Exploratory 8-day intravenous toxicity study in dogs (TT #06-6030)
Nineteen-day oral toxicokinetic study in mice (Study TT #05-0079)

One month oral toxicity study in rats (Study TT #06-6055)
Five week oral toxicokinetic study in mice (Study TT #05-1034)
Five-week oral toxicity study in rats (Study TT #04-0079)
Five week oral toxicity in dogs (Study TT #04-9811)
Fourteen-week oral range-finding study in mice (Study TT #05-1023)
Fourteen-week oral toxicity study in rats (Study TT #03-119-0)
Fourteen-week oral toxicity study in dogs (Study TT #03-118-0)
Twenty-seven-week oral toxicity study in rats (Study TT #04-6022)
A 53-week oral gavage toxicity study in the beagle dogs with a twenty-seven week interim necropsy (Study TT #04-9001)
Microbial mutagenesis assay (Study TT #03-8029)
In vitro alkaline elution/rat hepatocyte assay (Study TT #03-8381)
Assay for chromosomal aberrations *in vitro*, in Chinese Hamster Ovary Cells (Study TT #03-8681)
Assay for micronucleus induction in mouse bone marrow (Study TT #04-8619)
Oral fertility study in females rats (Study TT #04-7420)
Oral fertility study in male rats (Study TT#05-7180)
Oral developmental toxicity study in rats with prenatal and postnatal evaluation (Study TT #04-7090)
Oral range-finding reproductive study in female rats (Study TT #04-7095)
Oral developmental toxicity in study in rabbits (Study TT #04-7220)
Oral range-finding study in pregnant rabbits (Study TT #04-7225)
Oral juvenile study in rats (Study TT #05-7300)
Oral range-finding and toxicokinetic study in juvenile rats (Study TT #05-7305)
Local lymph node assay in mice (Study TT #04-5541)
Local lymph node assay in mice (Study TT #04-5545)
Acute dermal irritation study in rabbits (Study TT#04-5546)
Primary dermal irritation/corrosion in rabbits (Study TT#04-5550)
Bovine corneal opacity and permeability assay (Study TT #04-5510)
Bovine corneal opacity and permeability assay (Study TT #04-5551)
Skin irritation test (SIT) using skin model with optional IL-1 α (Study TT #04-5509)
Single-dose oral phototoxicity study in female mice (Study TT #06-2519)
Hemolytic assay (Study TT #06-4903 & TT #06-4905)

Studies not reviewed within this submission:

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Raltegravir (MK-0518; L-000900612) is a HIV-1 integrase inhibitor which blocks the insertion of the HIV-1 DNA into the host cell genome. This step is important for stable maintenance of the viral genome as well as efficient HIV-1 gene expression. Raltegravir has been shown to block HIV-1 replication in cell culture as well as SHIV replication in rhesus macaques. The detailed pharmacodynamic information can be found in the Dr. Sung Rhee's Microbiology review.

MK-0518 was studied in dogs, rats, and mice at doses up to 10 mg/kg IV or 120 mg/kg oral to evaluate its effects on cardiovascular/autonomic, respiratory, renal, and gastric acid secretion functions, gastrointestinal motility, and behavioral and other central nervous system. It was also tested for its inhibitory effect on human HERG ion channel

in vitro. Except for the increased gastric motility associated with 30 mg/kg oral dose in mice, no effect was associated with single doses of MK-0518 in any of the parameters monitored.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Please see Dr. Sung Rhee's Microbiology review.

Drug activity related to proposed indication: Please see Dr. Sung Rhee's Microbiology review.

2.6.2.3 Secondary pharmacodynamics

Please see Dr. Sung Rhee's Microbiology review.

2.6.2.4 Safety pharmacology

Neurological effects: No effect. Please see the study description below:

Functional observational battery assay (Study # TT #03-5720; GLP; with QA report; Conducted at Merck Research Laboratories, West Point, PA; Lot # L-000900612-003E009, ---, pure; dosing volume 2.5 ml/kg;

\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\tt035720\tt035720.pdf): Five rats/sex/dose received a single oral dose of 0 (80% PEG400), 30, 90, or 150 mg/kg L-000900612. Functional observational battery monitoring home cage observations, handling associated observations, open field observations, stimulus activity responses, and reflex measures were performed approximately 1 hour after dosing on all rats. Mortality checks, food consumption, and body weight measurement were also performed. No effect was associated with any of L-000900612 doses.

Behavioral and other CNS effects in conscious mice (Report # PD003; Non-GLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\pd003\pd003.pdf): Five female CD-1 mice/group received an oral dose of 0 (vehicle; 0.5% methylcellulose) or 100 mg/kg L-000900612 following 18-24 hours of fasting. The animals were observed individually for 120 minutes then group-housed by treatment with food and water. They were visually evaluated for any overt CNS signs from 5 to 24 hours after dosing. Evaluated parameters included motor signs (change in body or limb muscle tone or gait, abnormal posture of limbs or body, tremor, tonic or clonic convulsions, convulsions on handling, reduced or increased activity, impaired grip strength, and presence of psychomotor activation such as stereotype or headweaving), autonomic signs (writhing, dyspnea, exophthalmus, hypersalivation, piloerection, thermostasis such as hypothermia or hyperthermia > 1.5°C, abnormal skin color, lacrimation, eye closure when handled, and changes in pupil diameter), and

neural reflexes (righting when placed on back, pinna response, startle response to sudden noise, and blink response to the cornea touching). The L-000900612 treated group had mean core temperature of $37.36 \pm 0.23^\circ\text{C}$ which was significantly higher than that ($36.02 \pm 0.22^\circ\text{C}$) of the vehicle control group. However, the significant difference was probably due to the unusually low core temperature of the control group. No other effect was detected.

Cardiovascular effects: No effect. Please see the study descriptions below:

Cardiovascular telemetry assay in dogs (Study # TT #03-5642; GLP; QA report; Conducted at Merck Research Laboratories, West Point, PA; Lot # L-000900612-003E009 — , pure; dosing volume 5 ml/kg; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\tt035642\tt035642.pdf): Four female dogs received a single oral dose of 0 (vehicle; 0.5% methylcellulose), 5, 15, and 45 L-000900612 with ≥ 1 -week washout period between doses using a 4 X 4 Latin square crossover design. Body weights, physical signs, food consumption, arterial blood pressure, heart rate, PR interval, QRS interval, and QT interval were recorded. Cardiovascular telemetry data were collected for ≥ 18 hours prior to dosing and ≥ 24 hours postdose. In addition, blood samples were collected approximately ≥ 18 hours before dosing and 1 hours ± 15 minutes postdose in dose weeks 1, 2, 3, and 4 for plasma drug analysis. The plasma drug levels increased roughly proportional to doses. No effect in any parameters monitored was observed.

Cardiovascular effects in anesthetized dogs: rising cumulative dose study (Report # PD003; Non-GLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\pd003\pd003.pdf): Three sodium pentobarbital anesthetized male dogs received cumulative doses of 1, 3, and 5 mg/kg L-000900612 (dissolved in 20% N,N-dimethylacetamide) by intravenous infusion at a rate of 20 ml over a 30-minute period. Mean arterial pressure, heart rate, femoral arterial flow, and ECG activity were recorded. Another set of 3 similarly anesthetized dogs received vehicle alone with three 30 minute infusions. No effect on any parameter monitored was attributed to the L-000900612 treatment.

Cellular electrophysiological evaluation of a HIV integrase inhibitor on HERG (Study # TT #04-4721; non-GLP \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\tt044721\tt044721.pdf): The potential for L-000900612 to cause QT prolongation was tested on HERG channels heterologously expressed in CHO-K1 cells using standard whole-cell voltage-clamp techniques. The top L-000900612 concentration tested was 100 μM . At this concentration, there was a 16% inhibition of HERG current as compared to the vehicle control (DMSO). It was claimed that the magnitude of inhibition was not considered a real inhibition of HERG current since vehicle alone has been observed in the lab to cause this magnitude of change. However, no positive control was included in the assay and the validity of the test was thus questionable.

Pulmonary effects: No effect. Please see the study descriptions below:

Rat respiratory assay (Study # TT #03-5635; GLP; QA report; Conducted at Merck Research Laboratories, West Point, PA; Lot# L-000900612-003E009 —, pure; dosing volume 2.5 ml/kg; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\tt035635). A single oral dose of 0 (vehicle; 80% PEG400), 30, 90, or 120 mg/kg L-000900612 was given to 6 conscious male rats/dose. The effects on respiratory rate, tidal volume, minute ventilation, PenH, and body weight were monitored using Respiratory rate, tidal volume, minute ventilation, and PenH increased in all groups immediately following dosing which was probably an excitatory reaction to handling of the animals during dosing. No treatment-related change was reported.

Respiratory function, hemostasis, and platelet function in anesthetized dogs (Report # PD003; Non-GLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\pd003\pd003.pdf): Five mg/kg of L-000900612 was given to three spontaneously breathing, anesthetized dogs (2 males and 1 female) by intravenous administration over 5 minutes. The effects on respiratory function, ventilation, blood pressure, heart rate, hemostasis, and platelet function were monitored. No effect was detected. The average plasma concentration at the end of dosing was $69 \pm 5.5 \mu\text{M}$.

Renal effects: No effect. Please see the study description below:

Renal function and electrolyte excretion in conscious dogs (Report # PD003; Non-GLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\pd003\pd003.pdf): Three conscious female dogs received a single oral dose of 5 mg/kg L-000900612 followed by urine and plasma samples collection. Urine output, urinary sodium and potassium excretion, plasma electrolyte concentrations, glomerular filtration rate and filtration fractions were monitored and compared to the predose as well as historical control values. No effect on these parameters was detected.

Gastrointestinal effects: GI motility was significantly increased following a single oral dose of 30 mg/kg L-000900612. Please see the study description below:

Gastrointestinal motility in conscious mice (Report # PD003; Non-GLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\pd003\pd003.pdf): The effect of an oral dose of 0 (vehicle; 0.5% methylcellulose), 10, or 30 mg/kg L-000900612 on gastrointestinal motility was evaluated in 10 CF-1 female mice/dose by determining the distance a charcoal meal traveled in the intestine of conscious mice within 20 minutes. A positive control, subcutaneously administered neostigmine, was included. It was found that GI motility was significantly increased at 30 mg/kg dose as compared to the vehicle control. However, no behavior abnormality or diarrhea was seen in any of the L-000900612 treated groups.

Abuse liability: Not studied.

Other: None

2.6.2.5 Pharmacodynamic drug interactions

Please see Dr. Sung Rhee's Microbiology review.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetic profile of MK-0518 (L-000900612) was determined in CD-1 mice, Sprague-Dawley rats, beagle dogs, pregnant rabbits, and humans. MK-0518 was administered orally or intravenously. The potassium salt of MK-0518 was the most stable crystalline salt form, demonstrated good nonclinical as well as clinical pharmacokinetic profile, and was thus used in all nonclinical GLP studies as well as clinical studies. It is also the form proposed for registration. Several vehicles were used in the nonclinical pharmacokinetic studies. The vehicles used in the pivotal toxicology/toxicokinetic studies were 80% PEG 400 for rats and 0.5% methylcellulose in water for mice and dogs. However, many of the pharmacokinetic studies described in this section did not contain information on the vehicle used. The MK-0518 plasma concentrations were determined using LC-MS/MS that was able to detect MK-0518 concentrations as low as \sim ng/ml. The linear range of this detection method was from \sim ng/ml. Proposed clinical dosage is 400 mg B.I.D. by oral administration.

The pharmacokinetic data suggested that MK-0518 was absorbed rapidly following oral administration in all of the species studied, including humans. The oral bioavailability of 6 mg/kg MK-0518 was 62% in rats and 70% in dogs dosed with 1.5 mg/kg MK-0518. Systemic exposure was limited by the vehicle used. When 0.5% methylcellulose was used, saturation of absorption was reached at 120 mg/k/day in rats. MK-0518 was distributed rapidly and widely. It was demonstrated to penetrate blood-brain and blood-placental barriers and be secreted in milk, even though the penetration in brain was low. Metabolism of MK-0518 was mainly by UGT1A1 enzyme in liver to form a phenolic glucuronide derivative, M2, as the major metabolite in rats, dogs, and humans. The parent compound and its major metabolite were both excreted via feces and urine. MK-0518 was moderately bound in plasma.

The impurities specified in the final manufacturing specification are

The allowable levels of these impurities in the to-be-marketed clinical formulation were set based on the amount of impurities in the drug lots used in the pivotal toxicology studies. None of the lots in these studies contained all of the impurities in one single lot. Since these impurities are not expected to have synergistic toxicities, testing of the drug containing all of these impurities in a single lot is not necessary.

2.6.4.2 Methods of Analysis

Plasma concentrations of MK-0518 were determined using a validated LC-MS/MS assay which was able to detect the MK-0518 concentrations as low as ng/ml (nM). The linear range of detection fell between ng/ml . The structures of metabolites were determined by MS analysis by comparison to authentic standards.

2.6.4.3 Absorption

Pharmacokinetics of MK-0518 in Sprague-Dawley rats and beagle dogs after intravenous and oral administration (Study # PK-001; non-GLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4222-absorp\pk001\pk001.pdf): Two groups of 4 male Sprague-Dawley rats received a dose of 3 mg/kg ($\sim 50 \mu\text{Ci}$ of [^{14}C]MK-0518) via intravenous (IV) administration or 6 mg/kg ($\sim 50 \mu\text{Ci}$ of [^{14}C]MK-0518) via oral administration. For the dose proportionality study, groups of 4 male rats were administered orally with a single dose of 40, 80, 120, or 240 mg/kg of the potassium salt of MK-0518. Similarly, 4 male dogs received a 1.5 mg/kg IV dose ($\sim 40 \mu\text{Ci}$ of [^{14}C]MK-0518) and a 5 mg/kg oral dose ($\sim 40 \mu\text{Ci}$ of [^{14}C]MK-0518) in a crossover study design. For dose proportionality study groups of 2 male beagle dogs received a single oral dose of 5, 15, 45, or 135 mg/kg of potassium salt of MK-0518. Blood samples were collected at various time points postdose from both dogs and rats. Concentrations of MK-0518 in plasma were determined by LC-MS/MS in the positive ion mode using interface . The assay could determine MK-0518 concentration range of ng/ml with lower limit of quantitation at ng/ml .

Comparison of the mean AUC values for total radioactivity and MK-0518 in plasma showed that unchanged MK-0518 accounted for 36 and 37% of the total radioactivity after oral and IV administration, respective, in rats. In dogs, most of the plasma radioactivity ($>95\%$) recovered was the unchanged MK-0518. The pharmacokinetic profile indicated that saturation of absorption was reached at 120 mg/kg in rats with the 0.5% methycellulose as the vehicle. However, the systemic exposure was proportional to dose up to 135 mg/kg/day in dogs. The pharmacokinetic parameters for both species are listed in the following table:

	Rat						Dog					
	Oral	IV	Oral				Oral	IV	Oral			
Dose (mg/kg)	6	3	40	80	120	240	5	1.5	5	15	45	135
T_{max} (hr)	0.4	-	0.8	0.5	0.9	0.6	0.6	-	0.8	0.4	0.5	0.8
C_{max} ($\mu\text{g/ml}$)	1.7	-	10.5	12.8	29.8	28.9	11.9	-	8.0	29.2	92.3	127.8
$\text{AUC}_{0-\infty}$ ($\mu\text{M}\cdot\text{hr}$)	3.2	2.6	19.7	25.4	64.6	65.9	18.6	7.9	20.6	50.9	177.0	363.0
$t_{1/2}$ (hr)	-	0.2	-	-	-	-	-	1.6	-	-	-	-
Bioavailability (%)	61.6	-	-	-	-	-	70.0	-	-	-	-	-
CL_p (ml/hr/kg)	-	45.5	-	-	-	-	-	7.4	-	-	-	-
Vd_{ss} (L/kg)	-	2.2	-	-	-	-	-	0.4	-	-	-	-

Absorption kinetics of MK-0518 in rats and dogs (Study # PK004; nonGLP; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4222-absorp\pk004\pk004.pdf): The absorption of two forms (free phenol and potassium salt) of MK-0518 in various vehicles was studied in rats and dogs following oral administration. Doses of 20, 120, 160, and 320 mg/kg MK-0518 dissolved in 0.5%

aqueous methylcellulose, 0.5% aqueous methylcellulose containing 10% Tween 80, or 80% PEG400 and 20% water were evaluated in rats. The same doses of the free phenol form of MK-0518 dissolved in 0.5% aqueous methylcellulose containing 0.02% SDS and MK-0518 potassium salt dissolved in 0.5% aqueous methylcellulose were studied in dogs. It was found that potassium salt, in general, showed better bioavailability than the free phenol form in both rats and dogs. In rats, the 80% PEG400 formulation yielded dose-proportional exposures over the 20 to 120 mg/kg dose range.

2.6.4.4 Distribution

Tissue distribution of radioactivity after a single oral administration of [¹⁴C]-L-0612 to male rats (Study # PK-005; GLP; with QA report; [\\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4223-distrib\pk005\pk005.pdf](#)): The tissue distribution of radioactivity was determined by tissue excision following a single oral dose of 6 mg/kg [¹⁴C]-L-0612 to male Sprague-Dawley rats (n=12). Blood and tissues were collected from 3 rats/time point at 0.5, 2, 6, and 24 hours postdose. The tissues collected included adrenal glands, bladder, blood, blood cells, femur, bone marrow (femur), brain, cecum, cecum contents and wash, eyes, fat (reproductive), heart, kidneys, large intestine and its contents and wash, liver, lungs, mesenteric lymph nodes, thigh muscle, pancreas, pituitary gland, plasma, prostate, submandibular salivary gland, small intestine and its contents and wash, spleen, stomach and its contents and wash, testes, thymus, and thyroid/parathyroid. Maximum concentrations of [¹⁴C] L-0612-derived radioactivity were detected at 0.5 hours postdose in most of the tissues/plasma and were 30900 ng-eq/g, in stomach, 8970 ng-eq/g in small intestine, 3910 ng-eq/g in liver, 1720 ng-eq/g in kidneys, 437 ng-eq/g in urinary bladder, 601 ng-eq/g in plasma, 341 ng-eq/g in blood, and 157 ng-eq/g in blood cells. The radioactivity in plasma declined steadily to undetectable level by 24 hours postdose. The blood to plasma ratios were approximately 0.6 at all the time points, suggesting that [¹⁴C] L-0612-derived radioactivity partitioned preferentially into extracellular fraction of blood. The highest mean percentages of radioactivity following oral administration, excluding those from gastrointestinal contents and wash, were observed in liver (2.89%) and stomach (2.83%) at 0.5 hours postdose.

