CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-995

PHARMACOLOGY REVIEW

MEMORANDUM
Oct. 15, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-995

I concur with Drs. Todd Bourcier and Karen Davis-Bruno that the marketing application for Januvia (Sitagliptin) may be approved based on review of nonclinical data submitted by the sponsor.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

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/s/

Kenneth Hastings
10/16/2006 11:05:05 AM
PHARMACOLOGIST

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:	**21-995**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/16/05**
PRODUCT:	**Januvia (Sitagliptin)**
INTENDED CLINICAL POPULATION:	**Type 2 Diabetics**
SPONSOR:	**Merck**
DOCUMENTS REVIEWED:	**eCTD**
REVIEW DIVISION:	**Division of Metabolic and Endocrine Products**
PHARM/TOX REVIEWER:	**Todd Bourcier, Ph.D.**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Lina Aljuburi, Pharm. D., M.S.**

Date of review submission to Division File System (DFS): 31 August 2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
AP (Approval)
Pharmacology/Toxicology recommends approval of NDA 21,995 (Januvia®)

B. Recommendation for nonclinical studies
No additional nonclinical studies are required.

C. Recommendations on labeling

8. Use in Specific Populations
8.1 Pregnancy
Pregnancy Category B.
There are no adequate and well-controlled studies in pregnant women; [redacted] recommended for use in pregnancy unless clearly needed. Merck & Co., Inc. maintains a registry to monitor the pregnancy outcomes of women exposed to Januvia while pregnant. Health care providers are encouraged to report any prenatal exposure to Januvia by calling the Pregnancy Registry at (800) 986-8999.

Sitagliptin administered to pregnant female rats and rabbits [redacted] was not teratogenic at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100mg/day based on AUC comparisons. Higher doses [redacted] increased the incidence of [redacted] rib malformations in offspring at 1000 mg/kg, [redacted]

Sitagliptin administered to female rats [redacted] decreased the average body weight in male and female offspring at 1000 mg/kg [redacted]. No functional or behavioral toxicity was observed in offspring of rats.

[redacted] placental transfer was approximately 45% at 2 hours and 80% at 24 hours postdose. [redacted] placental transfer was approximately 66% at 2 hours and 30% at 24 hours.

8.3. Nursing Mothers

Sitagliptin is excreted in the milk of lactating rats at a milk to plasma ratio of 4:1. It is not known whether sitagliptin is excreted in human milk. Because many drugs are excreted in human milk [redacted] a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

13. Nonclinical Toxicology

13.1 Carcinogenesis, mutagenesis, impairment of fertility

A two year carcinogenicity study was conducted in male and female rats given oral doses of sitagliptin of 50, 150, and 500 mg/kg. There was an increased incidence of combined liver adenoma/carcinoma in males and females and of liver carcinoma in females at 500 mg/kg. This dose results in approximately 60 times the human exposure at the maximum recommended daily adult human dose (MRHD) of 100 mg/day based on AUC comparisons. Liver tumors were not observed at 150 mg/kg, approximately 20 times human exposure at the MRHD.

A two year carcinogenicity study was conducted in male and female mice given oral doses of sitagliptin of 50, 125, 250, and 500 mg/kg. There was no increase in the incidence of tumors in any organ up to 500 mg/kg, approximately 70 times human exposure at the MRHD.

Sitagliptin was not mutagenic or clastogenic with or without metabolic activation in the Ames bacterial mutagenicity assay, a Chinese hamster ovary (CHO) chromosome aberration assay, an *in vitro* cytogenetics assay in CHO, an *in vitro* rat hepatocyte DNA alkaline elution assay, and an *in vivo* mouse micronucleus assay.

In rat fertility studies with oral gavage doses of 125, 250, and 1000 mg/kg, males were treated for – weeks prior to mating and females were treated – weeks prior to mating through gestation day 7. No adverse effect on fertility was observed at 125 mg/kg (approximately 12 times human exposure at the MRHD of 100 mg/day based on AUC comparisons). Higher doses increased resorptions [redacted] in females at approximately 25 times human exposure at the MRHD based on AUC comparisons.

[redacted]

II. Summary of non-clinical findings

A. Brief overview of non-clinical findings

Pharmacology

MK-0431 (sitagliptin phosphate) is a competitive inhibitor of dipeptidyl peptidase 4 (DPP4), an enzyme principally responsible for degrading incretin peptides glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). MK-0431 prolongs incretin half-life and biological activity and thus potentiates glucose-dependent insulin release and delays gastric emptying. In non-clinical models of diabetes, MK-0431 moderates glucose excursion and improves insulin release and islet cell function/mass without provoking hypoglycemia. MK-0431 is body weight-neutral, unlike marketed glitazones (weight gain) and GLP-1 analogues (weight loss).

Immunomodulatory effects of DPP4 (aka CD26) are reportedly not altered by MK-0431, based on normal responses of murine T- and B-cells to antigens and mitogens. However, rodent DPP4/CD26 differs in some aspects from human DPP4/CD26 (e.g., binding of adenosine deaminase) and Merck's experiments did not directly test the T-helper memory function ascribed to CD26. Therefore, the non-clinical data do not adequately predict potential effects of MK-0431 on DPP4/CD26's role in human immunity.

Safety pharmacology assessment of neurological, renal, pulmonary, and gastrointestinal effects of MK-0431 did not identify any significant liabilities.

Absorption, Distribution, Metabolism, and Excretion

An oral dose of MK-0431 is rapidly absorbed and is 60-90% bioavailable in rats and dogs. MK-0431 distributes to most rat tissues with low amounts distributing to the brain, eyes, and bone. Plasma protein binding is moderate (30%). Metabolism of MK-0431 is minimal with 80% of unchanged parent compound being eliminated in the urine of rats, dogs, and humans. Oxidative metabolism by CYP3A4 and 2C8 is a minor metabolic pathway. MK-0431 has a longer plasma half-life in humans (13hrs) than in rats and dogs (2-5hrs) probably due to different rates of renal elimination. MK-0431 slightly accumulates in humans but not in dogs or rats after multiple dosing.

MK-0431 is a P-glycoprotein and hOAT3 substrate, but does not interfere in the shuttling of other substrates via these transporters *in vitro*. MK-0431 does not inhibit CYP450 enzymes or induce CYP3A4. The results predict a low probability for pharmacokinetic drug interactions via these pathways.

General Toxicology (MRHD, Maximum Recommended Human Dose, or 100mg)

Single dose studies identified minimum lethal doses of 2000mg/kg (200-400x MRHD) in mice and 3000mg/kg (150-300x MRHD) in rats. Little other toxicological information was obtained in these studies.

Repeat dose studies were conducted in Sprague-Dawley rats and Beagle dogs up to 6 months and 12 months duration, respectively.

A high-dose 3-month study in rats identified kidney and liver necrosis, myocardial degeneration, bone marrow necrosis, and death at 1500 and 2000mg/kg (150-200x MRHD). Kidney toxicity was also observed in mice at 500mg/kg. Note that exposure at these high doses is theoretically sufficient to inhibit off-target enzymes DPP8/9, proteases that are associated with these toxicities.

Administration of doses up to 20x the MRHD for 6 months in rats did not elicit significant toxicity.

Studies in dogs identified NOAEL doses based on clinical signs that consisted of reduced activity, hunched posture, ataxia, tremor, and sporadic emesis observed at 50mg/kg (20x MRHD). Respiratory distress, described as audible and labored breathing and open-mouthed breathing, was also reported. No consistent target organs were identified in these studies.

Administration of doses up to 5x the MRHD for up to 12 months in dogs did not elicit significant toxicity.

Special Toxicology

MK-0431 did not produce vascular/skin lesions in rhesus monkeys, as seen with some DPP4 inhibitors, after three months administration of doses up to 25x the MRHD. Mechanistic data provided by Merck suggests that inhibiting DPP4 activity alone is not sufficient to produce this toxicity.

The combination of MK-0431 and high-dose (50 mg/kg) but not low-dose (20 mg/kg) metformin in dogs may have resulted in more numerous and earlier deaths than observed with metformin alone. The lower dose of metformin (20 mg/kg) better approximates maximum human exposure to metformin (2500mg/day). Convincing evidence is provided by Merck that high-dose metformin is responsible for the deaths observed in combination with MK-0431. Nevertheless, there is a slight possibility of exacerbated toxicity in the setting of high metformin exposure and clinical exposure to MK-0431.

Reproductive Toxicology

Exposure to MK-0431 in the definitive studies ranged from 12x to 90x MRHD in the rat and 6x to 50x in the rabbit. Resorptions and post-implantation losses increased in females in a fertility study at ~25x MRHD; male fertility was not effected. MK-0431 was not teratogenic but increased the incidence of skeletal malformations in rat pups at maternally toxic doses. At maternally non-toxic doses, a single rat pup had multiple skeletal abnormalities (incidence within historical range), and a single rabbit pup had multiple cardiovascular abnormalities, but a relationship to drug treatment is not conclusive. MK-0431 crosses the placenta in rats and rabbits and is excreted in maternal milk at a 4:1 ratio to plasma. As with other oral hypoglycemic agents, MK-0431 should not be given to pregnant or nursing mothers and Merck will maintain a pregnancy register. Pregnancy Category 'B' is recommended.

There were no conclusive drug-related effects on embryonic/post-natal development in rats at 125mg/kg (12x MRHD) or in rabbits at 125mg/kg (20x MRHD).

Genetic Toxicology

MK-0431 was not mutagenic or clastogenic in three *in vitro* assays (Ames, hepatocyte alkaline elution, and chromosome aberration) and one *in vivo* assay (murine micronucleus induction).

Carcinogenicity

Carcinogenic potential of MK-0431 was evaluated in 2 year studies in mice and rats. Both studies adequately assessed carcinogenesis. MK-0431 significantly increased the incidence of combined liver adenoma/carcinoma in male and female rats, and increased liver carcinomas in female rats at 500mg/kg (62x MRHD). Non-genotoxic, chronic hepatotoxicity is the suggested etiological event but this is based on weak correlative evidence of liver toxicity. MK-0431 did not produce any drug-related tumors in CD-1 mice up to 500mg/kg (72x MRHD). MK-0431 poses a minimal carcinogenic risk to humans.

B. Non-clinical safety issues relevant to clinical use

1. DPP4 cleaves several substrates in addition to GLP-1. Therefore, MK-0431 may have undesirable effects related to inhibiting cleavage of non-incretin substrates. Effects on human immunity, specifically recall responses to antigens and immune cell trafficking, may be adversely effected by DPP4 inhibition. This risk is an unavoidable characteristic of MK-0431 and the drug class. There is currently no clinical evidence of such effects with Januvia.

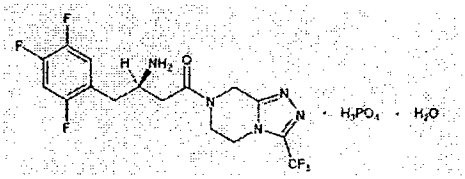
2. MK-0431 presents a marginal clinical risk of producing skin lesions with prolonged administration. This conclusion is based on the absence of skin findings in the 3-month monkey study, on mechanistic data suggesting that inhibiting DPP4 activity alone is not sufficient to produce this toxicity, and on the high DPP4 selectivity of MK-0431 at clinical exposure. Risk assessment for skin lesions must be done on a case-by-case basis and is not evidence of similar safety with other DPP4 inhibitors currently in clinical development.