Brain penetration of MK-0518 in CD-1 mice (Study # PK008; nonGLP; without QA report; [\\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4223-distrib\pk008\pk008.pdf](#)): The brain penetration of MK-0518 was evaluated in P-glycoprotein (P-gp) competent (+/+) and deficient (-/-) male CF-1 mice by determining the concentrations of MK-0518 in plasma and brain following intravenous administration of 3 mg/kg MK-0518. Blood and brain were collected at 0.25, 0.5, 1, 3, and 6 hours postdose from 3 animals/time point. Concentrations of MK-0518 in plasma and brain homogenate were determined by LC-MS/MS. The plasma concentrations were similar in both types of mice. The brain MK-0518 concentrations were below the limit of quantitation in the (+/+) mice at all time point while they were measurable in the brains of the (-/-) mice at only the first time point (0.25 hr postdose). At this time point, brain to

plasma concentration ratio was 0.07. The results suggested that MK-0518 penetrated the brain of mice poorly probably because it's a P-gp substrate in the mouse.

Oral toxicokinetic study in rats with evaluation of placental and lactational transfer (Study TT #05-7170; Merck Research Laboratories, West Point, PA & Laboratoires Merck Sharp & Dohme-cibret, centre de Recherche, Riom, France; Lot # L-000900612-003E014; GLP; With QA report; study date 3/18/2005-4/29/2005; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\tt057170\tt057170.pdf): Twenty-two CD(SD) pregnant females rats/dose received 300 or 600 mg/kg/day L-000900612 in 80% PEG 400 from gestation days 6 to 20. Mortality and body weight gain were monitored. Maternal blood samples were collected from 4 dams/time point at 0.5, 1, 2, 4, 6, 8, and 24 hours postdose for analysis of plasma drug concentrations on gestation day 20. Fetal blood samples were collected at 1 and 24 hours postdose by removing fetuses from uterus of each dam immediately following maternal blood collection. After parturition (starting on gestation day 21), each litter was reduced to 5 males and 5 females on postnatal day 5. On lactation day 14, the dams were dosed with a single dose of 300 or 600 mg/kg/day L-000900612 and blood samples collected at 2 hours postdose. As soon as possible after the blood collection, milk samples were collected from 4 dams/dose over a 10-minute period at 5 minutes after oxytocin injection and analyzed for drug concentrations. All animals survived until scheduled sacrifice. The calculated toxicokinetic parameters are presented below.

Absorption of L-000900612 in maternal plasma was rapid with maximum plasma concentration reached 0.5 hour after dose administration. Elimination was rapid and biphasic with mean trough concentrations that were less than 1% of the C_{max} values. The pharmacokinetic parameters measured were comparable to those in the non-pregnant rats at the same doses suggesting that pregnancy did not affect the pharmacokinetics of the drug. The results indicated that L-000900612 readily crosses rat placenta. Fetal drug concentrations were consistently higher than those in maternal plasma at the two time points monitored. L-000900612 was also excreted into rat milk. Higher drug concentrations were seen in milk than maternal plasma at 2 hours postdose.

Dose (mg/kg/day)	Gestation Day 20		Lactation day 14	
	300	600	300	600
T_{max} (hr)	0.5	0.5	-	-
C_{max} (μ M)	38.2 \pm 5.05	42.5 \pm 12.1	-	-
AUC_{0-24hr} (μ M-hr)	120 \pm 10.7	155 \pm 22.9	-	-
Maternal plasma conc. (μM)				
– 1 hr	17.4 \pm 4.85	23.1 \pm 11.7	-	-
– 2 hrs			12.6 \pm 1.94	15.8 \pm 4.41
– 24 hrs	0.146 \pm 0.0625	0.278 \pm 0.0908	-	-
Fetal plasma conc. (μM)				
– 1 hr	18.4 \pm 1.83	23.4 \pm 8.87	-	-
– 24 hrs	0.329 \pm 0.0798	0.601 \pm 0.184	-	-
Feta/maternal plasma ratio				
– 1 hr	1.21 \pm 0.217	1.91 \pm 0.709	-	-
– 24 hrs	2.67 \pm 0.375	2.48 \pm 0.468	-	-
Maternal milk conc. (μM) – 2 hrs	-	-	41.3 \pm 5.28	39.3 \pm 10.2
Milk/plasma ratio – 2 hrs	-	-	3.34 \pm 0.225	2.55 \pm 0.360

Oral toxicokinetic study in rabbits with evaluation of placental transfer (Study TT #05-7230; Merck Research Laboratories, West Point, PA & Laboratoires Merck Sharp & Dohme-cibret, centre de Recherche, Riom, France; Lot # ; GLP; With QA statement; Study dates 5/25/2005-6/15/2005; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\tt057230\tt057230.pdf):

Twelve pregnant Dutch-Belted rabbits, approximately 21 weeks old and weighed 1786-2350 g, received doses of 1000 mg/kg/day L-000900612 in 0.5% (w/v) methylcellulose from gestation days 7-20 with a dosing volume of 5 ml/kg. Mortality, clinical signs, and body weight gain were monitored. Blood samples were collected from 4 pregnant rabbits/time point at 0.25, 0.5, 1, 3, 6, 9, and 24 hours postdose on gestation day 20 for drug concentration analysis. Fetal blood samples were collected at 1 and 24 hours postdose by removing fetuses from uterus of each dam immediately following maternal blood collection. All animals survived until scheduled sacrifice. The calculated toxicokinetic parameters are presented as follows:

1000 mg/kg/day								
T _{max} (hr)	C _{max} (μM)	AUC _{0-24hr} (μM-hr)	Maternal plasma conc. (μM)		Fetal plasma conc. (μM)		Feta/maternal plasma ratio	
			1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs
0.25	37.4 ± 6.04	201 ± 19.5	23.8 ± 2.67	5.30 ± 2.33	0.513 ± 0.0819	0.123 ± 0.0550	0.0213 ± 0.00115	0.0232 ± 0.000559

Absorption of L-000900612 in maternal plasma was rapid with the maximum plasma concentration reached at 0.25 hour after dose administration. Elimination of drug from maternal plasma was rapid and biphasic with mean trough concentrations less than 15% of the C_{max} values. L-000900612 also readily crossed rabbit placenta, though the extent of placental transfer was much lower than that for rats. Fetal drug concentrations were consistently 2% those in maternal plasma at the two time points monitored. Elimination rate was similar to that observed in maternal plasma.

2.6.4.5 Metabolism

In vivo metabolism and excretion of MK-0518 in Sprague-Dawley rats and beagle dogs (Study # PK002; nonGLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4224-metab\pk002\pk002.pdf): The *in vivo* metabolism of MK-0518 was studied in bile-cannulated rats and dogs following a single IV dose. Doses of [¹⁴C]MK-0518 used were 3 mg/kg in rats and 1.5 mg/kg in dogs. Bile, urine, and plasma samples were collected and analyzed by LC-MS/MS-radiochromatography. MK-0518 was eliminated mainly by metabolism in both rats and dogs since unchanged MK-0518 accounted for 10 to 30% of administered dose. The metabolite profile in these two species was the same with the major metabolite, M2, being the phenolic glucuronide derivative of the parent compound, accounting for 62 and 31% of dose in rats and dogs, respectively. In humans, M2 is also the major metabolite. The glucose conjugate of the parent compound, M1, accounted for 0.3 and 0.1% of dose in rats and dogs, respectively. A minor metabolite, M3, the acetyl hydrazine derivative, was only detected in rats. The

plasma AUC ratio of the metabolites to parent compound was 0.6 for rats and 0.3 for dogs.

For mass balance studies, IV doses used were 3 mg/kg (~50 μ Ci; rats) and 1.5 mg/kg (~40 μ Ci; dogs) and oral doses were 6 mg/kg (~40 μ Ci; rats) and 5 mg/kg (~40 μ Ci; dogs). The recovery of total radioactivity was 70.2% (19.9% in urine and 50.3% in feces) for rats and 88.4% (30.8% in urine and 57.6% in feces) for dogs after IV administration. The value following oral administration was 87.7% (23.3% in urine and 64.0% in feces) for rats and 87.1% (13.2% in urine and 73.9% in feces) for dogs.

In vitro studies of MK-0518 (Study # PK003; non-GLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4224-metab\pk003\pk003.pdf): The *in vitro* metabolism of 5 and 50 μ M [14 C]MK-0518 was examined in liver microsomes and hepatocytes isolated from rats, dogs, and humans. The *in vitro* metabolic profile was similar across these three species with M2, the phenolic glucuronide conjugate, being the major metabolite and M1 (the glucose derivative) and M3 (an acetyl hydrazine derivative) existing in small quantity. No significant metabolism was seen in liver microsomes which had been fortified with NADPH. The metabolic pathways in hepatocytes from the three species were qualitatively and quantitatively similar. UGT1A1 was found to play a major role in the formation of M2 or the glucuronidation of MK-0518. MK-0518 was not a potent reversible inhibitor ($IC_{50} > 100 \mu$ M) of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP2B6, or of UGT1A1 and UGT2B7 ($IC_{50} > 50 \mu$ M) which mediated glucuronidation of β -estradiol (3-OH) and AZT, respectively. It did not induce CYP3A4 RNA expression or CYP3A4-dependent testosterone 6 β -hydroxylase activity.

MK-0518 was moderately bound to plasma proteins from rat, dog, and human, with the unbound fraction in plasma being 26, 30, and 17 %, respectively. The amount of MK-0518 distributed to red blood cells was minimal in rat and dog. The blood-to-plasma ratio was 0.7 for rat, 0.6 for human, and 0.9 for dog. MK-0518 was shown to be a substrate for human, rat, and mouse P-glycoprotein but did not inhibit human P-glycoprotein.

Metabolite profiles in urine, feces, and plasma of subjects administered [14 C]MK-0518 (Study # PK007; non-GLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4224-metab\pk007\pk007.pdf): The metabolic profile of MK-0518 was studied in 8 healthy male volunteers who had received a single oral dose of 200 mg [14 C]MK-0518 containing 200 μ Ci of radioactivity. There were two radioactive peaks which were identified as M2, the phenolic glucuronide derivative, and MK-0518 in urine collected from 0 to 8 hours postdose. They accounted for 23 and 9% of the dose, respectively. The only radioactive peak identified in feces was the parent compound, MK-0518, and accounted for 51% of the dose. The major circulating entity that accounted for 71% of radioactivity in plasma was MK-0518 with the remaining circulating radioactivity being M2. The data suggested that glucuronidation played a major role in the clearance of MK-0518 in humans.

In vivo metabolism of MK-0518 in CD-1 mouse (Report # PK009; non-GLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4224-

metab\pk009\pk009.pdf): Three intact (for plasma collection only) and bile duct-cannulated male mice/group/time point received a single oral dose of 20 mg/kg [¹⁴C]MK-0518 (28 µCi/mouse). Bile samples were collected at 0-4, 4-8, and 8-24 hours postdose and plasma samples collected at 0, 1, 3, 6, and 24 hours postdose and analyzed by HPLC-MS radiochromatography. The major metabolite detected in bile was the phenolic glucuronide derivative, M2, which accounted for about 96.3% of the radioactivity recovered in bile. It's also the major circulating species with the ratio to the parent compound, MK-0518, being 3.9, 2.1, and 2.6 at 1, 3, and 6 hours postdose, respectively.

2.6.4.6 Excretion

Radioanalysis of biological samples following a single oral administration of [¹⁴C]L-000900612 (Merck study protocol no. 011) to healthy male subjects (Study # GIA00060; GLP; With QA report;

01608; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4225-excr\pk006\pk006.pdf): The metabolic profile of MK-0518 was studied in 8 healthy male volunteers who had received a single oral dose of 200 mg [¹⁴C]MK-0518 (in 4 capsules) containing 200 µCi of radioactivity, under protocol no. 011. Urine and fecal samples as well fecal wipes were collected before dosing and at various time points up to 240 hours postdose. The recoveries of radioactivity in urine were comparable for each subject. However, the recovery of radioactivity from the fecal samples collected from one subject was notably lower (— % as compared to ————), resulting in the exclusion of the data from this subject from the data analysis. Radioactivity was eliminated rapidly and substantially in both urine and feces following a single oral MK-0518. Most of the radioactivity was recovered within 24 hours postdose. Mean (n=7) total recovery through 240 hours postdose was 51.11% in feces, 0.07% in fecal wipes, and 31.77% in urine.

2.6.4.7 Pharmacokinetic drug interactions

Pharmacokinetic interaction between MK-0518 and atazanavir in rats (Study # PK010; non-GLP; \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\422-pk\4226-pk-drug-interact\pk010\pk010.pdf): The pharmacokinetic interaction between MK-0518 and atazanavir was studied *in vitro* and *in vivo*. The *in vitro* system was rat liver microsome in which various concentrations of atazanavir were incubated in the presence of 200 µM MK-0518 to determine if atazanavir inhibited the metabolism of MK-0518. It was found that atazanavir was a potent inhibitor of the glucuronidation of MK-0518 with the estimated IC₅₀ value of 2.2 µM. The inhibitory potential of atazanavir was also studied in male Sprague-Dawley rats which were administered with oral doses of vehicle, 5, or 50 mg/kg atazanavir for 4 days and a single 10 mg/kg oral dose of MK-0518 on the 4th day. Blood samples were collected at various time points postdose on day 4. The concentrations of MK-0518 in rat plasma were determined by LC-MS/MS in the positive ion mode using ———— interface. It was found that coadministration with 50 mg/kg dose of atazanavir significantly increased all calculated pharmacokinetic

parameters (AUC, C_{max} , C_{min} , $t_{1/2}$) of MK-0518 by 1.5-3.5-fold. The results indicated that atazanavir inhibited the systemic clearance as well as first-pass metabolism of MK-0518 in rats, consistent with the inhibition of glucuronidation of MK-0518 observed *in vitro*.

2.6.4.8 Other Pharmacokinetic Studies

None.

2.6.4.9 Discussion and Conclusions

The ADME profile of raltegravir was adequately studied in several animal species. It was rapidly absorbed in both animals and humans, with the maximum plasma concentrations occurring as early as 0.5 hour postdose. It had a short plasma half-life (≤ 1.6 hr) and low (dog) to intermediate (rat) plasma clearance. The oral bioavailability ranged from 62 to 70% in rats and dogs. Pregnancy had no effect on the pharmacokinetic profile of raltegravir. In general, there was no gender effect on the pharmacokinetic parameters in all species studied. However, the systemic exposures of female rats were generally higher than those in males after 13 weeks of drug administration. The difference did not persist following 26 weeks of drug administration at the highest dose (600 mg/kg/day) studied. The systemic exposure generally increased in a dose-related manner across all species studied, though saturation of absorption was observed in dogs at 250 mg/kg/day and at 120 mg/kg/day in male rats and 300 mg/kg/day in female rats. Accumulation of raltegravir following multiple oral dosing was not apparent in all the species studied.

Raltegravir was extensively distributed into tissues and extravascular spaces. It was shown to be excreted into milk and cross the blood-brain and placenta barriers, though the extent of distribution was species-dependent. The results of placenta transfer and milk excretion studies indicated that fetuses and suckling pups as well as pregnant rats and rabbits received adequate systemic exposure to raltegravir in the reproductive toxicity studies.

Metabolism of raltegravir was via glucuronidation catalyzed likely by UGT1A1 enzyme in liver microsome to produce a phenolic glucuronidate derivative, M2, in all species studied including humans. *In vitro* drug interaction study with atazanavir showed that this metabolic process was inhibited by known UGT1A1 inhibitor like atazanavir. Drug-interaction was also expected when coadministration with known UGT1A1 inducers like rifampicin. However, *in vitro* study demonstrated that MK-0518 was a weak inducer or inhibitor of UGT1A1 and was unlikely to affect the metabolic clearance of drugs metabolized by UGT1A1. Another metabolite, M1, the glucose derivative, was detected in small amount in both rats and dogs, accounting for 0.3 and 0.1% of the administered dose, respectively. An additional metabolite M3, an acetyl hydrazine derivative, was detected in rats only. The plasma AUC ratio of the metabolites to parent compound was 0.6 for rats, 0.3 for dogs, and 3.9 for mice. MK-0518 was not a potent inhibitor of cytochrome P450 CYP isozymes. It's moderately bound to plasma proteins from rats, dogs, and humans with the unbound fraction in plasma being 26, 30, and 17%,

respectively. MK-0518 and M2 were excreted both in urine and feces, though fecal excretion of the parent compound was the major route of excretion in humans.