3. The combination of MK-0431 and high-dose metformin (50 mg/kg) in dogs may have resulted in more numerous and earlier deaths than observed with metformin alone. The combination of MK-0431 and a lower dose of metformin (20 mg/kg) that better approximates human exposure at 2500mg/day resulted in no deaths and yielded no evidence of exacerbated toxicity. Convincing evidence is provided that the deaths at 50 mg/kg is due to metformin toxicity and not to the combination. Nevertheless, there is a slight possibility of exacerbated toxicity in the setting of high metformin exposure ($\geq$ 400μM*h AUC) and clinical exposure to MK-0431 (~10μM*h AUC).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-995
Review number: 1
Sequence number/date/type of submission:
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Merck Research Laboratories
Manufacturer for drug substance:
Merck in Barceloneta, Puerto Rico, Vincenza, Italy, and Visp, Switzerland
Reviewer name: Todd Bourcier
Division name: Metabolic and Endocrine Products
Review completion date: 31 August 2006
Drug:
Trade name: Januvia
Generic name: Sitagliptin phosphate
Code name: MK-0431; L-000224715-010X
Chemical name:
7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate
CAS registry number: 654671-77-9
Molecular formula/ weight: $C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$ /523.32 MW
Structure:



Relevant INDs/NDAs/DMFs: 62,278 (Novartis); [redacted]; 63,634 (BMS); 67,369 (GSK); 65,495 (Merck); [redacted]; [redacted] 69,707 (PPD)

Drug class: dipeptidyl-peptidase IV (DPP-IV) inhibitor

Intended clinical population: Type 2 Diabetics

Clinical formulation: MK-0431 monohydrate phosphate salt (25, 50, 100 mg tablets)
Tablets contain microcrystalline cellulose, calcium phosphate dibasic, croscarmellose sodium, magnesium stearate, sodium stearyl fumarate. Tablets are pink, light beige, or beige depending on dosage strength.

Route of administration: Oral

Maximum Recommended Human Dose: Merck seeks approval of 25, 50, and 100mg. The 100mg qd strength provides an average AUC of 10 μM*h and a Cmax of 1 μM.

Disclaimer: Some Tables and Figures from the electronic NDA submission have been copied for use in this review

Studies reviewed within this submission:

Primary Pharmacodynamics
Affinity for human and animal DPP-IV Human, mouse, rat, dog In vitro
Activity in T cell activation assays Mouse In vitro
Acute efficacy in oral glucose tolerance test Mouse P.O.
Pharmacodynamics in oral glucose tolerance test Mouse P.O.
Acute efficacy in model of diet-induced obesity Mouse P.O.
Acute efficacy in db/db mice Mouse P.O.
Selectivity of MK-0431 for DPP-IV Human, cow, pig, rabbit, rat In vitro
Selectivity of comparator compounds for DPP-IV Human, pig In vitro

Safety Pharmacology
Respiratory assay Rat P.O. MK-0431 Tablets
Cardiovascular telemetry assay Dogs P.O.
Oral functional observational battery assay Rats P.O.
Cellular electrophysiological evaluation of MK-0431 on HERG CHO In vitro
Cardiovascular effects: rising dose study Dog IV
Renal function and electrolyte excretion Dog P.O.
Respiratory function, hemostasis, and platelet function Dog IV
Gastric acid secretion Dog P.O.
Gastrointestinal motility Mouse P.O.
Behavioral and Other CNS Effects

Pharmacokinetics
Absorption
Pharmacokinetics in rat and dog
Oral bioavailability and dose dependence in rat and dog

Distribution
Single-dose tissue distribution in rat
Placental transfer in rat and rabbit
Reversible plasma protein binding
Serum albumin and α1-acid glycoprotein binding
Blood-to-plasma partitioning
P-glycoprotein mediated transport, mouse and human
Uptake by renal transporters, human

Metabolism
Metabolites in plasma, mouse and rabbit
Metabolites in plasma, liver, kidney, urine, and bile in rat
Metabolites in plasma, urine, and bile in dog
Metabolites in plasma, urine, and feces in human
Identification of metabolites M2 and M5 in dog
Metabolism in liver microsomes, mouse, rat, rabbit, dog, monkey, human
Metabolism in hepatocytes, rat, dog, human
Metabolism in recombinant cytochromes P450 in human
Inhibition of cytochromes P450
Induction of cytochrome P450 3A4
Effect on MDR1 P-glycoprotein-mediated transport

Excretion
Mass balance in rats and dogs
Urinary and biliary excretion in rats and dogs
Excretion into milk in rat

General Toxicology
Single dose toxicity in mouse and rat (anhydrous and monophosphate salt formulations)
Repeat dose toxicity studies and their duration:

CD-1 Mouse:	1 and 3 months
Sprague Dawley Rat:	2 weeks, 3 months, 3 months high-dose, 6 months
Beagle Dogs:	2 weeks, 3, 6, and 12 months

Genetic Toxicology
Ames Assay (in vitro)
Primary rat hepatocytes (in vitro)
Chinese hamster ovary cells (in vitro)
Micronucleus induction in mice after single oral dose (in vivo)

Carcinogenicity
106 week oral gavage in CD-1 mice and toxicokinetic analysis
106 week oral gavage in SD rats and toxicokinetic analysis