2.6.4.10 Tables and figures to include comparative TK summary

Comparative Pharmacokinetics of Raltegravir in Animals and Humans Following Single and Multiple Oral Doses of Radiolabeled and Nonradiolabeled Raltegravir							
Species	Dose (mg/kg/day)	Dosing Duration	Vehicle	C _{max} ^a (μM)	T _{max} ^a (hr)	AUC ₀₋₂₄ ^a (μM-hr)	Dose-normalized AUC ^a
Humans	400 mg BID ^c	1 day	-	10	1.91	12.3	-
	400 mg BID ^d		-			14.3	-
CD-1 mice	50	5 weeks	0.5%	9.37	0.5	14.7	0.294
	500	18 days	methyl-cellulose	33.5	0.5	77.8	0.156
		5 weeks		21.5	0.5	45.2	0.0904
Rabbits ^b	50	13 days	0.5%	12.3	1.0	72.5	1.45
	250	13 days	methyl-cellulose	38.9	1.0	296	1.18
	1000	13 days		66.4	1.4	1023	1.02
Dogs	5	1 day	0.5% methyl-cellulose	8.98	0.6	18.2	3.640
		13 weeks		3.89	0.6	10.62	2.124
	15	1 day		18.8	0.4	56.3	3.753
		13 weeks		12.0	0.7	37.1	2.473
		26 weeks		11.9	0.5	33.9	2.260
		52 weeks		11.4	0.6	32.9	2.193
	45	1 day		51.5	0.6	106	2.356
		13 weeks		26.2	0.6	72.3	1.607
	90	13 weeks		54.1	0.7	171	1.900
		26 weeks		42.8	0.7	142	1.578
		52 weeks		61.8	0.6	159	1.767
	100	1 day		49.6	0.5	148	1.480
	125	1 day		79.3	0.5	226	1.808
		28 days		71.6	0.5	267	2.136
	135	1 day		128	0.8	363	2.689
	250	1 day		75.9	0.9	291	1.164
		28 days		91.4	0.6	367	1.468
360	26 weeks	77.6	0.5	230	0.639		
	52 weeks	87.4	0.6	273	0.758		
500	1 day	103	0.7	445	0.890		
	28 days	115	0.6	443	0.886		
1000	1 day	94.8	0.5	285	0.285		

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Species	Dose (mg/kg/day)	Dosing Duration	Vehicle	C _{max} ^a (μM)		T _{max} ^a (hr)		AUC ₀₋₂₄ ^a (μM-hr)		Dose- normalized AUC ^a	
				M	F	M	F	M	F	M	F
SD rats	6	1 day	80% PEG 400	1.7	-	0.4	-	3.2	-	0.533	-
	30	13 weeks		12.1	28.4	0.5	0.5	23.1	37.0	0.770	1.233
		26 weeks		12.8	17.1	0.5	0.5	21.9	36.4	0.730	1.213
	40	1 day		10.5	-	0.8	-	19.7	-	0.493	-
	80	1 day		12.8	-	0.5	-	25.4	-	0.318	-
	90	13 weeks		13.0	35.2	0.5	0.5	20.9	47.0	0.232	0.522
	120	1 day		29.8	-	0.9	-	64.6	-	0.538	-
		13 weeks		6.40	66.0	0.5	0.5	21.9	76.3	0.183	0.636
		26 weeks		10.6	30.4	0.5	1.0	37.4	77.7	0.312	0.648
	150	5 weeks		17.3	12.3	0.5	0.5	34.3	29.8	0.229	0.199
	240	1 day		28.9	-	0.6	-	65.9	-	0.275	-
	300	2 weeks ^b		-	38.2 ^b	-	0.5 ^b	-	120 ^b	-	0.400
		5 weeks		64.3	73.1	1.0	0.5	64.3	73.1	0.214	0.244
	450	5 weeks		37.6	40.4	1.0	1.0	77.5	80.8	0.172	0.180
	600	7 days		-	100	-	1.0	-	231	-	0.385
		2 weeks ^b		-	42.5 ^b	-	0.5 ^b	-	155 ^b	-	0.258
		5 weeks		91.5	46.8	2.0	0.5	234	104	0.390	0.173
	26 weeks	60.5	68.2	2.0	1.0	169	164	0.282	0.273		

a: Combined male and female data unless otherwise indicated
b: Pregnant females only
c: Raltegravir PK study in 20 healthy, young male and female volunteers with a mean weight of 78.73 kg (dose = 10.1 mg/kg)
d: Repeated dose study

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The nonclinical toxicological and toxicokinetic profiles of L-000900612, also known as MK-0518, have been studied in rats, mice, and dogs using oral and intravenous routes of administration. The toxicokinetic data indicated that the animals had received adequate systemic exposure to L-000900612. In general, there was no gender difference in the systemic exposures. L-000900612 did not accumulate after repeated daily administration. Similar to humans, the phenolic glucuronidate derivative, M2 (L-001277512), was the major metabolite and adequate exposure to this metabolite was achieved in the animal species used.

Single dose studies were performed in all three species at oral doses of 100, 250, 500, 1000, 1500, and 2000 mg/kg. The oral LD₅₀ was >2000 mg/kg in rats and mice and >1000 mg/kg (the highest dose studied) in dogs.

Single and multiple (up to 7) intravenous (IV) doses were studied in dogs and female rats. Deaths were associated with IV doses ≥ 200 mg/kg in rats and ~350 mg/kg in dogs. They were likely due to cardiac arrhythmia caused by the high amount of potassium in MK-0518 that was administered intravenously in a short amount of time.

At lower doses (≤ 100 mg/kg/day), i.e., lower amount of potassium, no cardiac effect or death was seen. However, MK-0518 caused irritation at the injection site and the surrounding area, marked increases in alanine aminotransferase and alkaline phosphatase without corresponding histopathological changes, and very slight renal cortex tubular dilatation and the associated small increase in blood urea nitrogen levels. The systemic exposure at IV dose of 100 mg/kg/day was approximately 5-fold higher than that at the same oral dose. It was approximately 3-fold higher than that for the high dose (360 mg/kg/day) used in the 52-week oral toxicity study in the same species.

Three-month oral toxicity studies were conducted in mice, rats, and dogs. Mice were given oral doses of 50, 500, 1000, 2500, and 5000 mg/kg/day. The maximum tolerated dose was definitely reached at 500 mg/kg/day since 20% of the animals died and body weight gain was 50% less than the concurrent controls. One of the notable histopathological changes was glandular multifocal mucosal erosion in stomach associated with the dose of 500 mg/kg/day. Similar histopathological change was seen in rats. There were a few other histopathological changes noted. The increases in their incidence rates and severity scores were small. Whether these histopathological changes persisted following longer term administration awaits the results from the 2-year carcinogenicity study in this species. The doses used in the 3-month studies in rats and dogs were considered not acceptable for meaningful toxicological assessment since the high doses were not a maximum tolerated dose, a maximum feasible dose, or a dose where saturation of absorption was reached. Since 26-week study in rats and 52-week study in dogs were ongoing using inadequate doses, high doses were increased during the studies after the results from the 5-week bridging studies which showed saturation of absorption at the high doses were performed. The doses used in the bridging study in rats were 150, 300, 450, and 600 mg/kg/day and in dogs were 125, 250, and 500 mg/kg/day. Emesis was the only adverse effect associated with the administration of MK-0518 in dogs. Rats exhibited dose-related increases in incidences of salivation, increased stomach inflammation, and nonglandular mucosal vacuolation. In a later study where adequate doses (30, 120, and 600 mg/kg/day) were given for one month in rats, the same effects to stomach were also observed. The no-adverse-effect levels (NOAELs) were 50, 120, and 500 mg/kg/day for mice, rats, and dogs, respectively.

In the longer-term studies (26 weeks in rats and 52 weeks in dogs), no additional toxicity was detected. In rats, the very slight nonglandular mucosal vacuolation progressed to glandular mucosal degeneration and erosion at the lower doses. In addition, inflammation of nose and nasopharynx was also seen and was believed to be associated with the irritation caused by aspiration of the drug formulation to nose and nasopharynx during gavage. The NOAEL for dogs was 360 mg/kg/day (the highest dose studied), and for rats was 30 mg/kg/day.

Genetic toxicology:

The genotoxic potential of L-000900612 was investigated in Ames test at concentrations up to 6000 $\mu\text{g}/\text{plate}$, *in vitro* alkaline elution/rat hepatocyte assay at concentrations up to 400 μM , chromosome aberration tests with Chinese Hamster Ovary cells at concentrations up to 1000 μM , and mouse micronucleus assay at 1500 mg/kg. Appropriate positive and negative controls were included. L-000900612 was found not to be mutagenic or genotoxic with or without metabolic activation.

Reproductive toxicology:

The effect of L-000900612 on fertility and early embryonic development was evaluated separately in male and female rats. Males treated with 100, 300, and 600 mg/kg/day L-000900612 were mated with untreated females. Females treated with 150, 300, and 600 mg/kg/day L-000900612 were mated with untreated males. No adverse effect in any of the reproductive parameters examined was observed. The NOAEL for both males and females was 600 mg/kg/day.

The effect of L-000900612 on the embryonic and fetal development was assessed in rats at oral doses of 100, 300, and 600 mg/kg/day and in rabbits at 100, 500, and 1000 mg/kg/day. In addition, maternal, fetal, and neonatal (through milk) exposures to L-000900612 were also evaluated in separate studies in pregnant rats and rabbits at similar doses. The results indicated that L-000900612 could cross placenta and be secreted in milk. Both fetuses and neonates were exposed to L-000900612 *in utero* and via milk. The study performed in rats was a combined Segments II and III reproductive toxicology study. *In utero* exposure to L-000900612 did not adversely affect embryo and fetal survival, fetal weight, and external, skeletal, and visceral development in rabbits at doses up to 1000 mg/kg/day (4-fold human exposure at 800 mg/day). Fetal plasma drug concentrations were about 2% of those in maternal plasma at 1 and 24 hours postdose, respectively. However, an increased incidence of supernumerary ribs in rat fetuses exposed *in utero* to 600 mg/kg/day (3-fold human exposure at 800 mg/day) raltegravir was observed. Mean drug concentrations in rat fetal plasma were approximately 1.5- to 2.5-fold greater than those in maternal plasma at 1 and 24 hours postdose, respectively. The NOAEL for the F1 generation was 1000 mg/kg/day for rabbits and <100 mg/kg/day for rats.

The results from the combined Segment II/III reproductive toxicology study in rats at oral doses of 0, 100, 300, and 600 mg/kg indicated that L-000900612 exerted no adverse effects on delivery, peri- and postnatal development, post-natal behavior, growth, sexual maturity, and fertility of the F₁ generation. The NOAEL for reproductive toxicity in F₁ generation was 600 mg/kg/day, providing a 3-fold safety margins for the clinical dose of 600 mg/day.

A juvenile oral toxicity study was also performed in 5 days old rats that were doses with 50, 200, and 600 mg/kg/day L-000900612 for 7 weeks. It was found that young rats had similar sensitivity to L-000900612 as adult rats. The same type of mucosal surface irritability was observed in 5-56 day-old rats administered the same dose range as adults. The NOAEL for juvenile rats was 200 mg/kg/day.

Local irritation and special toxicology:

The safety of L-000900612 was also investigated in a variety of *in vitro* and local tolerance studies. It was not a dermal sensitizer in the mouse local lymph node assay nor a skin irritant in *in vivo* rabbit dermal irritation model or *in vitro* Skin Model. It was not phototoxic in female rats nor hemolytic *in vitro* to blood cells isolated from rats, dogs, and humans. The irritation potential to bovine cornea was tested in two different testing laboratories and the results were equivocal. L-000900612 was classified as a severe irritant in the *in vitro* bovine corneal opacity and permeability test with an *in vitro* score higher than that for the positive control, imidazol. However, it was not considered a bovine corneal irritant in the same test performed in another lab.

Since it caused irritation to mucosal surfaces, it's not unexpected that it may be irritating to the cornea.

2.6.6.2 Single-dose toxicity

Study title: L-000900612: Exploratory acute oral-range finding study in mice. (Study TT #03-2616; non-GLP; Without QA statement; <\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4231-single-dose-tox\tt032616\tt032616.pdf>)

Results: The study was designed to support the dose selection for an *in vivo* micronucleus assay in mice. Three mice/sex/dose received a single oral dose of, 1000, 1500, or 2000 mg/kg L-000900612 and were observed for 3 days to determine the potential toxicity and approximate lethal dose (LD₅₀). One high dose male died on day 2. All of the high dose animals, 2 out of 3 mid dose males, and 1 out of 3 mid dose females exhibited treatment-related decreased activity, bradypnea, and ptosis within 10 minutes to 2 hours postdose and resolved on day 2. The no observed adverse effect level (NOAEL) for L-000900612 following a single oral dose in mice was 1000 mg/kg and LD₅₀ was >2000 mg/kg.

Study title: L-000900612: Acute oral toxicity study in rats (Study TT #03-2619; Merck Institute for Therapeutic Research, West Point, PA; Lot # L-000900612-003E009; GLP; With QA statement; study dates 10/24/2003- 11/10/2003; <\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4231-single-dose-tox\tt032619\tt032619.pdf>).

Results: Three female Sprague-Dawley rats, aged 44 days old, weighed 123-130 g received a single oral dose of 2000 mg/kg L-000900612 in a dosing volume of 20 ml/kg. The animals were observed daily for mortality and physical signs. Body weights were measured pretest, and on days 7 and 14. All rats were euthanized on day 14. No effects were observed on any of the parameters monitored. The lethal dose₅₀ in female rats was > 2000 mg/kg.

Study title: L-000900612: Exploratory single-rising dose oral tolerability study in dogs (Study TT#04-0080; Merck Institute for Therapeutic Research, West Point, PA; Lot #'s L-000900612-003E009 & L-000900612-003E013; non-GLP; Without QA report; Study dates 4/7/2004-5/19/2004; <\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4231-single-dose-tox\tt040080\tt040080.pdf>)

Results: Four female beagle dogs, 44-67 weeks old and weighed 8.5-10.3 kg, received single escalating oral doses of 0 (0.5% methylcellulose), 100, 250, 500, and 1000 mg/kg L-000900612 with a 7-day washout period between each dose. The dosing volume was 5 ml/kg. The animals were observed daily for clinical signs, weighed daily, and received blood collection at 0.25, 0.5, 1, 3, 6, 8, and 24 hours postdose. There was no mortality associated with any of the doses. Emesis was seen approximately 5 to 44 minutes postdose in 3 out of 4 dogs received 500 mg/kg dose and in all animals received 1000 mg/kg dose. Body weight gain was unaffected by the treatment. The toxicokinetic data are presented in the following table:

Dose (mg/kg)	100	250	500	1000
T _{max} (hr)	0.5	0.9 ± 0.1	0.7 ± 0.2	0.5 ± 0.0
C _{max} (µM)	49.6	64.7 ± 11.9	75.2 ± 21.9	94.8 ± 10.2
AUC _{0-24h} (µM•hr)	148	277 ± 31.0	353 ± 180	285 ± 44.5

Absorption was rapid with the mean C_{max} occurring within 1 hour postdose. Plasma elimination was also rapid with the mean trough concentrations less than 2% of their respective C_{max} values. Saturation of absorption occurred between 250 to 1000 mg/kg. Compared to rats, the maximum systemic exposure value for dogs was approximately 4-fold greater.

Study title: MK-0518: Exploratory single dose intravenous toxicity study in female rats (Study TT#06-2521; Merck Research Laboratories, West Point, PA; Lot #'s L-000900612-003E040; non-GLP; Without QA report; Study dates 3/22/2006-3/28/2006; \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\tt062521\tt062521.pdf)

Results: Nine female Sprague-Dawley rats, 52 days old and weighed 140-164 g, received a single intravenous dose of 100 (n=3), 200 (n=3), 400 (n=1), 800 (n=1), or 1600 (n=1) mg/kg MK-0518 with a dosing volume of 2.5, 5, 10, 20, or 40 ml/kg, respectively, and an infusion rate of about 2 ml/minute. The animals were observed daily for clinical signs for 7 days, weighed pretest and on day 7, and sacrificed on day 7. There was no effect on the body weight. However, mortalities and a variety of clinical signs as described in the following table were observed.

Dose (mg/kg)	100	200	400	800	1600
Total number examined	3	3	1	1	1
Deaths	0	1	1	1	1
Time of death	-	8 min postdose	4 min postdose	During dosing	During dosing
Missing tail	1	0	-	-	-
- # of days affected	2	-	-	-	-
Tail sign*	3	2	-	-	-
- # of days affected	7	6	-	-	-
Decreased activity	0	3	-	-	-
- # of days affected	-	-	-	-	-
Labored breathing	0	2	-	-	-
- # of days affected	-	1	-	-	-
Gasping	0	2	1	-	-
- # of days affected	-	1	1	-	-
Recumbency	0	2	1	-	-
- # of days affected	-	1	1	-	-

* Including: Purple, red, or black colored tail; sores.

Deaths were associated with ≥ 200 mg/kg/day. The observed mortalities and clinical signs were likely due to excessive amount of intravenously administered potassium associated with MK-0518 salt (a potassium salt). There was no NOAEL for the study.

2.6.6.3 Repeat-dose toxicity

Study title: Exploratory 7-day intravenous rising-dose tolerability study in dogs

Key study findings: One male and one female dog received escalating doses of 40, 100, and 400 mg/kg/day MK-0518 potassium salt by intravenous injection. Three doses were administered for each dose level. Only 358 mg/kg of the intended 400 mg/kg dose was administered before the male dog died. Emesis and unformed stool were associated with the 1st 100 mg/kg/day dose. However, no clinical signs were recorded prior to the death of the male dog. It was believed that the death was caused by cardiac arrhythmia induced by excessive high amount of potassium (88 mEq administered as one bolus slow intravenous infusion as compared to 1.25 mEq level that is recommended for safe intravenous infusion) rather than by the toxicity of MK-0518 molecule. Because of the death, the female dog was sacrificed without receiving the 400 mg/kg/day dose. At doses \geq 40 mg/kg/day, the female dog had one episode of scant feces and several episodes of intermittent trembling. No or partial weight bearing in the forelimb was observed after the administration of the 1st dose of 100 mg/kg/day dose to the female dog. The symptom was thought to be caused by the irritation that was induced by partial administration of dose subcutaneously. Excessive high levels (2-66-fold above pre-treatment ones) of alanine transferase, aspartate transferase, and alkaline phosphatase were associated with 100 mg/kg/day dose in both animals, though the male was more affected. These clinical chemistry changes did not have the corresponding histopathological effect on the liver. There was no NOAEL for the study.

Study no.: TT #06-1036

Volume # and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\tt061036\tt061036.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 3/21/2006

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: L-000900612 (monopotassium salt), lot# L000900612-003E040 — by HPLC

Method:

Doses: Escalating doses of 40, 100, and 400 (male only) mg/kg/day in which only 358 mg/kg/day was administered before death

Species/strain: Beagle dogs

Number/sex: 1

Route, formulation, volume, and infusion rate: Intravenous, dissolved in 0.9% (w/v) NaCl, injection rate of 30 ml/min, dosing volume of 10 ml/kg

Age: 38 and 47 weeks old

Weight: 10.5 and 8.2 Kg

Treatment duration: 3/dose

Frequency of dosing: Once a day

Observation and Times:

Clinical signs: Daily for mortality

Body weights: Pretest, days 3 and 6

Food consumption: Qualitative measure at pretest, days 2, 3, 5, and 6

Serum biochemistry: Pretest, days 4 and 7

Gross pathology: At termination on day 7 which was done because of the death of the male that died when 358 mg/kg of the intended 400 mg/kg was administered.

Histopathology: See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: The male died during the administration of intended 400 mg/kg on day 7.

Only 358 mg/kg was administered. No treatment-related physical signs were seen prior to death. MK-0518 is a potassium salt. The death was thought to be related to the potassium not the MK-0518 molecule itself. The maximum potassium concentration that can be administered safely by intravenous route is thought to be 0.125 mEq/kg/hour. Since the male dog weighed around 10 kg, the amount of potassium allowable per hour should be about 1.25 mEq to avoid heart arrhythmias. To administer 400 mg/kg dose of MK-0518 potassium salt, the male dog received approximately 8.8 mEq of potassium (in 358 mg/kg) as a slow bolus before it died. This level was 7 times the recommended safe level. Female was not dosed at this level.

Clinical signs: Emesis (yellow foamy or brown food) was seen in days 3 (3rd 40 mg/kg/day dose) and 4 (1st 100 mg/kg/day dose) and unformed stool observed on day 4 in the male. Intermittent trembling was recorded for the female dog 3 hours after the 40 mg/kg/day dose was administered. The trembling resolved prior to the administration on day 2 but persisted after the 2nd dose until pre-dose on day 5 (prior to the administration of the 2nd 100 mg/kg/day dose). The female dog also had scant feces and a non-weight bearing or partial-bearing forelimb which were observed immediately after the first dose at 100 mg/kg/day on day 4 but were resolved on the following day. The observation on the limb was considered not drug treatment-related but was secondary to irritation caused by a partial subcutaneous dose on day 4.

Body weights: No effect.

Food consumption: No effect.

Serum biochemistry: Alanine transferase, aspartate transferase, and alkaline phosphatase levels for the male dog when treated with intravenous doses of 100 mg/kg/day MK-0518 potassium salt were greatly elevated. Only alanine transferase in the female was elevated at the same dose level. The enzyme elevation was not accompanied by any histopathological changes in the liver.

Dose (mg/kg/day)	Male		Female	
	40	100	40	100
Alanine transferase	No change	+ 6655 %	No change	+ 906%
Aspartate transferase	No change	+ 678%	No change	No change
Alkaline phosphatase	No change	+ 279%	No change	No change

Histopathology: No effect.

Study title: Eight-day oral toxicokinetic study in female rats

Key study findings: Female rats were dosed with 600 mg/kg once or twice a day. Plasma samples were collected for toxicokinetic analysis. The results showed that absorption was rapid after the first daily dose, similar to those observed in other species and studies. Absorption after the 2nd daily dose was 6 times lower than that after the 1st

daily dose, resulting in similar AUC values obtained for the once and twice daily dosing regimens. Steady state for systemic absorption was probably achieved after 7 days of dosing since the AUC values for this study were similar to those attained in female rats received the same dose level for 4 and 26 weeks.

Study no.: TT #05-6030

Volume # and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt056030

Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret, Riom, France

Date of study initiation: 9/19/2005

GLP compliance: French GLP

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot# L000900612-003E017. — o by HPLC

Method:

Doses: 600 mg/kg once daily or twice daily (4 hours between doses)

Species/strain: Female rats/Sprague-Dawley, — CD(SD))

Number/dose: 15

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 80% PEG 400 (w/w), dosing volume 2.5 ml/kg

Age: 39 to 40 days old

Weight: 112-147 g

Treatment duration: 7 days

Frequency of dosing: Once or twice a day

Observation and Times:

Clinical signs: Daily for mortality

Body weights: Once in week one for drug calculation

Toxicokinetics: Blood samples collected from 5 rats/group/time point at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose for the once daily dose group and at 1, 2, 5, 6, 8, 12, and 24 hours after the first daily dose for the twice daily dose group on day 7.

Results:

Mortality: One female in the once daily dose group died after its last blood collection (6-hour time point) on day 7. The cause of death was probably relating to bleeding procedure, not to the drug treatment.

Toxicokinetics: Absorption was rapid in the once a day dose group, with mean T_{max} at 1 hour postdose. Plasma drug elimination was biphasic with the rapid phase between 1 and 4 hours postdose and the slow phase between 4 and 24 hours postdose. In the twice daily dose group, absorption rate after the first daily dose was similar to that in the once daily dose group. Absorption of the 2nd dose, however, was greatly reduced with the C_{max} value six times lower than that after the 1st daily dose. Plasma drug elimination was also biphasic with the rapid phase between 1 and 5 hours postdose and slow phase between 6 and 24 hours after the first daily dose (the 2nd daily dose was administered 4 hours after the 1st daily dose, making the 5-, 6-, and 24-hour time points corresponded respectively to 1, 2, and 20 hours after the 2nd daily dose). Drug elimination was rapid with C_{min} values less than 0.3-0.4% of the C_{max} ones. There were no substantial differences in the systemic exposure (AUC) or maximum plasma concentration (C_{max}) between once and twice a day dose groups, reflecting limited

absorption after the 2nd daily dose. The AUC and C_{max} values were similar to those obtained for females that had been dosed at the same dose for 4 and 26 weeks (Study TT #04-0079 and TT #04-6022), suggesting that steady state was attained on drug day 7 and was maintained until week 26 of dosing.

Dose (mg/kg)	600 once daily	600 twice daily
T _{max} (hr)	1.0	1.0
C _{max} (μM)	100 ± 27.7	68.9 ± 31.5
AUC _{0-24h} (μM•hr)	231 ± 44.6	267 ± 34.1

Study title: Exploratory 8-day intravenous toxicity study in dogs

Key study findings: Intravenous doses of 0, 30, and 100 mg/kg/day MK-0518 (potassium salt) were administered to 2 dogs/sex/dose. The effects on mortality, physical signs, clinical chemistry, and urinalysis parameters were monitored. Histopathological examination was performed on kidneys and liver only. In a previous study, mortality was associated with an intravenous dose of 358 mg/kg and thought to be caused by the high amount of potassium rather than the MK-0518 molecule itself. In this study, no mortality was observed. However, salivation and emesis/retching were seen in the MK-0518-treated groups, usually during the first 2-3 days of dose administration. It's unclear what caused these observations since MK-0518 was not administered orally. MK-0518 also caused local reactions (transient swelling and/or induration, and/or red/purple discoloration) at injection sites on forelimbs. These reactions likely caused small increases in total protein, globulin, and albumin/globulin ratio. One- to 10-fold increases in cholesterol, alanine aminotransferase, and alkaline phosphates levels were seen in the high dose animals without any corresponding histopathological changes. And small increases in the serum urea nitrogen levels were likely associated with the very slight dilatation of renal cortex tubules. Except for salivation, none of the effects were seen in dogs dosed orally with MK-0518 and were likely reflective of the toxicity of MK-0518 at high systemic exposure which was 5-fold higher than that at 100 mg/kg/day given orally.

Study no.: TT #06-6030

Volume # and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4237-other-tox-stud\42377-other\tt066030\tt066030.pdf

Conducting laboratory and location: Laboratoires Merck-Sharp & Dohme-Chibret, Merck Research Laboratories, Riom, France

Date of study initiation: 5/10/2006

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: L-000900612 (monopotassium salt), lot# L000900612-003E027 % by HPLC

Method:

Doses: 0, 30, and 100 mg/kg/day

Species/strain: Beagle dogs

Number/sex/dose: 2

Route, formulation, volume, and infusion rate: Intravenous, dissolved in 0.9% (w/v)

NaCl, injection rate of 30 ml/min, dosing volume of 5 ml/kg

Age: 34 and 45 weeks old

Weight: 5.8-8.8 kg for females and 7.4-11.0 kg for males

Treatment duration: 7 days

Frequency of dosing: Once a day

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest and day 7

Food consumption: Days 2, 3, and 7

Hematology: Pretest and day 6

Serum biochemistry: Pretest and day 6

Urinalyses: Overnight urine collections on day 6

Toxicokinetics: Blood samples collected at 0.25, 0.5, 1, 3, 6, 8, and 24 hours postdose on day 7

Gross pathology: At termination on day 7

Histopathology: Only kidneys from all animals and liver from control and 100 mg/kg/day animals were examined.

Results:

Mortality: None.

Clinical signs: Emesis/retching and postdose salivation were seen during the early period of the study but stopped mostly after 3 days of dosing. Changes at injection sites were observed started after 3 days of intravenous administration. The clinical signs at forelimb were seen a day later, suggesting that they were likely related to the swelling, induration, and discoloration at the injection site.

Dose (mg/kg/day)	Male			Female		
	0	30	100	0	30	100
Number examined	2	2	2	2	2	2
Emesis/retching						
- # affected	0	1	2	0	0	1
- Days affected	-	Day 1	Days 1-3	-	-	Days 2, 7
Salivation						
- # affected	0	0	1	0	0	1
- Days affected	-	-	Days 2-3	-	-	Days 2-3
Injection site changes*						
- # affected	0	1	2	0	1	2
- Days affected	-	Days 3-6	Days 3-8	-	Days 3-7	Days 3-8
Forelimb swelling						
- # affected	0	0	2	0	0	1
- Days affected	-	-	Days 4-8	-	-	Days 4-8
Forelimb discoloration						
- # affected	0	0	2	0	0	1
- Days affected	-	-	Days 4-7	-	-	Days 3-8

* Including: induration, swelling, red and/or purple discoloration

Body weights: One high dose male and one high dose female showed a 6 to 7% body weight loss at the end of study.

Food consumption: No effect.

Hematology: One high dose male had increased total leukocyte, absolute neutrophil, and absolute monocyte counts (+149%, +275%, and +152%, respectively) as compared to the control. These changes were considered secondary to the treatment-related local changes (consisted of persistent swelling and red/purple discoloration of the left forelimb) in this animal starting on day 4.

Serum biochemistry:

Dose (mg/kg/day)	Male						Female					
	0		30		100		0		30		100	
Animal number	0218	0022	0034	0062	0012	0046	0049	0103	0043	0045	0013	0101
Urea nitrogen	-	-	-	-	↑31%	-	-	-	-	-	↑64%	↑47%
Total protein	-	-	-	-	-	↑13%	-	-	-	-	-	↑18%
Globulin	-	-	-	-	-	↑26%	-	-	-	-	-	↑33%
A/G ratio	-	-	-	-	-	↓21%	-	-	-	-	-	↓23%
Alanine amino-transferase	-	-	-	-	↑1537%	↑1967%	-	-	-	-	↑237%	↑313%
Alkaline phosphatase	-	-	-	-	↑374%	↑124%	-	-	-	-	-	↑314%
Cholesterol	-	-	-	-	-	-	-	-	-	-	-	↑102%

There were small increases of urea nitrogen levels relative to the pretest values in 3 out of 4 high dose animals. The mean urea nitrogen levels in the high dose were also higher than those of the concurrent control. The correlated histomorphological change was slight tubular dilatation in kidneys. The small changes in total protein and globulin levels and A/G ratio (as compared to the pretest values) were probably secondary to the marked treatment-related local changes (persistent swelling and red/purple discoloration of the forelimbs) observed from day 4 onward in these animals. The marked increases in alanine aminotransferase and alkaline phosphatase levels (as compared to both pretest and concurrent control values) were seen in all of the high dose animals. However, no corresponding histopathological change was observed in the liver of these animals.

Urinalysis: No effect.

Histopathology: At 100 mg/kg/day, treatment-related very slight multifocal tubular dilatation in renal cortex was seen in 3 out of 4 dogs. The tubular dilatation was characterized by dilated proximal tubules lined by cuboid or flattened basophilic epithelium.

Toxicokinetics: There was no gender difference in systemic exposure. Plasma elimination was rapid and biphasic. The mean plasma concentrations at 24 hours postdose were less than 1% of their respective $C_{0.25hr}$ concentrations.

Dose (mg/kg)	Male		Female	
	30	100	30	100
$C_{0.25hr}$ (µM)	100	295	91.8	273
AUC_{0-24hr} (µM•hr)	180 ± 13.4	722 ± 242	209 ± 13.6	609 ± 200

Study title: L-000900612: Nineteen-day oral toxicokinetic study in mice

Key study findings: To find the best formulation with the best pharmacokinetic profile, 500 mg/kg L-000900612 in three different vehicles, 0.5% methycellulose, 80% PEG 400, and 20% sucrose/4% hydroxypropylcellulose/0.19% sodium lauryl sulfate, were administered to mice for 18 days and their pharmacokinetics analyzed. It was found that there were no substantial gender differences in the pharmacokinetic parameters amongst the three formulations. The absorption and elimination profiles were also similar. The AUC and C_{max} values were lowest for the 80% PEG 400 vehicle group, however, the differences were not substantial (less than 1 fold).

Study no.: TT #05-0079

Volume # and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt050079\tt050079.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, Merck Research Laboratories, West Point, PA

Date of study initiation: 6/16/2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612; lot# L-000900612-003E017 — % pure by HPLC

Methods:

Doses: 500 mg/kg

Species/strain: Mouse — CD1@(ICR)

Number/sex/dose (main study): 22

Route, formulation, volume, and infusion rate: Oral gavage, 0.5% (w/v) methylcellulose in water; 80% (w/w) PEG400 in water; 20% (w/w) sucrose/4% (w/w) hydroxypropyl cellulose-super low/0.19% (w/w) sodium lauryl sulfate in water, dosing volume of 10 ml/kg except for the 80% PEG400 group which received 2.5 ml/kg as dosing volume

Age: 64 days old

Weights: Male: 30.4-43.0 g; Female: 21.9-32.2 g

Treatment duration: 18 days

Frequency of dosing: Once a day

Observation and Times:

Clinical signs: Daily for mortality

Body weights: Pretest, once in week 1, and once per week thereafter for the purpose of dose calculation

Toxicokinetics: Blood samples collected from 3-4 mice/sex/group/time point at approximately 0.5, 1, 3, 8, 12, and 24 hours postdose in week 3

Results:

Mortality: One animal in the 0.5% methylcellulose group and one in the 20% sucrose/4% HPC/0.19% SLS group were found dead in days 16 and 18, respectively.

Body weights: No effect

Toxicokinetics: There were no substantial sex-related differences in systemic exposure or C_{max} values among the three formulations. In addition, the absorption and elimination profiles for the three formulations were also similar. Maximum plasma concentrations occurred at 0.5 hr postdose, indicating rapid absorption. Elimination from plasma was biphasic, with rapid phase between 0.5 to 3 hours postdose and slow phase between 3 and 12 hours postdose. Mean trough levels were less than 1% of their respective C_{max} values. The AUC and C_{max} values for the 20% sucrose/4% HPC/0.19% SLS vehicle group were highest, those for 0.5% methylcellulose vehicle group intermediate, and those for 80% PEG 400 the lowest. The high values for the 20% sucrose/4% HPC/0.19% SLS vehicle group were the results of high concentrations from three animals within the early sampling time points. Thus, they were not considered different from the other groups. 0.5% methylcellulose was chosen as the vehicle for future toxicology studies in mice since it was the vehicle

used in the dog toxicology studies and the toxicokinetic parameters showed the least gender differences.

Dose (mg/kg)	500					
Vehicle	0.5% methylcellulose		80% PEG 400		20% sucrose/4% HPC/0.19% SLS	
Sex	M	F	M	F	M	F
T _{max} (hr)	0.5	0.5	0.5	0.5	0.5	1.0
C _{max} (µM)	31.8 ± 3.29	35.1 ± 5.06	18.4 ± 3.32	26.3 ± 2.37	50.8 ± 32.7	48.1 ± 40.6
AUC _{0-24h} (µM•hr)	80.1 ± 7.56	75.5 ± 8.83	48.3 ± 5.32	63.3 ± 5.46	83.3 ± 20.6	143 ± 52.2

Study title: MK-0518: One-month oral toxicity study in rats

Key study findings: Sprague-Dawley rats were administered 0 (80% PEG400), 30, 120, and 600 mg/kg/day MK-0518 for one month. There was no clear drug-related effect on all of the parameters monitored in this study except for slightly increased stomach inflammation and nonglandular mucosal vacuolation in stomach. Dose-related post-dose salivation was also observed in the doses ≥ 120 mg/kg/day. These effects were very slight in nature and not dose-limiting. Maximum tolerated dose was not achieved in this study. The no-effect level for the study was 30 mg/kg/day.

Study no.: TT #06-6055

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4237-other-tox-stud\42376-imp\tt066055\tt066055.pdf

Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret, Riom, France

Date of study initiation: 10/18/2006

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: MK-0518; Lot # L-000900612-003E038: — % pure by HPLC

Methods:

Doses: 0, 30, 120, and 600 mg/kg/day

Species/strain: Sprague-Dawley rats

Number/sex/dose (main study): 10

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 80:20 (w/w) PEG 400 to water, dosing volume of 2.5 ml/kg

Age: 37-38 days old

Weight: 100-134 g for females & 118-166 g for males

Treatment duration: 4 weeks

Frequency of dosing: Once a day

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest, once in week 1, and twice per week thereafter

Food consumption: Cageside examination for the presence of food twice a week at time of dosing

Ophthalmoscopy: Control and high dose groups in week 4

Hematology: Weeks 2 and 4

Serum biochemistry: Weeks 2 and 4

Urinalysis: Samples obtained from 10 rats/sex/dose in week 4

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, mid dose, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: None.

Clinical signs: There were no tables with summary or individual animal data included in the study report. Incidence of post-dose (within 0.5 hours) salivation increased in a dose-related manner and was probably attributed to the palatability of the formulation.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effects were claimed. However, no summary or individual animal data were included in the study report.

Hematology: No effect.

Serum biochemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: Dose-related very slight increases in inflammation and nonglandular mucosal epithelial vacuolation in the stomach were observed. The effects on stomach were observed in rats treated with similar dose range for a similar duration.

Dose (mg/kg/day)	Male				Female			
	0	30	120	600	0	30	120	600
Number examined	10	0	10	10	10	0	10	10
Stomach --								
<i>Inc. inflammation</i> % affected	0	-	0	20	0	-	0	30
Mean severity	0.0	-	0.0	1.0	0.0	-	0.0	1.0
<i>Nonglandular mucosal epithelial vacuolation</i> % affected	0	-	0	60	0	-	0	50
Mean severity	0.0	-	0.0	1.0	0.0	-	0.0	1.0

Severity score: 1 = very slight; 2 = slight or small; 3 = moderate; 4 = marked; 5 = severe

Study title: L-000900612: Five week oral toxicokinetic study in mice

Key study findings: Toxicokinetic parameters were investigated for doses of 50, 500, 1000, and 2500 mg/kg L-000900612. Deaths were seen at 500 (2 out of a total of 78), 1000 (5 out of a total of 78), and 2500 (7 out of a total of 34). All of the mice in 1000 and 2500 mg/kg groups were sacrificed without collecting plasma samples for toxicokinetic determination. There were no sex differences in either the C_{max} or AUC values for the 50 and 500 mg/kg groups. Absorption was rapid occurring 0.5 hour after dosing. The initial phase of plasma drug elimination was rapid between 0.5 and 2 hours and slower between 2 and 8 hours. The overall plasma drug elimination was rapid with the trough concentrations that were less than 1% of their respective C_{max} values. The systemic exposure and C_{max} values were less than dose proportional between 50 and 500 mg/kg/day.

Study no.: TT #05-1034

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt051034\tt051034.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, West Point, PA

Date of study initiation: 2/17/2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612-003E017; — % pure by HPLC

Methods:

Doses: 0, 50, 500, 1000, and 2500 mg/kg/day

Species/strain: Mouse/—:CD1@(ICR)

Number/sex/dose (main study): 34

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 0.5% (w/v) methylcellulose in water, dosing volume of 10 ml/kg

Age: 38-39 days old

Weight: Male: 25.7- 33.2 g; Female: 15.8-28.1 g

Treatment duration: up to 30 days

Frequency of dosing: Once a day

Sampling times for TK study: 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose in week 5

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest and once or twice per week thereafter for dose calculation only

Results:

Mortality: Animals in the 1000 and 2500 mg/kg group were sacrificed during drug week 3 because of the high rate of mortality and no collection of plasma samples were performed for them.