Reproductive/Developmental Toxicology
Male and female fertility in rat
Rat Embryonic Development (dose-ranging and definitive studies)
Rabbit Embryonic Development (dose-ranging and definitive studies)
Rat Post-natal Development

Special Toxicology Studies
Dermal sensitization in mice, rabbits, and humans [redacted]
Ocular toxicity in bovine cornea (in vitro) and in rabbits (in vivo)
Intravenous administration of MK-0431 for 16 consecutive days in rats and dogs
Skin lesion assessment of sitagliptin in a 14-week oral toxicity study in monkeys
Skin lesion assessment of L-000000826 in a 12-week oral toxicity study in monkeys
Interim Report: Skin lesion assessment of L-000233357 in a 14-week oral toxicity study in monkeys
MK-0431 + Metformin: Combination Toxicity Studies in Dogs: Summary
MK-0431 + Metformin: 14 week oral toxicity study in dogs
Exploratory 5-week oral tolerability study with Metformin in female dogs
MK-0431 + Metformin: 16 week oral toxicity in female dogs

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

MK-0431 is a triazolopiperazine-based competitive inhibitor of dipeptidyl peptidase 4 (DPP4). MK-0431 selectively inhibits DPP4 activity in serum from humans, rodents, and dogs with high potency (IC_{50}, 18-69nM; Ki, 9nM). Inhibitory activity against closely related proteases, including DPP8/9, and a panel of unrelated enzymes and ion channels is minimal (IC_{50}, 48μM to >100 μM) and not relevant at clinical drug concentrations (~1.0 μM at a 100mg dose). The DPP4 selectivity of MK-0431 is superior to vildagliptin, a DPP4 inhibitor being developed by Novartis. The selectivity of MK-0431 for DPP4 minimizes the potential for toxicities associated with inhibition of DPP8/9.

MK-0431 bound to serotonin receptors with a Ki of 2-5 μM, but was devoid of agonist activity; it is not known if MK-0431 interferes with endogenous serotonergic activity. Merck states that MK-0431 distributes poorly to the brain ($1/10^{th}$ plasma) and that 5HT2A antagonists are used clinically.

DPP4, also known as CD26, contributes to the co-activation of memory/helper T-cells to recall antigens. MK-0431 did not suppress murine T- and B-cell activation in a series of *in vitro* activation assays. Other selective DPP4 inhibitors did not suppress reactivity of human peripheral lymphocytes, but MK-0431 was not specifically tested. These experiments did not address the memory T-cell function of CD26 and are of uncertain value in predicting the effect of MK-0431 on human immunity.

MK-0431 showed efficacy in lean mice, diet-induced obese mice, and in db/db mice. MK-0431 inhibited plasma DPP4 activity, increased plasma GLP-1, and reduced blood glucose excursion in a dose-dependent manner. Efficacious plasma drug concentrations were 200-700nM, sufficient to inhibit plasma DPP4 activity more than 90%. For comparison, the Cmax at the 100mg clinical dose is 1000nM.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

MK-0431 inhibits DPP4 in vitro: MK-0431 inhibits activity of human recombinant DPP4 by 50% at 17.9 nM (IC_{50}, **Figure 1**). The range for inhibitory activity is ~5nM to 1000nM, representing ~20% to 99% inhibition of DPP4 activity against a fluorogenic dipeptide substrate (Gly-Pro-AMC). Inhibitory activity of MK-0431 was competitive and reversible.
MK-0431 inhibits activity of native DPP4 from humans and from species used for toxicology testing with similar potency (16-69nM, **Table 1**). MK-0431 inhibits free DPP4 in serum as well as membrane-bound enzyme (CACO-2 extracts).

Figure 1: *In vitro* inhibition of human recombinant DPP4 by MK-0431

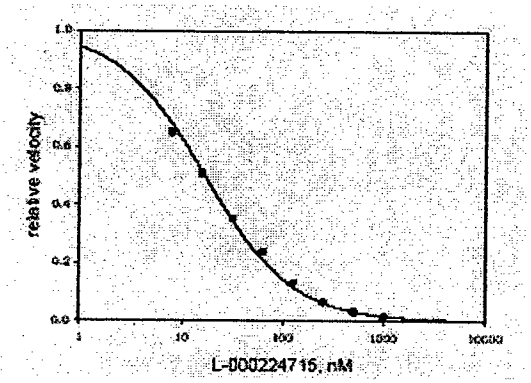


Table 1: Inhibition of DPP4 from various sources by MK-0431

DPP-IV Source	IC_{50}, nM	SD (n)
Human recombinant	17.9	7.4 (3)
Human serum	12.9	0.6 (3)
CACO-2 extract	20.3	1.2 (3)
Rat serum	52.4	6.2 (3)
Mouse serum	69.3	7.0 (3)
Dog serum	16.3	4.2 (5)

MK-0431 is selective for DPP4 in vitro: MK-0431 selectively inhibits activity of DPP4 relative to closely-related proline specific serine proteases (**Table 2**), although FAPα was not assayed. MK-0431 inhibits DPP8 activity with ~2,500 fold less potency compared to DPP4, based on IC_{50} values.

MK-0431 was also screened for activity against a panel of unrelated proteases and ion channels (**Tables 3 & 4**). Granzyme B and gamma-secretase were inhibited with an IC_{50} $\geq$ 10μM, and L-type calcium channels with an IC_{50} of 22μM. These concentrations are approximately 500-fold and 1000-fold higher than the IC_{50} for DPP4 activity.

MK-0431 bound to rat serotonin receptors 5HT2 (Ki, 5.8μM) and 5HT2A (Ki, 2.1μM), but no agonist activity was observed up to 10μM concentration.

The potential for MK-0431 to exhibit off-target inhibitory activity at clinically relevant concentrations is minimal. The low inhibitory activity against related DASH members (**D**PP4 Activity & **S**tructural **H**omologs) minimizes the toxicities associated with DPP8/9 inhibition in rats and dogs (e.g., thrombocytopenia, mortality in rats, gastrointestinal toxicity in dogs). Despite the minimal off-target potential of MK-0431, substrate promiscuity of DPP4 activity and its possible sequalae is an unavoidable characteristic of MK-0431 and the drug class.

Table 2

Activities of L-000224715 in Assays for Proline Specific Enzymes

Screening Target	IC_{50}, µM (n)
DPP8	48 ± 20 (4)
DPP9	>100 (3)
QPP	>100 (3)
APP	>100 (2)
PEP	>100 (2)
Prolidase	>100 (3)

Table 3:
MK-0431 inhibition of selected proteases

Screening Target	IC_{50}, µM
Cathepsin B	>100
Cathepsin H	>100
Caspases 1-10, 13	>100
Granzyme B	>10
Gamma-secretase	>10
Beta-secretase	>50
Thrombin	>100
Trypsin	>100
Factor Xa	>100
TAFI	>100

Table 4:
MK-0431 inhibition of selected ion channels

Screening Target	IC_{50}, µM (n)
IKr (MK-0499)	67 ± 39 (4)
L-type Ca Channel	22 ± 5 (2)
Na Channel Site II	52 ± 7 (2)

MK-0431 selectivity vs. comparator compounds: MK-0431 (**L-000224715 in Table 5**) exhibits a superior selectivity profile compared to a panel of other DPP4 inhibitors, including the Novartis compound LAF237 (vildagliptin) currently in Phase 3 clinical trials. The threo-, allo- and DPP8/9 selective compounds produced toxicity in rats and dogs, including thrombocytopenia, anemia, multiple organ histopathology, and mortality ([1]Lankas 2005). The threo- and allo-Ile non-selective compounds also produced similar toxicity in DPP4 deficient mice. MK-0431 did not produce these toxicities in this study, indicating that several toxicities are associated with inhibition of DPP8/9 but not DPP4. A highly selective inhibitor of DPP4 would therefore avoid such DPP8/9-related toxicities.

[1]Lankas GR, et al. (2005) Diabetes (10):2988-94.
This study was conducted by the Dept. of Safety Assessment, Merck Research Laboratories

Table 5: *In vitro* selectivity of comparator DPP4 inhibitors (IC_{50}, μM)

Compound	DPP-IV	DPP8	DPP9	QPP	PEP	APP	Prolidase
threo-Ile thia	0.42	2.2	1.6	14	100	>100	>100
allo-Ile thia	0.46	0.22	0.32	18	>100	>100	>100
QPP selective	1.9	22	31	0.019	100	>100	>100
DPP8/9 selective	30	0.038	0.055	14	>100	>100	>100
L-000224715	0.018	48	>100	>100	>100	>100	>100
DPP-728	0.01	1.9	0.067	3	>100	>100	>100
LAF237	0.038	5.9	0.28	>100	>100	>100	>100

MK-0431 activity in murine T cell activation assays in vitro: DPP4, also known as CD26, is thought to contribute to co-activation of memory/helper T-cells. MK-0431 was therefore evaluated over a concentration range of 12nM to 50μM in several *in vitro* activation assays with murine T- and B-cells. MK-0431 did not inhibit T-cell proliferation in the mixed splenic lymphocyte reaction (MLR) or in response to antigen, and did not alter lipopolysaccharide-induced proliferation of B cells. The Lankas article[1] reported that DPP4-selective compounds do not suppress *in vitro* proliferation of human peripheral blood lymphocytes in response to phytohemagglutinin or staph enterotoxins but less selective compounds do have inhibitory activity. MK-0431 was not evaluated in that experiment.

DPP4/CD26 in mice and rats differs in some aspects from the human form[1,2], despite ~85% homology across species (e.g., ADA binding). In addition, the *in vitro* assays done by Merck do not clearly test the helper functions ascribed to CD26 on memory T-cells (e.g., human T-cell response to tetanus toxoid-loaded antigen presenting cells). At least in mice, MK-0431 does not suppress T- and B-cell activation, but the possible effect on human immunity is unknown.

[1]Lankas GR, et al. (2005) Diabetes (10):2988-94.
This study was conducted by the Dept. of Safety Assessment, Merck Research Laboratories