Dose (mg/kg/day)	Male				Female			
	50	500	1000	2500	50	500	1000	2500
# of animals	34	34	34	-	34	34	34	34
# of deaths	0	1	3	-	0	1	2	7
Week of death	-	4	2 & 3	-	-	2	1 & 3	2

Toxicokinetics: No toxicokinetic parameters were calculated for 1000 and 2500 mg/kg dose group because of the high mortality rate. There were no sex differences in either the C_{max} or AUC values for the 50 and 500 mg/kg groups. Absorption was rapid, occurring 0.5 hour after dosing. The initial phase of plasma drug elimination was rapid between 0.5 and 2 hours and slower between 2 and 8 hours. The overall plasma drug elimination was rapid with the trough concentrations that were less than 1% of their respective C_{max} values. The systemic exposure and C_{max} values were less than dose proportional between 50 and 500 mg/kg/day.

Dose (mg/kg/day)	Male		Female	
	50	500	5	500
T_{max} (hr)	0.5	0.5	0.5	0.5
C_{max} (μ M)	8.14 \pm 1.13	25.5 \pm 12.7	10.6 \pm 0.684	17.5 \pm 1.28
AUC _{0-24h} (μ M-hr)	14.6 \pm 2.60	40.0 \pm 7.01	14.7 \pm 1.12	50.4 \pm 3.46

Study title: L-000900612: Five-week oral toxicity study in rats

Key study findings: Fifteen rats/sex/dose received oral doses of 0, 150, 300, or 600 mg/kg/day L-000900612 for 4 weeks. The study was used as a bridging study since the 3-month toxicology study in the same species did not employ adequate high dose. There was no clear drug-related effect on any of the parameters monitored in this study. Dose-related salivation and very slight increases in stomach inflammation and stomach nonglandular mucosal epithelial vacuolation were observed. These effects were very slight in nature and not dose-limiting. The maximum tolerated dose was not achieved in this study. The toxicokinetic results suggested that saturation of absorption may not have been reached in the doses studied. The sponsor claimed that the top dose of 600 mg/kg was the maximum feasible dose because of the difficulties in achieving a consistent solution in the vehicle chosen.

Study no.: TT #04-0079

Volume #, and page #: \\Cdseub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt040079\tt040079.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 6/22/04

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612; Lot # L-000900612-003E014: ———% pure by HPLC

Methods:

Doses: 0, 150, 300, 450, and 600 mg/kg/day

Species/strain: Sprague-Dawley rats

Number/sex/dose (main study): 15

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 80:20 (w/w) PEG 400 to water, dosing volume of 2.5 ml/kg

Satellite groups used for toxicokinetics or recovery: No additional rats were included for TK blood sampling. Blood samples were taken from 3-4 rats/sex/dose/time point (each rats bled no more than twice) that were assigned to the main study.

Age: 38 days old

Weight: 106-139 g for females & 136-177 g for males

Treatment duration: 4 weeks

Frequency of dosing: Once a day

Sampling times for TK study: From 3-4 rats/sex/dose/time point at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose in week 4

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest, once in week 1, and twice per week thereafter

Food consumption: Cageside examination for the presence of food twice a week at time of dosing

Ophthalmoscopy: Control and high dose groups in week 4

Hematology: Weeks 2 and 4

Serum biochemistry: Weeks 2 and 4

Urinalysis: Samples obtained from 10 rats/sex/dose in week 4

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: Two females in the 450 mg/kg/day group were found dead in weeks 2 and 4. The cause of these two deaths was associated with complication of bleeding procedure. One female in the 600 mg/kg/day group had to be sacrifice during week 4 because of the inflammation in brain. This death was also deemed not related to treatment since this particular finding has been observed in the same testing facility. There were no summary or individual animal data included in the report.

Clinical signs: There were no tables with summary or individual animal data included in the study report. Salivation was increased in drug-treated animals as compared to controls and was probably attributed to the palatability of the formulation.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effect was claimed. However, no summary or individual animal data were included in the study report.

Hematology: No effect.

Serum biochemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology:

Dose (mg/kg/day)	Male					Female				
	0	150	300	450	600	0	150	300	450	600
Number examined	15	15	15	15	15	15	15	15	15	15
Stomach –										
<i>Inc. inflammation</i>										
% affected	0	0	20	53	53	0	0	0	27	33
Mean severity	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	1.0	1.0
<i>Nonglandular mucosal epithelial vacuolation</i>										
% affected	0	13	47	40	40	0	7	13	40	33
Mean severity	0.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	1.0
Harderian gland –										
<i>Increased pigmentation</i>										
% affected	0	-	-	-	7	0	-	-	-	0
Mean severity	0.0	-	-	-	1.0	0.0	-	-	-	0.0
Eye –										
<i>Unilateral phthisis bulbi</i>										
% affected	0	-	-	-	0	0	-	-	-	27
Mean severity	0.0	-	-	-	0.0	0.0	-	-	-	2.0
<i>Unilateral retina atrophy</i>										
% affected	0	-	-	-	0	0	-	-	-	7
Mean severity	0.0	-	-	-	0.0	0.0	-	-	-	2.0
Severity score: 1 = very slight; 2 = slight or small; 3 = moderate; 4 = marked; 5 = severe										

Dose-related increases in the incidence and severity of increased inflammation and nonglandular mucosal epithelial vacuolation in the stomach were observed. Some of the high dose females also had slight unilateral phthisis bulbi. One high dose female

has slight unilateral retina atrophy and one high dose male had increased pigmentation in Harderian gland.

Toxicokinetics: There were no consistent and substantial sex-related differences in the systemic exposures to L-000900612 and L-001277512 (glucuronide metabolite, M2) or C_{max} values between 150 mg/kg/day and 450 mg/kg/day. But at 600 mg/kg/day, the values were 2-fold greater in males than in females. This observation was in contrast to the findings of the previous 13-week study where the AUC value in females was three times higher than that in males at a dose of 120 mg/kg. It's unclear what the reason for the inconsistencies. The C_{max} and AUC values were, in general, dose proportional for both the parent and the metabolite.

Absorption and metabolism to the major glucuronide derivative were rapid in all doses and in both sexes. Peak plasma concentration for L-001277512 was reached within 2 hours of dosing. Decreases in plasma concentrations of both the parent compound and the glucuronide metabolite were generally biphasic and rapid.

Based on the body surface conversion, the high dose of 600 mg/kg in rats was approximately similar to the dose of 250 mg/kg in dogs. The systemic exposures at these dosages in both species were also similar.

Dose (mg/kg/day)		Male				Female			
		150	300	450	600	150	300	450	600
L-000900612	T_{max} (hr)	0.5	1.0	1.0	2.0	0.5	0.5	1.0	0.5
	C_{max} (μ M)	17.3 \pm 4.75	47.3 \pm 19.7	37.6 \pm 17.6	91.5 \pm 35.0	12.3 \pm 3.00	42.3 \pm 32.0	40.4 \pm 30.0	46.8 \pm 27.6
	AUC _{0-24h} (μ M-hr)	34.3 \pm 3.92	64.3 \pm 15.6	77.5 \pm 17.6	234 \pm 60.3	29.8 \pm 2.61	73.1 \pm 22.1	80.8 \pm 24.9	104 \pm 25.7
L-001277512	T_{max} (hr)	0.5	1.0	1.0	2.0	0.5	1.0	1.0	1.0
	C_{max} (μ M)	24.4 \pm 2.48	40.6 \pm 10.3	33.8 \pm 11.4	98.0 \pm 44.5	19.0 \pm 3.91	34.7 \pm 15.4	33.8 \pm 17.1	41.9 \pm 17.7
	AUC _{0-24h} (μ M-hr)	60.4 \pm 6.31	97.9 \pm 12.7	92.6 \pm 12.9	235 \pm 76.6	52.1 \pm 3.72	84.9 \pm 17.2	90.9 \pm 14.4	116 \pm 18.5

Study title: L-000900612: Five week oral toxicity in dogs

Key study findings: Three dogs/sex/dose received oral doses of 0, 125, 250, or 500 mg/kg/day L-000900612 for 4 weeks. The study was designed to find a high dose that was a maximum tolerated dose or one that has reached the saturation of absorption. The results of the previous single rising dose study seemed to suggest that the saturation of absorption occurred around 250 mg/kg. Thus, the high dose of this multiple dose study was set at 500 mg/kg. At this dose, no effect was observed on all the parameters monitored. Emesis after dosing was seen in the dose groups that received doses 250 mg/kg and higher. However, it was transient and subsided after 2 weeks of dosing. Thus, the effect was clearly not dose-limiting. The toxicokinetic data suggested a trend to plateau of exposures to both the parent compound, L-000900612, and its phenolic glucuronidation metabolite, L-001277512, at 500 mg/kg/day. On day 1, the AUC value for 500 mg/kg dose increased 1.6 fold over that for the 250 mg/kg while on week 4 the increase was only 1.2 fold. At 500 mg/kg, the AUC value in dogs was approximately 8-folds over that for the proposed clinical dose of 800 mg/day.

Study no.: TT #04-9811

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt049811

Conducting laboratory and location: Tsukuba Safety Assessment Laboratories, Banyu Pharmaceutical Co., Ltd., Tsukuba, Ibaraki, Japan

Date of study initiation: 7/21/04

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612 potassium salt, lot# L-000900612-003E017;
— pure by HPLC

Methods:

Doses: 0, 125, 250, and 500 mg/kg/day

Species/strain: Beagle dogs

Number/sex/dose (main study): 3

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 0.5% (w/v) methycellulose in water, dosing volume of 5 ml/kg

Satellite groups used for toxicokinetics or recovery: No separate dogs were used for TK blood sampling.

Age: 39-40 weeks old

Weight: 7.2-9.8 kg for females & 8.6-10.8 for males

Treatment duration: 5 weeks

Frequency of dosing: Once a day

Sampling times for TK study: 0.25, 0.5, 1, 3, 6, 9, and 24 hours postdose in day 1 and week 4.

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest and once per week thereafter

Food consumption: Pretest and 4 times per week, except in weeks 2 and 4 when blood collection was performed, then three times per week

Ophthalmoscopy: Pretest and week 4

EKG: Pretest, weeks 2 and 4

Hematology: Weeks 2 and 4

Serum biochemistry: Weeks 2 and 4

Urinalysis: Week 4

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: None

Clinical signs: Emesis was observed in all dogs in the mid and high dose groups and 2 males at the low dose group. It occurred within 30 minutes after dosing and was first observed in week 1. The incidence decreased thereafter. The mid and high dose females did not exhibit sign of emesis starting on week 3. There was no line listing of individual animal or summary data in the report.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effect was claimed. However, no summary or individual animal data were included in the study report.

Hematology: No effect.

Serum biochemistry: One male dog in the high dose group had an approximately 8-fold increase in alanine aminotransferase (ALT) value in week 2 as compared to controls. By week 4, ALT value in this dog had returned to near baseline. Systemic exposure to L-000900612 in this dog was higher than its dose cohorts in week 2, yet decreased to a value comparable to the dose group cohort in week 4. The increase in ALT was not associated with any gross or histomorphologic changes in liver.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: No effect.

Toxicokinetics: The results of this toxicokinetic study confirmed most of the findings from the previous 13-week toxicity study. There were no sex differences in either the C_{max} or AUC values. Absorption was rapid in all doses and in both sexes. Plasma drug elimination was generally biphasic and rapid and repeated dosing did not impact on the C_{max} or AUC values. The AUC values in day 1 increased dose proportionally between doses of 250 and 500 mg/kg, but less than dose proportional after 4 weeks of continuous daily drug administration. The AUC value for 500 mg/kg dose increased 1.6-fold over that for the 250 mg/kg while on week 4 the increase was only 1.2-fold, suggesting that saturation of absorption may be reached at 500 mg/kg/day. Thus, the 500 mg/kg should be acceptable as the high dose for the 9-month toxicity study. The toxicokinetic profile for its major metabolite, M2 (L-001277512), was similar to that of the parent compound. It appeared rapidly in the plasma and reaching the maximum concentrations within 2 hours. Decreases in the plasma concentration were also rapid for all doses, with the mean trough levels no greater than 5% of their respective C_{max} values. The systemic exposure to this metabolite increased less than dose proportionality, reaching a plateau at the dose of 250 mg/kg/day. The individual AUC values from 2 out of 6 dogs in the 500 mg/kg/day group overlapped with those of several dogs in the 250 mg/kg/day group.

Dose (mg/kg/day)		Male			Female			
		125	250	500	125	250	500	
L-000900612	T_{max} (hr)	Day 1	0.4 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.5 ± 0.0	0.8 ± 0.2	0.4 ± 0.1
		Week 4	0.4 ± 0.1	0.5 ± 0.0	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
	C_{max} (µM)	Day 1	72.8 ± 17.3	98.5 ± 32.9	116 ± 21.3	85.7 ± 8.50	75.7 ± 15.5	143 ± 9.21
		Week 4	82.4 ± 9.96	86.4 ± 7.17	124 ± 10.7	60.7 ± 9.05	96.3 ± 4.67	106 ± 21.8
	AUC _{0-24h} (µM-hr)	Day 1	213 ± 66.9	355 ± 135	524 ± 267	238 ± 50.2	293 ± 115	550 ± 117
		Week 4	316 ± 3.94	368 ± 47.9	432 ± 54.9	228 ± 33.7	365 ± 26.1	453 ± 102
M2	T_{max} (hr)	Week 4	1.0 ± 0.0	1.0 ± 0.0	1.7 ± 0.7	0.8 ± 0.2	0.8 ± 0.2	2.3 ± 0.7
	C_{max} (µM)	Week 4	8.67 ± 0.814	10.4 ± 0.167	15.1 ± 1.68	5.76 ± 0.612	9.46 ± 1.66	20.6 ± 7.32
	AUC _{0-24h} (µM-hr)	Week 4	41.6 ± 2.42	51.6 ± 6.87	77.0 ± 10.3	28.2 ± 1.78	56.4 ± 13.6	98.9 ± 33.3

Study title: L-000900612: Fourteen-week oral range-finding study in mice

Key study findings: Fifteen mice/sex/dose received oral doses of 0 (0.5% methylcellulose), 50, 1000, 2500, and 5000 mg/kg/day L-000900612 for 13 weeks. Excessive mortality was associated with doses ≥ 1000 mg/kg/day (33-60%). Mortality was likely associated with gastrointestinal bloating that was caused by the irritating effect of the drug which was also evident in the 500 mg/kg/day dose group. Three males at this dose group were either found dead or had to be sacrificed early because of the clinical signs associated with gastrointestinal bloating. At 500 mg/kg/day, the body weight gain after 13 weeks of drug administration was 58% and 71% that for the control. Thus, 500 mg/kg/day was considered the maximum tolerated dose. The no adverse effect level for this study was 50 mg/kg/day.

Study no.: TT #05-1023

Volume #, and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt051023\tt051023.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, West Point, PA

Date of study initiation: 1/18/2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot# L-000900612-003E017, \longrightarrow % pure by HPLC

Methods:

Doses: 0, 50, 500, 1000, 2500, and 5000 mg/kg/day

Species/strain: Mouse/ \longrightarrow CD1®(ICR)

Number/sex/dose (main study): 15

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 0.5% (w/v) methylcellulose in water, dosing volume of 5 ml/kg

Age: 45 days old

Weight: 20.3-28.1 g for females and 24.5-35.3 g for males

Treatment duration: 13 weeks

Frequency of dosing: Once a day

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest, once in week 1, and twice per week thereafter

Food consumption: Once per week over a 4-day period

Ophthalmoscopy: Pretest on all animals, week 6 on 1000 mg/kg animals, and week 12 on all surviving 500 and 1000 mg/kg animals.

Hematology: At termination (week 13)

Serum biochemistry: At termination (week 13)

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, 50, and 500 mg/kg/day animals only; See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality and clinical signs: The deaths at 5000 mg/kg/day group occurred on days 2 to 6. All the animals in this group that were sacrificed early or found dead exhibited distended abdomen, labored breathing, audible respiratory noises, decreased activity, hunched posture, eye partially closed, pale, cool to touch, decreased skin turgor, intermittent trembling, and/or sternally recumbent. In general, most of these treatment-related signs were first observed on day 2 and increased in incidence and severity with increased dosing days. Because of the high mortality and excessive physical signs, all of the remaining animals in this dose group were sacrificed on day 8.

The deaths at the 2500 mg/kg/day group occurred between days 4 to 37. Majority of animals in this group exhibited distended abdomen, labored breathing, audible respiratory noises, decreased activity, hunched posture, eye partially closed, and/or intermittent trembling by week 2 and 5 in males and females, respectively. In general, these signs increased in incidence and severity prior to early termination of this dose group on days 27 and 38 for males and females, respectively.

The deaths at the 1000 mg/kg/day group occurred between days 14 to 44. Distended abdomen, labored breathing, audible respiratory noises, decreased activity, eye partially closed, pale, cool to touch, and/or decreased skin turgor were observed beginning on day 11 and were seen in the majority of males and females by week 3 and 5, respectively. Because of the high mortality and increased in incidence and severity of these treatment-related clinical signs, the rest of the animals in this dose group were sacrificed on day 45.

At 500 mg/kg/day group, distended abdomen, labored breathing, audible respiratory noises, decreased activity, hunched posture, and/or eye partially closed were seen in a few females in weeks 4 to 13 and in majority of males in weeks 5 to 11. These signs were recorded in the males as early as day 20.

No mortality or treatment-related clinical signs were associated with 50 mg/kg/day dose group.

Dose (mg/kg/day)	Male					Female				
	50	500	1000	2500	5000	50	500	1000	2500	5000
# of animals	15	15	15	15	15	15	15	15	15	15
# of deaths	0	3	5	9	7	0	0	7	8	6
% mortality	0	20	33	60	47	0	0	47	53	40
Week of death	-	3, 6, 10	2, 4, 7	1, 2, 3, 4	1, 2	-	-	2, 4, 5, 6, 7	2, 4, 5, 6	1

Body weights: The body weight gain for doses ≥ 500 mg/kg was reduced as compared to the vehicle control. The reduction was apparent and dose-related starting on week 4. After 13 weeks of dosing, the body weight gain for the 500 mg/kg/day group was 58% and 71% of the control for males and females, respectively. 500 mg/kg/day was considered the maximum tolerated dose based on this parameter.

Dose (mg/kg/day)	Male					Female				
	0	50	500	1000	2500	0	50	500	1000	2500
Δ in body wt. (g)										
Week 4	4.1	4.0	3.2	1.0	0.6	-	-	-	-	-
Week 5	-	-	-	-	-	4.2	4.9	3.5	0.8	2.8
Week 7	6.0	5.9	3.8	2.4	-	4.5	5.2	3.9	0.5	-
Week 13	7.4	7.6	4.3	-	-	6.9	7.8	4.9	-	-

Food consumption: The food consumption was reduced at doses ≥ 500 mg/kg/day which may have caused the reduced body weight gain at the same doses.