[2]Iwaki-Egawa S, et al. (1997) Cellular Immunology (178):180-186

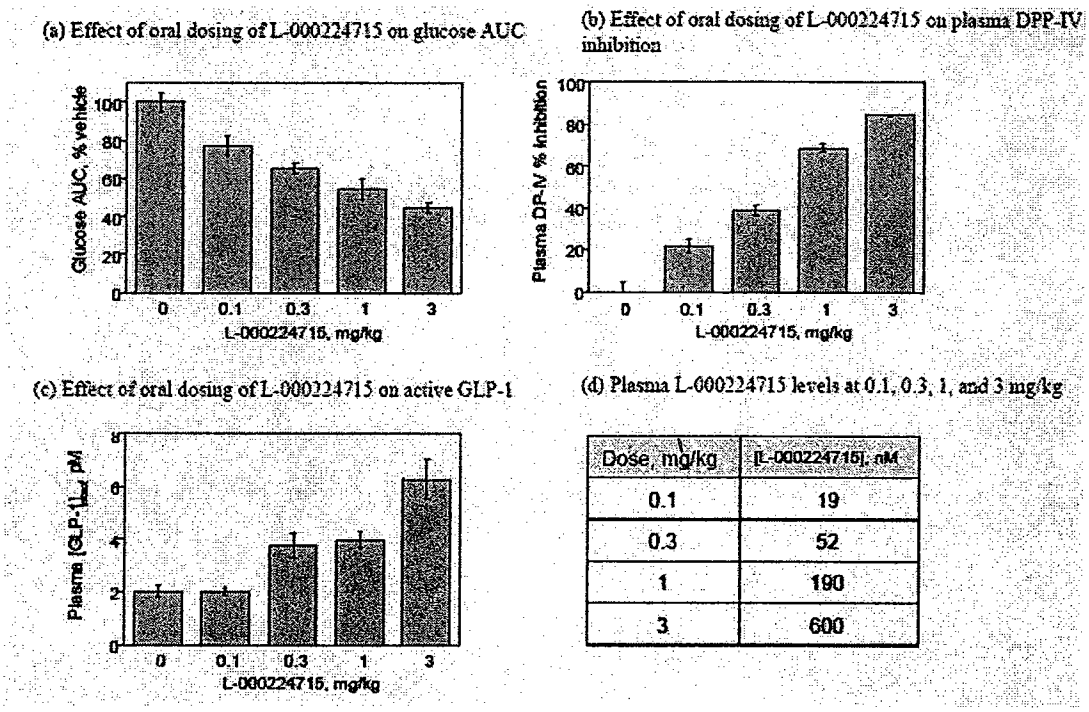
Drug activity related to proposed indication:

Non-clinical efficacy of MK-0431 was assessed in lean mice, diet-induced obese mice, and in db/db mice. MK-0431 inhibited plasma DPP4 activity, increased plasma GLP-1, and reduced blood glucose excursion in a dose-dependent manner. Efficacious plasma drug concentrations were 200-700nM, sufficient to inhibit plasma DPP4 activity more than 90%. Merck suggests that clinical efficacy will be achieved by maintaining 80% DPP4 inhibition and 2-fold GLP-1 elevation at trough plasma drug levels.

Lean mice were treated orally with MK-0431 (0.1, 0.3, 1, 3 mg/kg) and then challenged with dextrose (5g/kg, 10ml/kg) 1 hour post-dose **(Figure 3)**. The blood glucose excursion profile from 0 to 120 minutes was used to integrate an area under the curve (AUC) for each treatment. MK-0431 inhibited blood glucose excursion in a dosage-dependent manner achieving maximum efficacy at 1 mg/kg (46% inhibition).

In a separate experiment, plasma was collected at 20 minutes post-dose for measurement of plasma DPP4 inhibition, active GLP-1, and compound. Maximal efficacy, corresponding to plasma DPP4 inhibition of 70% and plasma concentrations ≥190 nM, resulted in a 2- to 3-fold increase in active GLP-1, analogous to what is observed upon glucose challenge in DPP4 deficient mice.

Figure 3: Pharmacodynamics of MK-0431 in lean mice



Dose, mg/kg	[L-000224715], nM
0.1	19
0.3	52
1	190
3	600

High fat diet-induced obese mice (DIO) mice (**Figure 4**) develop obesity, hyperglycemia, and hyperinsulinemia and have impaired blood glucose tolerance in response to a dextrose challenge. Following oral administration of 0.3, 3, and 30mg/kg MK-0431, dextrose-induced blood glucose excursion was significantly inhibited by 68, 90, and 82% (normalized to the dextrose-challenged lean controls), respectively. Maximum efficacy was seen at the 3 mg/kg dose in this study, corresponding to a plasma concentration of approximately 700nM based on a parallel PK study in DIO mice.

The db/db mouse is a murine model of type 2 diabetes (**Figure 5**) characterized by severe insulin resistance and marked hyperglycemia. Oral administration of MK-0431 (3, 10, and 30 mg/kg) to diabetic *db/db* mice (9 to 10 weeks of age) resulted in near normalization of blood glucose to lean controls. Maximal efficacy was observed at 3 mg/kg (76% correction of hyperglycemia at 4 hours postdose), corresponding to a maximum plasma concentration of approximately 400 nM based on a parallel PK study in *db/db* mice.

Figure 4:
Glucose AUC in DIO mice with MK-0431

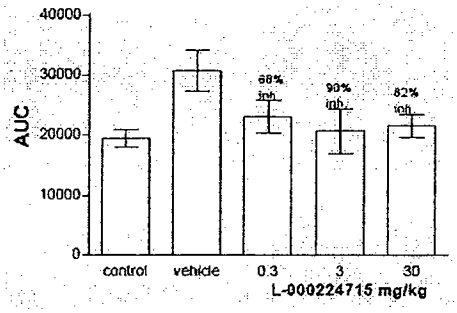
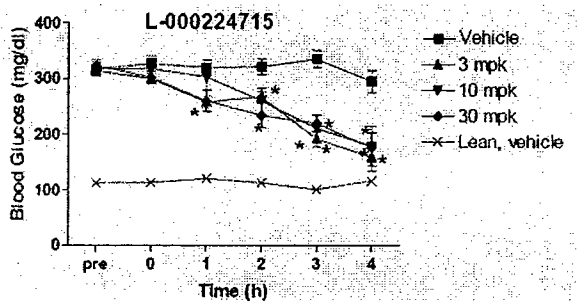


Figure 5:
Blood glucose in db/db mice with MK-0431



2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Brief Summary:
Safety assessment of neurological, renal, pulmonary, and gastrointestinal effects of MK-0431 did not identify any significant liability. MK-0431 clearly inhibits hERG potassium current *in vitro* at concentrations that markedly exceed human exposure, but nevertheless represents a potential cardiac conduction liability. Further cardiac telemetry and dose-rising studies did not identify a treatment-related change in QT or other ECG interval in dogs up to 50 mg/kg, reducing the importance of the hERG results. Other cardiovascular findings include a 56mmHg decrease in blood pressure and slight increase in heart rate in vagotomized dogs at 30mg/kg i.v., and a slight rise in heart rate in conscious dogs at 50mg/kg oral dose.

Neurological effects:
NOEL > 180 mg/kg (rats), >100 mg/kg (mice)

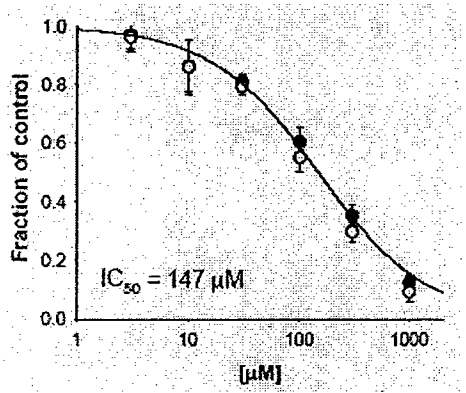
For CNS activity measurements, Sprague-Dawley rats were subjected to a functional observational battery assay (home cage, hand-held, and open-field observations, stimulus activity responses, and grip strength, foot splay, and body temperature measurements). There were no treatment-related effects after a single dose at 20, 60 or 180 mg/kg. CNS

function, behavior, motor activity, and thermoregulatory effects of MK-0431 (100mg/kg P.O) were also evaluated in 10 conscious mice. MK-0431 had no meaningful effect on these parameters when compared to vehicle-dosed animals.

Cardiovascular effects:
NOEL in vivo, 10mg/kg (dogs)

hERG activity: MK-0431 inhibits hERG potassium current with an IC_{50} of 147μM and an IC_{20} of ~50μM (**Figure 6**). Complete inhibition is achieved at 1000μM. Inhibitory activity is 80% reversible upon removal of MK-0431.

Figure 6: MK-0431 inhibition of hERG Current



Cardiovascular Rising Dose Study: Anesthetized and vagotomized dogs (n=3) were given an *intravenous* infusion of MK-0431 yielding cumulative doses of 1, 3, 10, and 30mg/kg over a 10 minute period (**Table 6**). No important changes in mean arterial pressure or heart rate were observed up to 10mg/kg, but at 30mg/kg blood pressure decreased 56 mmHg and heart rate decreased 40 bpm near the end of the 10 minute infusion.

Heart rate-corrected (Bazzet's) QT interval did not change at any dose. PR interval increased 7.4% at 30mg/kg without a change in QRS width or R-wave amplitude.

Plasma concentration was 202μM at 30mg/kg, and ≤ 59μM at 10mg/kg and lower. For comparison, the clinical Cmax is 1μM at 100mg.

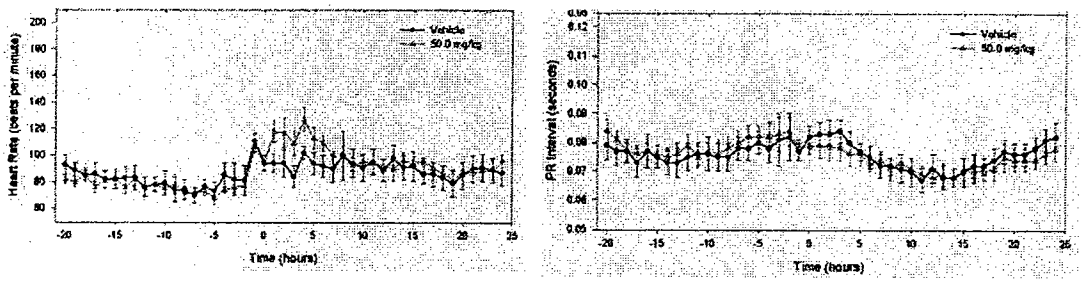
Table 6: Cardiovascular effects of MK-0431 in dose-rising study

CUMULATIVE DOSE (mg/kg IV)	0	1	3	10	30
Heart Rate (beats/minute)	163 ± 7	160 ± 7	162 ± 8	155 ± 9	123 ± 8
Mean Arterial Pressure (mm Hg)	111 ± 7	113 ± 9	115 ± 4	110 ± 2	55 ± 11
ECG PR Interval (msec)	95 ± 9	97 ± 6	91 ± 9	95 ± 9	102 ± 11
ECG QT_C Interval (msec)	368 ± 7	375 ± 9	373 ± 13	369 ± 11	372 ± 9
Plasma Concentration (μM)	—	6.02 ± 0.32	14.21 ± 2.37	58.70 ± 1.62	202.00 ± 38.58

Cardiovascular Telemetry Study: Conscious, telemetered dogs (n=4) were given single *oral* doses of MK-0431 at 2, 10, and 50mg/kg, or vehicle. All animals received all doses with at least a one-week washout period. There were no treatment-related effects on blood pressure or QT and QRS intervals at any dose. Between 4 and 6 hours after the 50mg/kg dose, heart rate increased ~30bpm with a concomitant shortening of the PR interval in 3/4 dogs (**Figure 7**). Emesis was noted in one dog after the 50mg/kg dose.

Plasma concentration at one hour post-dose was 1.6, 7, and 34μM at 2, 10, and 50mg/kg respectively. For comparison, the clinical Cmax is 1μM at 100mg MK-0431.