Dose (mg/kg/day)	Male						Female					
	0	50	500	1000*	2500*	5000*	0	50	500	1000*	2500*	5000*
Mean food consumption (g)	5.2	5.4	4.5	4.5	4.6	4.6	4.7	4.8	4.4	3.84	4.4	4.0

* Food consumption was based on the mean of weeks 1-6, 1-5, and 1 for doses of 1000, 2500, and 5000 mg/kg, respectively

Ophthalmoscopy: No effect was claimed. However, no summary or individual animal data were included in the study report.

Hematology: No effect (only the 50 and 500 mg/kg/day groups were monitored).

Serum biochemistry: No effect (only the 50 and 500 mg/kg/day groups were monitored).

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: Histopathological changes were seen in various organs and tissues.

Similar to rats, mice were sensitive to the irritation to the mucosal surface caused by L-000900612. The irritation was manifested in mice as stomach glandular mucosal multifocal erosion and focal inflammation of trachea. One male in the 500 mg/kg/day had very slight osteoarthritis. Although only one out 30 animals was affected, this was an unusual finding and thus noted for comparison when carcinogenicity study results become available. There was also only 1 out of 30 animals noted with Harderian gland cellular infiltration. However, the rats that were administered with oral doses of 600 mg/kg/day L-000900612 for 4 weeks also had some histopathological findings in their eyes and Harderian gland. Increased incidence of thymus depletion was also observed. The toxicological significance of these two histopathological changes was unclear and required confirmation by the results of the two-year carcinogenicity study.

Dose (mg/kg/day)	Male			Female		
	0	50	500	0	50	500
Number examined	15	15	15	15	15	15
<i>Bone -</i>						
Osteoarthritis	% affected	0	0	7	0	0
	Mean severity	0.0	0.0	1.0	0.0	0.0
<i>Eye -</i>						
Harderian gland cellular infiltration	% affected	0	0	0	0	7
	Mean severity	0.0	0.0	0.0	0.0	2.0
<i>Stomach -</i>						
Glandular mucosal multifocal erosion	% affected	0	0	13	0	0
	Mean severity	0.0	0.0	2.5	0.0	0.0
<i>Trachea -</i>						
Focal inflammation	% affected	-	-	-	0	7
	Mean severity	-	-	-	0.0	2.0
<i>Thymus -</i>						
Depletion	% affected	13	0	20	7	7
	Mean severity	1.5	0.0	1.7	1.0	3.0
Severity score: 1 = very slight; 2 = slight or small; 3 = moderate; 4 = marked; 5 = severe						

Study title: L-000900612: Fourteen-week oral toxicity study in rats

Key study findings: Fifteen rats/sex/dose received 0 (80% PEG 400), 30, 90, or 120 L-000900612 for 13 weeks. This was not a well-designed study. There were missing (summary or individual animal) data. Even though no effects were associated with the doses studied, the toxicokinetic data indicated that the doses used for this study were inappropriately selected. At the doses studied, the systemic exposure to L-000900612 was flat with respect to doses in males. The systemic exposure in females was 3-times that of the males at the same dose. The maximum tolerated dose was not found and the toxicity profile not defined in this study.

Study no.: TT #03-119-0

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt031190\tt031190.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 9/29/2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot# L-000900612-003E009: ———, pure by LCAP

Methods:

Doses: 0, 30, 90, and 120 mg/kg/day

Species/strain: Sprague-Dawley rats —: CD®(SD)IGS BR

Number/sex/dose (main study): 15

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 80% PEG400, dosing volume of 2.5 ml/kg

Satellite groups used for toxicokinetics or recovery: No separate rats were used for TK blood sampling. Blood samples were taken from 3-4 rats/sex/dose/time point (each rats bled no more than twice) that were assigned to the main study.

Age: 43 days old

Weight: 119-154 g for females & 176-222 g for males

Treatment duration: 13 weeks

Frequency of dosing: Once a day

Sampling times for TK study: 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose during week 12/13

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest, once in week 1, twice/week thereafter except for weeks 4, 8, 9, and 12

Food consumption: Twice weekly except for week 12 when no recording took place

Ophthalmoscopy: Control and high dose in weeks 6 and 12

EKG: None

Hematology: Weeks 4, 8, and 12 from all surviving rats

Serum biochemistry: Weeks 4, 8, and 12 from all surviving rats

Urinalysis: Weeks 8 and 12 from 10 rats/sex/dose

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: All of the deaths were caused by gavage or bleeding accidents.

Doses (mg/kg/day)	Male				Female			
	0	30	90	120	0	30	90	120
# animals/group	15	15	15	15	15	15	15	15
# rats died/sacrificed	1	1	0	0	2	0	0	0

Clinical signs: There was treatment-related pre- and/or postdose salivation that lasted 1-35 minutes in all dose groups beginning in week 1 and continuing until study termination. However, neither the summary or individual animal data were included.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effect was claimed. However, no summary or individual animal data were included in the study report.

Hematology: No effect.

Serum biochemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: No effect.

Toxicokinetics: There was a clear sex difference in the systemic exposure to L-000900612. At the same dose, the AUC value in females was three times higher than that in males. At the doses selected for the males, the systemic exposures were similar and not increasing with increased doses. Absorption was rapid in all doses and in both sexes. Plasma drug elimination was generally biphasic and rapid with the mean trough concentrations less than 3% of their respective C_{max} values. Saturation of absorption was reached for males at 30 mg/kg/day.

Dose (mg/kg/day)	Male			Female		
	30	90	120	30	90	120
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (μ M)	12.1 \pm 4.61	13.0 \pm 4.13	6.40 \pm 1.23	28.4 \pm 6.86	35.2 \pm 9.60	66.0 \pm 25.6
AUC_{0-24h} (μ M-hr)	23.1 \pm 3.14	20.9 \pm 2.60	21.9 \pm 2.01	37.0 \pm 4.86	47.0 \pm 8.95	76.3 \pm 18.4

Study title: L-000900612: Fourteen-week oral toxicity study in dogs

Key study findings: Four dogs/sex/dose received oral doses of 0 (0.5% methylcellulose), 5, 15, or 45 mg/kg/day L-000900612 for 13 weeks. No effect on the parameters monitored was associated with oral administration of L-000900612. The systemic exposures increased roughly linearly with the increasing doses. There was no sex difference in the pharmacokinetic parameters. However, it's clear that the maximum tolerated dose was not achieved in this study. The AUC value at high dose of 45 mg/kg/day was similar to that for the 800 mg dose in humans.

Study no.: TT #03-118-0

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt031180\tt031180.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA & Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France

Date of study initiation: 9/16/2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot # L-000900612-003E009, — pure by HPLC

Methods:

Doses: 0, 5, 15, and 45 mg/kg/day

Species/strain: Beagle dogs

Number/sex/dose (main study): 4

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 0.5% methylcellulose in water, dosing volume of 5 ml/kg

Satellite groups used for toxicokinetics or recovery: No additional rats were included for TK blood sampling. Blood samples were taken from 3-4 rats/sex/dose/time point (each rats bled no more than twice) that were assigned to the main study.

Age: 38-41 weeks old

Weight: 6.9-9.5 kg for females & 7.6-10.8 kg for males

Treatment duration: 13 weeks

Frequency of dosing: Once a day

Sampling times for TK study: 0.25, 0.5, 1, 3, 6, 9, and 24 hours postdose after the 1st dose and during week 13

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest and once per week thereafter

Food consumption: Four times per week at approximately 15 minutes prior to dosing

Ophthalmoscopy: Pretest, weeks 6 and 11

EKG: Pretest, weeks 4, 8, and 12

Hematology: Pretest, weeks 4, 8, and 12

Serum biochemistry: Pretest, weeks 4, 8, and 12

Urinalysis: Overnight collection in weeks 8 and 12

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory; Fresh liver and bile samples were collected at necropsy and processed to determine copper content by ICP-OES.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: None.

Clinical signs: No effect.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effects were claimed. However, no summary or individual animal data were included in the study report.

EKG: No effect.

Hematology: No effect.

Clinical chemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: No effect.

Toxicokinetics: There were no sex differences in either the C_{max} or AUC values. The systemic exposures increased linearly with increasing doses. Absorption was rapid in all doses and in both sexes. Plasma drug elimination was generally biphasic and rapid with the mean trough concentrations less than 0.4% of their respective C_{max} values. Repeated dosing for 13 weeks did not impact on the C_{max} or AUC values which was consistent with rapid elimination of the drug.

Dose (mg/kg/day)	Male			Female		
	5	15	45	5	15	45
T_{max} (hr)						
Day 1	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.2
Week 13	0.6 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.5 ± 0.2
C_{max} (μM)						
Day 1	9.07 ± 2.18	15.3 ± 2.22	28.9 ± 4.37	6.95 ± 1.36	12.0 ± 0.70	33.3 ± 3.89
Week 13	6.60 ± 1.21	13.1 ± 2.82	29.6 ± 2.49	5.36 ± 0.73	14.0 ± 1.51	22.8 ± 3.72
AUC _{0-24h} (μM-hr)						
Day 1	17.8 ± 2.94	39.1 ± 2.82	70.0 ± 6.35	15.8 ± 3.04	37.4 ± 4.48	72.2 ± 5.1
Week 13	15.6 ± 2.09	30.5 ± 5.02	72.8 ± 9.20	13.6 ± 0.60	42.5 ± 3.19	71.7 ± 5.56

Study title: L-000900612: Twenty-seven-week oral toxicity study in rats

Key study findings: The rats were treated with 0, 30, 120, or 90/600 mg/kg/day of L-000900612. The high dose animals received 90 mg/kg/day dose for 9 weeks and 600 mg/kg/day for 18 weeks. The treatment effects that can be attributed to the dosing with L-000900612 were mortality, a reduction in the body weight gain, and audible respiratory sounds in the high dose male group, post-dose salivation in the mid and high dose groups, dose-related increases in the incidences of very slight degeneration of stomach glandular mucosa and very slight to slight inflammation of nose and nasopharynx in the mid and high dose groups. There were 3 deaths in males attributed to the drug treatment, in addition to a 14% reduction in body weight gain as compare to the concurrent control group. Thus, the maximum tolerated dose was reached for males at 600 mg/kg/day. A death in the 600 mg/kg/day females was seen without the accompanied reduction in food consumption and weight loss. Irritation of the stomach was seen in many high dose animals, however, since the samples showed autolysis, it's unclear whether the death was caused by the irritation to the stomach. In addition, the incidence and severity of the stomach histopathological findings did not increase as compared to those in the 5-week study at the same dose. The difference in the level of toxicity between the sexes cannot be explained by the exposure to L-000900612 since there was no sex difference in the systemic drug exposures. The adverse events seen in the treated females were not considered dose limiting toxicities. Since the irritation to the nose and nasopharynx was dose-related and more severe in males than females, the death in female may have been caused by inhalation of excess drug into the respiratory

tract. Thus, the maximum tolerated dose was probably not reached in the high dose females. The no adverse effect level (NOAEL) for this study was 120 mg/kg/day.

Study no.: TT #04-6022

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt046022\tt046022.pdf

Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France

Date of study initiation: 6/15/2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot #'s L-000900612-003E014 (weeks 1-11) & L-000900612-003E017 (weeks 11-27), — pure by HPLC

Methods:

Doses: 0, 30, 120, and 600/90 mg/kg/day; high dose rats received 90 mg/kg/day of L-000900612 from days 1-58 (week 9) and 600 mg/kg/day from day 59 to the scheduled termination

Species/strain: Sprague-Dawley rats/—CD®(SD)IGS BR

Number/sex/dose (main study): 20

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 80% PEG400, dosing volume of 2.5 ml/kg

Satellite groups used for toxicokinetics or recovery: No separate rats were used for TK blood sampling. Blood samples were taken from 3-4 rats/sex/dose/time point (each rats bled no more than twice) that were assigned to the main study.

Age: 36-37 days old

Weight: 87-122 g for females; 119-161 g for males

Treatment duration: 26 weeks

Frequency of dosing: Once a day

Sampling times for TK study: 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose in week 26

Observation and Times:

Clinical signs: Daily for mortality and physical signs

Body weights: Pretest, once in week 1, twice/week in weeks 2-13, and once per week thereafter

Food consumption: Twice weekly in week 1-13, twice per week every 4 weeks thereafter at the time of dosing to determine whether any food remained

Ophthalmoscopy: Control and high dose animals only in weeks 12 and 25

Hematology: Weeks 3, 13, and 26 from all surviving rats

Serum biochemistry: Weeks 3, 13, and 26 from all surviving rats

Urinalysis: Weeks 13 and 26 from 10 rats/sex/dose

Gross pathology: At termination

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: The female rat died in week 14. The 4 male rats died in weeks, 12, 21, 22, and 26. One male died in week 12 because of dosing error while the cause of death for the others was unknown. It was claimed that on the days preceding death, the animals exhibited weight losses, unfinished food, staining on the abdominal and/or thoracic zones, weakness, decreased motor activity and/or irregular and/or labored breathing. However, these clinical observations cannot be verified since summary or individual animal data containing the clinical observations are not included in the report.

Doses (mg/kg/day)	Male				Female			
	0	30	120	600/90	0	30	120	600/90
# animals/group	20	20	20	20	20	20	20	20
# rats died/sacrificed	0	0	0	4	0	0	0	1

Clinical signs: It was described in the text under “Results” section that there was treatment-related pre- and/or postdose salivation that lasted less than 15 minutes in the 90/600 and 120 mg/kg/day dose groups beginning in day 1 and continuing until study termination. The number of affected animals was dose-related. In addition, abnormal respiratory sounds or whistling sounds were heard in the 90/600 mg/kg/day group starting from week 10 (a week after dosing with 600 mg/kg/day) with up to a maximum daily incidence of 10/38 affected animals in week 20. However, no summary or individual animal data were included for verification.

Body weights: The body weight gain of the high dose males was significantly reduced (~14.5%) as compared to the control. No effect on the body weight gain was observed in any of treated female groups.

Doses (mg/kg/day)	Male				Female			
	0	30	120	90/600	0	30	120	90/600
Body wt. change (g)	327	320	310	280***	153	154	152	149

Food consumption: It is claimed that the high dose males had increased incidences of uneaten food in weeks 17, 21, and 25. No effect was claimed for the female groups. The actual data as presented in summary or individual animal table were not included in the report.

Ophthalmoscopy: No effect was claimed. No actual and verifiable data were included in the report.

Hematology: No effect.

Serum biochemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: Percentage of animals and mean severity score for glandular mucosal degeneration in the stomach and inflammation in nose and nasopharynx were increased with increasing dose of L-000900612. One high dose male also exhibited stomach glandular mucosal erosion. The findings in nose and nasopharynx may be a result of irritation cause by aspiration of drug into the nasal passages during the dosing procedures.

The histological samples for the stomach of the animals that died before scheduled sacrificed (3 males and 1 female) showed autolysis thus, the cause of death was not determined for them. However, liver necrosis, adrenal cortex hypertrophy,

cardiomyopathy, lymphoid depletion in spleen and thymus, kidney tubular degeneration, decreased secretion in prostate and seminal vesicle, and histiocytosis in the lymph node were common findings in these animals. These findings may be related to the degradation of the general condition of the animals.

Doses (mg/kg/day)	Male				Female			
	0	30	120	90/600	0	30	120	90/600
Number examined	20	20	20	20	20	20	20	20
<i>Stomach –</i>								
Glandular mucosal degeneration								
% animal affected	0	0	10.0	35	0	0	10.0	30
Mean severity	0.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00
Glandular mucosal erosion								
% animal affected	0	0	0	5	0	0	0	0
<i>Nose –</i>								
Inflammation								
% animal affected	0	0	20.0	95.0	0	-	10.0	50.0
Mean severity	0.00	0.00	1.00	1.84	0.00	-	1.00	1.30
<i>Nasopharynx –</i>								
Inflammation								
% animal affected	5.00	-	10.0	80.0	0	-	0	25.0
Mean severity	1.00	-	1.00	1.50	0.00	-	0.00	1.00

The severity scores are: 1 = very slight; 2 = slight; 3 = moderate; 4 = marked; 5 = severe

Toxicokinetics: There were no sex differences in the systemic exposures to L-000900612 or its metabolite, L-001277512. The systemic exposures, in general, increased with increasing doses between mid and high dose groups. Absorption was rapid in all doses and in both sexes with the maximum plasma concentrations occurring between 0.5 to 2 hours. Plasma drug elimination was generally biphasic and rapid at mid and high dose with the mean trough concentrations less than 3% of their respective C_{max} values. The terminal elimination was slow (between 12 to 24 hours).

Dose (mg/kg/day)	Male			Female		
	30	120	90/600	30	120	90/600
L-000-900612	T_{max} (hr)	0.5	0.5	2.0	0.5	1.0
	C_{max} (μ M)	12.8 \pm 7.43	10.6 \pm 3.43	60.5 \pm 18.5	17.1 \pm 7.83	30.4 \pm 20.3
	AUC_{0-24h} (μ M-hr)	21.9 \pm 3.83	37.4 \pm 9.56	169 \pm 31.5	36.4 \pm 4.86	77.7 \pm 27.3
L-00-1277512	T_{max} (hr)	0.5	0.5	2.0	1.0	0.5
	C_{max} (μ M)	6.79 \pm 2.55	6.60 \pm 1.75	21.5 \pm 3.60	9.11 \pm 2.04	10.3 \pm 2.37
	AUC_{0-24h} (μ M-hr)	18.9 \pm 1.97	26.5 \pm 4.24	79.7 \pm 6.74	25.2 \pm 2.35	32.7 \pm 5.47

Study title: L-000900612: A 53-week oral gavage toxicity study in the beagle dogs with a twenty-seven week interim necropsy

Key study findings: Dogs was treated with 0 (0.5% methycellulose), 15, 90, or 360 mg/kg/day L-000900612 orally for 27 weeks. In the original study design, doses of 0, 5, 15, and 90 mg/kg/day were used. However, the results of the 5-week bridging toxicity study suggested that 90 mg/kg/day was not a maximum tolerated dose, thus, the animals in the 5 mg/kg/day group were dosed with 360 mg/kg/day starting from week 15. The dogs at this dose group were exposed to this higher dose for a total of 38 weeks. Except for emesis seen in animals dosed with 360 mg/kg/day, there was no treatment-related effect in any of the parameters monitored. The toxicokinetic data

indicated that 360 mg/kg/day dose was approaching saturation of absorption. The NOAEL for this study was 360 mg/kg/day.

Study no.: TT #04-9001

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\tt049001\tt049001.pdf

Conducting laboratory and location: _____

Date of study initiation: 5/7/2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot # L-000900612-003E017, _____, pure by LCAP, L-000900612-003E013 _____, pure by LCAP, and L-000900612003E015, _____, pure by LCAP

Methods:

Doses: 0, 5/360, 15, and 90 mg/kg/day

Species/strain: Beagle dogs

Number/sex/dose: 8 (4 assigned to interim necropsy and 4 to final necropsy)

Route, formulation, volume, and infusion rate: Oral gavage, dissolved in 0.5% methylcellulose in water, dosing volume of 5 ml/kg

Satellite groups used for toxicokinetics or recovery: None

Age: 28-30 weeks old

Weight: 5.9-8.8 kg for females & 6.5-10.5 kg for males

Treatment duration: 52 weeks

Frequency of dosing: Once a day

Sampling times for TK study: 0.25, 0.5, 1, 3, 6, 9, and 24 hours postdose after the 1st dose and during week 13

Observation and Times:

Clinical signs: Three times/day for mortality and physical signs; detailed physical examination performed weekly

Body weights: Pretest and once per week thereafter

Food consumption: Qualitative food consumption monitored daily

Ophthalmoscopy: Pretest, weeks 12, 26, 38, and 52

EKG: Pretest, weeks 12, 26, 38, and 52

Hematology: Pretest, weeks 4, 12, 25, 39 and 51

Serum biochemistry: Pretest, weeks 4, 12, 25, 39 and 51

Urinalysis: Overnight collection during pretest and weeks 12, 25, 39, and 51

Gross pathology: Interim sacrifice at week 27 and final sacrifice at week 53

Organ weights: See histopathology inventory in Appendix

Histopathology: Control, high dose, and found-dead/moribund animals; See appendix for the histopathology inventory.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: None.

Clinical signs: Emesis following dosing, ranging from slight to severe, was noted in the animals that had their dose increased from 5 to 360 mg/kg/day in week 15. In

most of the animals, emesis was noted for about 7 days following the increase in dose level and was occasionally observed throughout the study.

Body weights: No effect.

Food consumption: No effect.

Ophthalmoscopy: No effect was claimed. No actual and verifiable data were included in the report.

EKG: No effect.

Hematology: No effect.

Clinical chemistry: No effect.

Urinalysis: No effect.

Gross pathology: No effect.

Organ weights: No effect.

Histopathology: No effect.

Toxicokinetics: There were a no sex differences in either the C_{max} or AUC values. The systemic exposures increased linearly with increasing doses up to 90 mg/kg/day but less than dose proportionality between 90 and 360 mg/kg/day suggesting near maximum exposure at 360 mg/kg/day. Absorption was rapid in all doses and in both sexes. Plasma drug elimination was generally biphasic and rapid with the mean trough concentrations less than 1% of their respective C_{max} values. Repeated dosing for 52 weeks did not impact on the C_{max} or AUC values which was consistent with rapid elimination of the drug and maintenance of steady state.

Dose (mg/kg/day)	Male				Female			
	5*	15	90	360*	5*	15	90	360*
T_{max} (hr)								
Week 13	0.5 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	-	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	-
Week 26	-	0.6 ± 0.1	0.7 ± 0.1	0.4 ± 0.0	-	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
Week 52	-	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	-	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.0
C_{max} (µM)								
Week 13	3.77 ± 0.44	10.5 ± 1.07	45.9 ± 4.53	-	4.01 ± 0.42	13.5 ± 2.08	62.2 ± 4.35	-
Week 26	-	11.2 ± 1.61	40.1 ± 2.75	63.5 ± 5.89	-	12.6 ± 1.20	45.4 ± 4.49	91.6 ± 6.10
Week 52	-	9.77 ± 2.10	61.5 ± 5.05	75.7 ± 4.77	-	13.1 ± 1.81	62.1 ± 8.32	99.1 ± 10.3
AUC_{0-24h} (µM-hr)								
Week 13	9.64 ± 0.80	31.8 ± 3.17	143 ± 5.53	-	11.6 ± 1.12	42.4 ± 3.51	199 ± 18.3	-
Week 26	-	30.9 ± 2.99	127 ± 5.39	204 ± 18.3	-	36.8 ± 2.20	156 ± 24.4	255 ± 31.1
Week 52	-	29.2 ± 4.2	143 ± 7.56	266 ± 28.7	-	36.6 ± 4.18	174 ± 27.6	280 ± 31.1

* Animals were treated at 5 mg/kg/day for 14 weeks and received 360 mg/kg/day starting from week 15. Thus, the TK values for this group at week 13 were for the 5 mg/kg/day dose while those at week 26 were for the 360 mg/kg/day.

**Appears This Way
On Original**

Histopathology inventory

Study	#06-1036	#04-0079	#04-9811	#05-1023	#06-6055	#03-1190	#03-1180	#04-6022	#04-9001
Species	Dogs	Rats	Dogs	Mice	Rats	Rats	Dogs	Rats	Dogs
Adrenals		X*	X*	X	X*	X*	X*	X*	X*
Aorta					X				X
Bone marrow smear	X	X	X	X	X	X	X	X	X
Bone		X (femur)	X (rib)	X (femur)	X (femur)	X (femur)	X (rib)	X (femur)	X (femur)
Brain	X	X*	X*	X*	X*	X*	X*	X*	X*
Cecum	X				X				X
Cervix					X				X
Colon	X	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X	X
Epididymis		X	X	X	X	X	X	X	X*
Esophagus		X	X	X	X	X	X	X	X
Eye		X	X	X	X	X	X	X	X
Fallopian tube									
Gall bladder	X		X	X			X		X*
Gross lesions		X	X	X	X	X	X	X	X
Harderian gland		X		X	X	X		X	
Heart	X	X*	X*	X*	X*	X*	X*	X*	X*
Hypophysis									
Ileum	X	X	X	X	X	X	X	X	X
Injection site	X								
Jejunum	X	X	X	X	X	X	X	X	X
Kidneys	X	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland									
Larynx									X
Liver	X	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X	X	X	X	X
Lymph nodes, cervical		X		X	X	X		X	
Lymph nodes, submandibular									
Lymph nodes, mesenteric		X	X	X	X	X	X	X	X
Mammary Gland		X	X	X	X	X	X	X	X
Nasal cavities								X	
Optic nerves		X	X	X	X	X	X	X	X
Ovaries		X*	X*	X	X*	X*	X*	X*	X*
Pancreas		X	X	X	X	X	X	X	X
Parathyroid		X	X	X	X	X	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 glands		X	X	X	X	X	X	X	X
Sciatic nerve		X	X	X	X	X	X	X	X
Seminal vesicles									
Skeletal muscle	X	X	X	X	X	X	X	X	X
Skin		X	X	X	X	X	X	X	X
Spinal cord		X	X	X	X	X	X	X	X
Spleen	X	X*	X*	X*	X*	X*	X*	X*	X*
Sternum									X
Stomach	X	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
Trachea					X				X
Urinary bladder		X	X	X	X	X	X	X	X
Uterus		X	X	X	X	X	X	X	X
Vagina					X				X
Zymbal gland									
Peyer's patch					X				
Nasopharynx								X	
Lymph node, retropharyngeal			X				X		X

X, histopathology performed
* organ weight obtained

2.6.6.4 Genetic toxicology

Study title: L-000900612: Microbial mutagenesis assay.

Key findings: Not mutagenic.

Study no.: TT #03-8029, TT #03-8059, TT #03-8063, and TT #03-8068

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\tt038029\tt038029.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, West Point, PA 19486.

Date of study initiation: 6/10/2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000900612-000Y008 for study TT #03-8029 & L-000099612-003E009 for studies TT #03-8059, TT #03-8063, & TT #03-8086: — pure by HPLC

Methods:

Strains/species/cell line: *Salmonella* strains TA1535, TA97a, TA98, & TA100

E. coli strains WP2 uvrA pKM101

Doses used in definitive study: 30, 100, 300, 1000, 3000, & 6000 µg/plate

Basis of dose selection: 6000 µg/plate was the highest dose used in the exploratory assay.

Negative controls: DMSO

Positive controls: 1 or 2 µg/plate and 2 or 5 µg/plate 2-aminoanthracene with and without S-9 metabolic activation, respectively, for *Salmonella* strains and *E. coli* strain WP2 uvrA pKM101; 0.75 µg/plate sodium azide for strains TA100 & TA1535, 1.5 µg/plate ICR-191 for strain TA97a, 1.0 µg/plate 2-Nitrofluorene for strain TA98, 1 µg/plate 4-nitroquinoline-N-oxide for strain WP2 uvrA pKM101 without S-9 metabolic activation.

Incubation and sampling times: 48 hours

Results:

Study validity: Three replicate cultures per dose were used. Two independent experiments were performed. The assay per experiment was considered positive if the number of revertant colonies induced was at least 2-fold higher than the solvent negative control with an evident dose-related increase. The positive controls showed appropriate S-9 and strain-dependent increase in revertants. A precipitate was seen on plates at a test concentration of 6000 µg/plate. Inhibition of bacterial lawn growth and revertant growth was noted at ≥ 3000 µg/plate in certain strains. In study numbers TT #03-8063 and TT #03-8068, only *E. coli* strain was used because the control values were outside of the acceptable range for *E. coli* in study number TT #03-8059.

Study outcome: The increases in revertants relative to negative control either with or without S9 metabolic activation did not exceed 2-fold. L-000900612 was considered not mutagenic.

Study title: L-000900612: *In vitro* alkaline elution/rat hepatocyte assay.

Key findings: Not mutagenic.

Study no.: TT #03-8381 and TT #03-8394

Volume #, and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\tt038381\tt038381.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, West Point, PA 19486.

Date of study initiation: 10/21/2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000099612-003E00^o,  pure by HPLC

Methods:

Strains/species/cell line: Primary rat hepatocytes isolated from male Sprague-Dawley rats

Doses used in definitive study: 100, 200, 300, and 400 μ M

Basis of dose selection: Drug has to be soluble in the tested medium for this assay.

The results from the range-finding study showed that L-000900612 precipitated from culture medium at 450 μ M but was soluble at \leq 400 μ M.

Negative controls: Water

Positive controls: 1 mM dimethylnitrosamine and 3 Gy γ -radiation

Incubation and sampling times: 3 hours

Results:

Study validity: Duplicate cultures per dose and positive control were used.

Quadruplicate samples were used for the negative control. Separate plates of culture medium, without cells, were also prepared at some compound concentrations incubated at 37°C for 15 minutes and 3 hours and examined using dark-field microscopy to check for the presence of insoluble precipitate. L-000900612 was soluble up to 400 μ M.

Cytotoxicity was determined for each sample by the trypan-blue exclusion method (immediately after harvest and after a 3-hour recovery) and luciferin-luciferase chemiluminescence assay to measure the cellular ATP content (immediately after harvest). The relative survival measured immediately after treatment with L-000900612 ranged from 93-102% and from 94-101% when measured after 3-hour recovery incubation over the concentration range of 100 to 400 μ M. The cellular ATP content as a percentage of the control ranged from 81-99% over the same concentration range. Thus, the cytotoxicity of the measured samples was considered acceptable.

Induction of DNA strand breaks was measured by lysing rat hepatocytes on the filter for 30 minutes, eluting the DNA from the filter with an alkaline solution, precipitating the DNA, and quantitating the amount of DNA as the fluorescent product of 3,5-diaminobenzoic acid and deoxyribose. The elution slope values were calculated from semi-logarithmic plots of the fraction of DNA retained on the elution filter (log axis) versus elution time (linear axis) and expressed as the absolute value of the average rate of elution per 3-hour fraction for the terminal phase (3 to 9 hours) of alkaline elution. The theory for the assay was that the rate of elution of single-stranded (resulting from genotoxin-induced DNA strand breaks), alkaline-denatured DNA through 2.0 μ m pore filters was different from that of the double-stranded DNA.

The criteria for a positive result were (1) the compound, at concentrations soluble in the culture medium, produced an induced elution slope (the mean treatment slope

minus the mean negative control slope) which is ≥ 0.020 ; (2) any induced slope of ≥ 0.020 should not be associated with significant cytotoxicity which was defined as when cell viability, as measured by trypan blue exclusion either immediately after harvest or after a 3-hour recovery incubation, was reduced to less than 70% of the control values and/or when the cellular ATP content measured immediately after harvest was reduced to less than 50% of the control.

Study outcome: The results indicated that L-000900612 did not produce an induced slope of ≥ 0.020 at any concentration tested. The positive controls, dimethyl-nitrosamine and 3 Gy γ -radiation, produced an induced elution slope of 0.316 and 0.127, respectively, with 102-106% relative viability and 8-100% relative cellular ATP content immediately after the treatment and 98-100% relative viability after a 3-hour recovery incubation. The results from the positive control indicated that the assay was performing as expected. Thus, L-000900612 was not considered genotoxic in this assay.

Study title: L-000900612: Assay for chromosomal aberrations *in vitro*, in Chinese Hamster Ovary Cells

Key findings: Not mutagenic.

Study no.: TT #03-8681 and TT #03-8687

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\tt038681\tt038681.pdf

Conducting laboratory and location: Merck Institute for Therapeutic Research, West Point, PA 19486.

Date of study initiation: 10/10/2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000099612-003E009; — pure by LCAP

Methods:

Strains/species/cell line: Chinese hamster ovary cells, sub clone WBL, at ≤ 15 passages since cloning

Doses used in definitive study:

3-hour treatment time: 100, 200, 400, 600, 800, and 1000 μM with S-9 activation (200, 400, and 600 μM scored);
100, 200, 400, 600, 800, and 1000 μM without S-9 activation (100, 200, and 400 μM scored);
20-hour treatment time: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 μM without S-9 activation (50, 250, and 550 μM scored)

Basis of dose selection: Precipitation was clearly visible in the cultures with or without S-9 activation at concentrations ≥ 750 μM . The cell growth after the 3-hour treatments was 81% and 67% of the control with and without S-9 activation, respectively, at 750 μM . The cell growth was 54 and 38% of the control at 250 and 500 μM , respectively, after 20 hour treatment period. Thus, the top dose of 600 μM was chosen for the definitive chromosomal aberration assay.

Negative controls: Distilled water

Positive controls: 0.5, 1.0, and 4.0 μ M cyclophosphamide with S-9 activation; 0.05 and 0.15 μ M mitomycin without S-9 activation

Incubation and sampling times: 3 hours with and without S-9 activation and 20 hours without S-9 activation

Results:

Study validity: A single culture per dose was used. One of the studies was a range finding study and the other a definitive chromosomal aberration assay. Chromosomal analysis was performed only in the definitive assay. Two hundred metaphase cells were scored. However, if 200 acceptable cell spread was not available, the maximum number of usable cells was scored. A test was considered positive if there were statistically significant increases ($p \leq 0.05$) over concurrent solvent controls in the percentages of cells with chromosomal aberrations at 2 or more separate concentrations of test article without greatly exceeding a 50% reduction in growth. The high-dose positive controls with and without S-9 activation induced significant increase in aberrations over the concurrent negative controls, however, the aberration level at the low dose mitomycin C group was not statistically increased. The sponsor claimed that such an increase was not a criterion for an acceptable assay and deemed the assay valid.

Study outcome: There were no statistical increases in the percentages of cells with aberrations at any dose of L-000900612 as compared to the negative control. The test was considered negative by the criteria set for the assay.

Study title: L-000900612: Assay for micronucleus induction in mouse bone marrow

Key findings: Not genotoxic.

Study no.: TT #04-8619

Volume #, and page #: [\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\tt048619\tt048619.pdf](#)

Conducting laboratory and location: Merck Research Laboratories, West Point, PA.

Date of study initiation: 3/23/2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000099612-003E009; \checkmark pure by HPLC

Methods:

Strains/species/cell line: Male \checkmark CD-1®(ICR) BR mouse, 6 weeks old, weighed 25.1-33.7 g

Doses used in definitive study: 375, 750, and 1500 mg/kg

Basis of dose selection: In an exploratory acute oral range-finding study in mice, 1 out of 3 males and no female died after receiving a single 2000 mg/kg dose. Decreased activity, ptosis, and bradypnea were associated with 1500 and 2000 mg/kg doses in both males and females. Thus, 1500 mg/kg dose was selected to be the high dose. No effect was associated with 375 and 750 mg/kg doses.

Negative controls: 80% PEG 400/20% deionized water

Positive controls: 0.35 mg/kg and 2 mg/kg mitomycin C

Incubation and sampling times: 24 and 48 hours postdose

Results:

Study validity: A single dose of vehicle, L-000900612, or positive controls were administered to male rats orally with a dosing volume of 20 ml/kg (10 ml/kg for positive controls). Bone marrow cells were harvested from 5 mice/group/time point at 24 or 48 (vehicle and L-000900612 dose groups only) hours postdose. Two thousand polychromatic erythrocytes (PCE)/mouse were counted and analyzed for micronuclei, and the frequencies of PCE and normochromatic erythrocytes (NCE) were recorded based on 1000 erythrocytes per mouse. The assay was considered positive if a significant increase in the frequency of micronucleated PCE ($p < 0.05$) occurred at a minimum of 2 dose levels when compared with the concurrent vehicle control mean (either 2 doses at a given sacrifice time or 1 dose at each of 2 sacrifice times). Mitomycin at 0.35 and 2 mg/kg doses induced 4 and 59-fold increases, respectively, in the number of micronucleated PCE.

Study outcome: All mice treated with 1500 mg/kg dose exhibited decreased activity, bradypnea, and ptosis with 45 minutes of dosing but the clinical signs subsided to normal at 3 hours postdose. There were no statistically significant increases in micronucleated PCE in any of the L-000900612 treatment groups in either 2 sampling time as compared to the vehicle control. The positive control, mitomycin C induced highly significant increases in micronucleated PCE in bone marrow of mice treated with mitomycin C.

2.6.6.5 Carcinogenicity

Two-year carcinogenicity studies in rats and mice are ongoing. The study will be completed in the 4th quarter of 2007 expected. The doses selected for the rat study were 0, 50, 150, and 300 mg/kg/day in males and 0, 50, 300, and 600 mg/kg/day in females. The high doses were selected as the maximum tolerated dose and the maximum feasible dose, respectively. The doses selected for the mice study were 0, 50, 100, and 250 mg/kg/day in males and 0, 50, 250, and 400 mg/kg/day in females. The high doses were the maximum tolerated doses based on data from the 13-week toxicity study. The doses were approved by the Executive Carcinogenicity Assessment Committee and the meeting minutes are attached in Appendix I.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: L-000900612: Oral fertility study in females rats

Key study findings: Oral administration with L-000900612 at doses up to 600 mg/kg/day from 14 days before mating to gestation day 7 did not affect the mating performance and fertility of the treated females. It did not affect any of the parameters of the early embryonic development. The NOAEL for this study was 600 mg/kg/day.

Study no.: TT #04-7420

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\tt047420\tt047420.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 8/27/2004

GLP compliance: Yes.

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot # L-000900612-003E017, —, pure by HPLC

Methods:

Doses: 0, 150, 300, and 600 mg/kg/day

Species/strain: Sprague-Dawley rats (— CD@[SD]IGS BR)

Number/group: 24

Route, formulation, and volume: Oral gavage, PEG 400 in water (80:20) (w/w), 2.5 ml/kg

Duration of dosing: Once per day for 14 days prior to cohabitation, during cohabitation, and through gestation day 7

Study design: Each F₀ female was mated with one untreated proven breeder male on pre-mating day 14 with cohabitation period limited to 20 nights. The presence of sperm in the vaginal lavage (performed daily) or a seminal plug in the vagina was considered evidence of a positive mating. The day of confirmed mating was considered gestation day 0.

Parameters and endpoints evaluated: Body weights, clinical observation, food consumption, mating performance and fertility of F₀ generation, embryonic/fetal survival, and necropsy of F₀ generation.

Results

Mortality: None.

Clinical signs: None.

Body weight: No effect.

Food consumption: No effect.

Necropsy: No effect.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
No effect.

Study title: L-000900612: Oral fertility study in males rats

Key study findings: Oral administration of L-000900612 to male rats at doses up to 600 mg/kg/day from 4 weeks prior to mating to gestation day 17 (approximately 8 weeks of treatment) did not affect any of the fertility parameters measured. The NOAEL for male fertility was 600 mg/kg/day.

Study no.: TT #05-7180

Volume #, and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\tt057180\tt057180.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 4/20/2005

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000900612, lot # L-000900612-003E015, —, pure by HPLC

Methods:

Doses: 0, 100, 300, and 600 mg/kg/day

Species/strain: Only male Sprague-Dawley rats (CD-1 [SD]IGS BR) were treated.

Number/group: 24

Route, formulation, and volume: Oral gavage, PEG 400 in water (80:20) (w/w), 2.5 ml/kg

Duration of dosing: Once per day for 29 days prior to cohabitation, during cohabitation, and through drug week 8.

Study design: Males were treated for 4 weeks before cohabitation with untreated females in a ratio of 1 female:1 male. Cohabitation period was limited to a maximum of 10 nights. The presence of sperm in the vaginal lavage (performed daily) or a seminal plug in the vagina was considered evidence of a positive mating. The day of confirmed mating was considered gestation day 0. Following the 5th night, any female that was apparently not mated was removed and replaced with a virgin female. There were 2, 0, 3, and 3 females in the control, 100, 300, and 600 mg/kg/day groups, respectively that were replaced.

Parameters and endpoints evaluated: Body weights, clinical observation, food consumption, numbers of corpora lutea, implantation sites, live and dead fetuses, and resorption, fetal weights, sex ratio, necropsy of males, testis weights, and histology (sperm motility analysis and epididymal sperm head quantitation) for males only.

Results

Mortality: Two high dose and one mid dose males died prior to scheduled termination. One high dose male died of intubation error. The other high dose animal was sacrificed during drug week 1 because of coarse tremors which were observed prior to dosing. A mid dose animal had to be sacrificed in drug week 8 because of physical signs relating to a trauma-induced fracture of the hard palate. None of the deaths was considered treatment related.

Clinical signs: No effect.

Body weight: No effect.

Food consumption: No effect

Fertility parameters:

Mating performance and fertility: The fertility indices were 100, 96, 92, and 91% for the control, low, mid, and high dose groups, respectively. The value for the high dose group was slightly lower as compared to that of the control group but was within those of the historical controls found in the performing laboratory. Thus, there was no treatment effect on mating performance and fertility.

Embryonic/fetal survival: No effect.

Sperm analyses: No effect.

Necropsy: No effect.

Testis weights: No effect.

Histopathology: No effect.

Embryofetal, Prenatal, and postnatal development

Study title: L-000900612: Oral developmental toxicity study in rats with prenatal and postnatal evaluation.

Key study findings: This study was a combined Segments II and III study in rats. L-000900612 was orally administered during gestation days 6 to lactation day 20 at doses of 0, 100, 300, and 600 mg/kg/day. Maternal toxicity was absent. All of the reproductive and development parameters in F₀, F₁, and F₂ were normal. However, dose-related increases in the numbers of fetuses and litters with supernumerary ribs were observed. Thus, the NOAEL for maternal toxicity is > 600 mg/kg/day and that for developmental toxicity was 300 mg/kg/day.

Study no.: TT #04-7090

Volume #, and page #: [\\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\it047090\it047090.pdf](#)

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 9/2/2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000900612-003E017,  pure by HPLC

Methods:

Doses: 0, 100, 300, and 600 mg/kg/day

Species/strain: Sprague-Dawley rats (← CD®[SD]IGS BR)

Number /group: 44 females mating with untreated males

Route, formulation, and volume: Oral gavage, PEG400 in water (80:20) (w/w), 2.5 ml/kg

Period of dosing: Gestation days 6 to 20 for those scheduled for Caesarian-section and gestation day 6 to lactation day 20 for those scheduled for natural deliveries

Study design: Only female F₀ generation was dosed with L-000900612. Mating of F₀ generation was done on site. After confirmation of mating (gestation day 0), female rats were placed in individual housing and received dosing from gestation day 6 to 20 or lactation day 20 (rats that were scheduled for natural delivery). Dams in the process of delivering pups did not receive dosing for that day. F₁ generation pups were allowed to grow and develop and did not receive directly any dosing but were exposed to L-dT *in utero* or via maternal milk during the lactation period. Developmental milestones, like sexual organ maturation (2/litter), learning, long- and short-term memory, overt coordination, and auditory startle responses (1/aex/litter) were evaluated in each selected F₁ generation. In addition, 22 F₁ generation rats/sex/dose were selected to mate at approximately 90 days of age, one male per one female rat, based on a random unit table. Sibling mating was excluded.

Parameters and endpoints evaluated:

F₀ generation: *Mortality:* Daily.

Clinical observation: Weekly during acclimation, on gestation day 0, daily before dosing and within 60 minutes of dosing, and on the day of sacrifice.

Body weights: Gestation day 0, every other day on gestation 6-20, gestation days 21 and 22, and on lactation days 0, 3, 7, 10, 14, 17, and 21.

Food consumption: Gestation days 3-5, 6-8, 10-12, 14-16, 18-20, and lactation days 1-5 and 8-12.

Ophthalmic examination: Once between gestation days 0 and 6 to select out animals with possible heritable lesions.

Caesarian section: Twenty-two/dose assigned to be C-sectioned on gestation day 21; uterine examination including recording the numbers of implantation sites, live or dead fetuses, resorption, and corpora lutea, evaluating placental morphology and fetal sex, external, visceral, and skeletal morphology.

Parturition: Twenty-two/dose scheduled for natural delivery; examined for abnormal behaviors during delivery, recorded duration of gestation, litter sizes (all pups delivered), and pup viability, sex, and weights at birth for those scheduled for natural delivery.

Necropsy: Gross examination of thoracic, and abdominal viscera, on lactation day 21 for animals delivered naturally.

F₁ generation: Preweaning:

Mortality: Daily.

Clinical observation: Daily.

Body weight: Postnatal days (PND) 0, 7, 14, and 21.

External examinations: PND 0 for gender and external malformations and variations, PND 3, 7, 14, and 21 to confirm gender.

Culling: PND 3 to standardized litter size of 4 pups/sex.

Postweaning:

Culling: PND 21 to 2 pups/sex/litter.

Clinical signs: Daily for mortality and twice weekly for physical signs.

Body weights: Between PND 25-28, weekly except for females that were mated and pregnant where body weights were recorded on gestations days 0, 7, 14, 20, and 24 and on lactation day 0.

Food consumption: Weekly during postweaning period except during cohabitation for the F₁ males; weekly during postweaning period (except during cohabitation) and on gestation days 0, 7, 10, 14, 17, and 21.

Sexual maturity: Observed daily for vaginal patency in females from PND 28-38; daily for preputial separation in males from PND 39-54.

Ophthalmic examinations: On control and 600 mg/kg/day groups between PND days 47-50 to select out animals with possible heritable lesions.

Behavior evaluation: Passive avoidance test for learning, short-term retention, and long-term retentions starting at PND days 34 or 35 and one week later in one pup/litter (tested twice/rat); Auditory startle habituation test to assess sensorimotor reflexes and habituation to redundant, nonsignificant stimuli between PND 62 and 64 in one pup/sex/litter; Open field test to evaluate

the general activity levels between PND 70 and 71 with animals previously tested in the auditory startle habituation test.

Mating and fertility: Twenty-two/sex/litter selected for cohabitation, one male paired with one female during PNW 12; a maximum duration of cohabitation set for 21 days.

Parturition and length of gestation: Observed up to 4 times/day from gestation day 21 until completion of delivery (lactation day 0).

Necropsy: Necropsy performed on those females used for mating from 22 litters/dose within 1 week after parturition; uterus examined and the number of metrial glands recorded.

F₂ generation: Body weight, sex determination, and gross external alterations evaluated.

Results

F₀ generation:

Mortality: One female in the 600 mg/kg/day group that delivered a litter of 4 pups was sacrificed with all of her pups on lactation day 4. This death was not related to treatment.

Clinical observation: No effect.

Body weights: No effect.

Food consumption: No effect.

Length of parturition: No effect.

Placental morphology: No effect.

Number of pups/litter: No effect.

F₁ generation:

Preweaning:

Mortality: No effect.

Clinical observation: No effect.

Body weight: No effect.

Fetal examinations: No treatment effects on external and visceral morphology as well as ossification. However, there were dose-related increases in the incidence of supernumerary rib in fetuses exposed to L-000900612 *in utero*.

Incidence of supernumerary rib				
Dose (mg/kg/day)	0	100	300	600
Total # of affected fetuses	14	22	28	46
% affected per litter	5.7 ± 12.0	7.2 ± 10.4	8.7 ± 15.9	15.0 ± 24.9

Postweaning:

Mortality: No effect

Clinical signs: No effect.

Body weights: No effect.

Food consumption: No effect.

Sexual maturity: No effect.

Ophthalmic examinations: No effect.

Behavior evaluation: No effect.

Mating and fertility: The fertility indices were 95, 95, 100, and 81% for the control, low, mid, and high dose groups, respectively. The fertility index for the high dose

group was lower than that for the control group. However, the value was within those of the historical controls in the performing laboratory. Thus, no treatment effect on the mating and fertility was detected.

Paturition and length of gestation: No effect

Necropsy: No effect.

F₂ generation:

Body weight: No effect.

Sex ratio: No effect

External morphology: No effect.

Study title: L-000900612: Oral range-finding reproductive study in female rats.

Key study findings: Treatment with L-000900612 during gestation days 6-20 at doses up to 600 mg/kg/day did not cause any maternal or developmental toxicity. Thus, the NOAEL for maternal and developmental toxicity is > 600 mg/kg/day.

Study no.: TT #04-7095

Volume #, and page #: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\tt047095\tt047095.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 6/24/2004

GLP compliance: No

QA reports: yes () no (X)

Drug, lot #, and % purity: L-000900612-003E015,  % pure by HPLC

Methods:

Doses: 0, 150, 300, 450, and 600 mg/kg/day

Species/strain: Sprague-Dawley  CD®(SD)IGS BR

Number /dose: 10

Route, formulation, and volume: Oral gavage, 80% PEG400 (w/w), 2.5 ml/kg

Period of dosing: Gestation day 6 to lactation day 20

Observation and time:

F₀ generation:

Mortality: Daily.

Clinical observation: Daily.

Body weights: Gestation days 0, every other day from gestation days 6-24, and lactation days 0, 3, 7, 10, 14, 17, and 21.

Food consumption: Gestation days 3-5, 6-8, 10-12, 14-16, 18-20, and lactation days 1-5 and 8-12.

Hematology: Gestation day 14

Serum biochemistry: Gestation day 14.

Reproductive performance: Length of gestation (4 times per day starting on gestation day 21), mean percent postimplantation survival, and mean numbers of implants per female.

Necropsy: On lactation day 21; record number of metrial gland.

F₁ generation:

Physical examinations: Daily for mortality.

Body weights: PND 0, 7, 14, and 21.

External examinations: PND 0 for malformation and gender which were confirmed on PND 3, 7, 14, and 21.

Culling: PND 3 down to 8/litter.

Necropsy: PND 21

Results:

F₀ generation:

Mortality: None related to the treatment. One female each in 150 and 300 mg/kg/day groups were sacrificed on gestation day 9 because of signs related to gavage errors. One female in 450 mg/kg/day group was sacrificed on lactation day 1 because of only one surviving pup in the litter.

Clinical signs: Abnormal respiratory sounds, likely related to the poor palatability and viscosity of the dosing formulation, were noted starting on the first week of dosing in 4 and 5 females of the 450 and 600 mg/kg/day groups, respectively.

Body weight (dams): No effect.

Food consumption (dams): No effect.

Hematology: No effect.

Serum biochemistry: No effect.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No effect

F1 generation:

Mortality: No effect.

Body weights: No effect.

External examinations: No effect.

Study title: L-000900612: Oral developmental toxicity study in rabbits.

Key study findings: L-000900612 was administered orally to pregnant rabbits at doses of 0, 100, 500, and 1000 mg/kg/day from gestation days 7 to 20. The formulation used was 0.5% methylcellulose, the same one used in the toxicity studies in mice and dogs. Treatment with L-000900612 at doses up to 1000 mg/kg/day did not cause any maternal toxicity. There were increases in the incidence of increased ossification of hyoid in the treatment groups as compared to the control group, however, they were not dose proportional and were within historical control values for the performing lab. The NOAEL for maternal and developmental toxicity was > 1000 mg/kg/day.

Study no.: TT #04-7220

Volume #, and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\tt047220\tt047220.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 8/16/2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: L-000900612-003E017,  pure by HPLC

Methods:

Doses: 0, 100, 500, and 1000 mg/kg/day

Species/strain: Dutch belted rabbits

Number /group: 18

Route, formulation, and volume: Oral gavage, 0.5% methylcellulose (w/v), 5 ml/kg

Period of dosing: Gestation days 7 to 20

Observation and time:

Physical examinations: Daily from gestation days 1 or 2 to 28; prior to dosing and 1 to 5 hours after dosing from gestation days 7 to 20.

Body weights: Gestation days 0, 7, 9, 11, 13, 15, 17, 19, 21, 24, and 28.

Food consumption: Gestation days 4, 7, 8, 9, 10, 12, 14, 16, 18, 20, 23, and 27.

Reproductive performance: Gestation day 28 on pregnancy status, placenta morphology, uterine implants, fetal survival, fetal sex ratio, live fetal weight

Necropsy of F₀ females: Gestation day 28

Fetal examinations: Gestation day 28 on external, visceral and coronal, and skeletal morphology as well as ossification status of fetuses.

Results:

Mortality (dams): None.

Clinical signs (dams): No effect.

Body weight: No effect.

Food consumption (dams): No effect.

Necropsy (dams): No effect.

Reproductive performance (dams): No effect.

Placental morphology (offsprings): No effect.

Offspring (malformations, variations, etc.): The incidences of increased ossification of hyoid were higher in the treatment groups as compared to the control, though the increases were not dose proportional. The incidence rate for this finding was within the historical control range and thus not considered treatment-related.

Incidence of increased ossification of hyoid				
Dose (mg/kg/day)	0	100	500	1000
Total # of affected fetuses	5	9	23	10
% affected per litter	3.3 ± 6.1	7.0 ± 11.1	19 ± 20.7	7.8 ± 15.4

Study title: L-000900612: Oral range-finding study in pregnant rabbits.

Key study findings: L-000900612 was administered orally to pregnant rabbits at doses of 0, 100, 500, and 1000 mg/kg/day from gestation days 7 to 20. The formulation used was 0.5% methylcellulose, the same one used in the toxicity studies in mice and dogs. No effect was detected in any of the parameters monitored. The NOAEL for maternal and developmental toxicity was > 1000 mg/kg/day.

Study no.: TT #04-7225

Volume #, and page #: \\Cdsesub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\tt047225\tt047225.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA

Date of study initiation: 6/24/2004

GLP compliance: No

QA reports: yes () no (X)

Drug, lot #, and % purity: L-000900612-003E015. — pure by HPLC

Methods:

Doses: 0, 125, 250, 500, and 1000 mg/kg/day

Species/strain: Dutch belted rabbits

Number /group: 10

Route, formulation, and volume: Oral gavage, 0.5% methylcellulose (w/v), 5 ml/kg

Period of dosing: Gestation days 7 to 20

Observation and time:

Physical examinations: Daily from gestation days 1 or 2 to 28; prior to dosing and 1 to 5 hours after dosing from gestation days 7 to 20.

Body weights: Gestation days 0, 7, 9, 11, 13, 15, 17, 19, 21, 24, and 28.

Food consumption: Gestation days 4, 7, 8, 9, 10, 12, 14, 16, 18, 20, 23, and 27.

Hematology: Gestation day 21.

Serum biochemistry: Gestation day 21.

Reproductive performance: Gestation day 28 on pregnancy status, placenta morphology, uterine implants, fetal survival, fetal sex ratio, live fetal weight

Necropsy of F₀ females: Gestation day 28

Fetal examinations: Gestation day 28 on external morphology.

Results:

Mortality (dams): No treatment effect. One female in 1000 mg/kg/day group died on gestation day 7 after the first dose due to the gavage error.

Clinical signs (dams): No effect.

Body weight: No treatment effect.

Food consumption (dams): No effect.

Hematology: No effect.

Serum biochemistry: No effect.

Necropsy (dams): No effect.

Reproductive performance (dams): No effect.

Placental morphology (offsprings): No effect.

Offspring (malformations, variations, etc.): No effect.

Juvenile studies

Study title: L-000900612: Oral juvenile toxicity study in rats.

Key study findings: Five-day old rats (44/sex/dose) were administered with oral doses of 0 (80% PEG 400), 50, 200, or 600 mg/kg/day L-000900612 for 7 weeks (until PND 52 to 54). The effects on the general toxicological parameters as well as the sexual maturity, behavioral parameters, and reproductive performance were evaluated. The only treatment effects were those related to the irritation of the stomach mucosal surface at doses \geq 200 mg/kg/day. The same effects were observed in adult rats at similar doses. There was no difference between the adult and juvenile rat toxicokinetic parameters. The NOAEL for this study was 50 mg/kg/day, although, the maximum tolerated dose was not achieved.

Study no.: TT #05-7300

Volume: \\Cdsub1\evsprod\NDA022145\0000\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42354-juv\tt057300\tt057300.pdf

Conducting laboratory and location: Merck Research Laboratories, West Point, PA and Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France

Date of study initiation: 9/29/2005

GLP compliance: Yes

QA report: yes (X) no ()