Figure 7: Heart rate (left) and PR interval (right) in telemetered dogs after



Pulmonary effects: *NOEL >180 mg/kg (rats), >10 mg/kg (dogs)*
Respiratory function (respiratory rate, tidal volume, minute ventilation) was evaluated in conscious rats using whole body plethysmography. There were no treatment-related effects on indices of respiratory function following single oral administration of 20, 60, or 180 mg/kg MK-0431.

In spontaneously breathing anesthetized dogs (n=3), the effect of intravenous MK-0431 (10mg/kg) on respiratory function, hemostasis, and platelet was evaluated. MK-0431 had

no meaningful effects on measured respiratory parameters, including peak expiratory flow, intrapulmonary pressure, tidal volume, lung compliance, airway resistance, and respiration rate. Blood pH, blood gases, hemostasis, and platelet function were not meaningfully changed. A decrease in blood pressure and increase in heart rate lasting less than 15 minutes were observed in 2 dogs.

Renal effects: *NOEL >10 mg/kg (dogs)*
The doses of 1 mg/kg and 10 mg/kg MK-0431 were administered orally to 6 conscious dogs (n=3 per dose) to assess renal function. No consistent changes in renal function, including glomerular filtration rate, effective renal plasma flow, electrolyte excretion, plasma electrolyte concentrations, and filtration fraction, were seen at either dose. No behavioral or emesis effects were seen after dosing.

Gastrointestinal effects: *NOEL >10mg/kg (dogs)*
MK-0431 at 10 mg/kg via gastric fistula cannula had no significant effect on basal gastric acid secretion or gastrin-stimulated gastric acid output in 6 conscious, female beagle dogs. No behavioral effects or emesis were seen after dosing. The dose of 10 mg/kg p.o. MK-0431 was evaluated in conscious mice for potential effects on GI motility as determined by the advancement of a charcoal meal in the small bowel. MK-0431 had no significant effect on intestinal transit and did not alter demeanor during the 80-minute exposure period.

Abuse liability: No abuse liability studies were performed.

Other: None

2.6.2.5 Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed. Combination studies with metformin are described in the *Special Toxicology Studies* section.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

An oral dose of MK-0431 is rapidly absorbed and is bioavailable (60-90%) in rats and dogs, with AUC and plasma concentration increasing generally in proportion with dose. MK-0431 distributes widely to tissues and tissue concentration of drug generally exceeds that in plasma except in the brain, eyes, and bone. Binding to plasma proteins is 32%-38% and does not differ between species. Clearance is moderate in dogs and high in rats, consistent with a longer plasma half-life and higher dose-normalized exposure in dogs vs. rats. Plasma half-life in humans is 3- to 6-fold longer due to slower clearance than in dogs and rats. MK-0431 is excreted primarily in urine but also in feces partly via biliary secretion.

In vivo metabolism of MK-0431 is minimal (≤ 20%) and oxidative conversion is mediated primarily by CYP3A4 and secondarily by CYP2C8. Six metabolites identified in human plasma are also present in either rat or dog plasma in equal or greater quantity, except for M4 (present at <1% in human plasma). Parent MK-0431 accounts for 80% to 90% of total exposure after an oral dose in rats, dogs, and humans; no metabolite in human plasma exceeded 7% of total exposure. Glutathione metabolites were also identified, but are not present in plasma.

In humans, MK-0431 and its metabolites are excreted predominately in the urine (87%) and less in the feces (13%). MK-0431 may be actively secreted into the urine by organic anion transporters in the renal tubules, based on *in vitro* data (*vide infra*). Nearly all of an administered radioactive dose is recovered in the feces and urine combined, indicating minimal retention of MK-0431 in tissues. Excretion in dogs and rats is similar to human, though the percent excreted in urine is higher in humans.

MK-0431 does not interfere in p-glycoprotein-mediated transport, despite itself being a substrate. MK-0431 does not appear to inhibit CYP450 enzymes nor induce CYP3A4. MK-0431 is a substrate for hOAT3 transport, but potential interference in transport of other organic ions appears low given the high Km for transport saturation (162μM). The results predict a low probability for pharmacokinetic drug interactions via the aforementioned pathways.

MK-0431 crosses the placenta in pregnant rats and is excreted in milk in lactating rats, ensuring drug expsoure to fetuses and newborns.

2.6.4.2 Methods of Analysis

Plasma concentrations of MK-0431: LC-MS/MS assay with a lower limit of quantification of 1.0 and 5.0 ng/ml in rat and dog plasma, respectively.

Radioactivity measurements: Liquid scintillation counting of relevant samples.

Metabolite identification: LC-MS and comparison with authentic synthetic standards.

2.6.4.3 Absorption

Intravenous and oral dosing produces linear increases in AUC exposure in male rats and dogs (**Table 1**). Bioavailability of an oral dose was high in rats (> 61%) and dogs (≥ 90%), and Cmax was reached within 1-2 hours. The dose-normalized $AUC_{0-\infty}$ values after oral administration were 4 to 6 times higher in dogs than rats, consistent with slower plasma clearance in dogs. Similarly, plasma half life was longer in dogs (4-5 hrs) than in rats (2-4 hrs). The Vd in dogs (3 ml/kg) was also lower than in rats (8 to 9 l/kg).

MK-0431 does not accumulate with multiple dosing in rats and dogs, but slightly accumulates ~15-30% in humans because of a 13 hour plasma half-life in humans.

Table 1: Pharmacokinetic properties of MK-0431 in rats and dogs

Summary of Nonclinical Pharmacokinetic Parameters of L-000224715[a]

I. Intravenous					
Species	Dose[b] (mg/kg)	$AUC_{0-\infty}$ (μM-hr)	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (hr)
Male Rat (n=4)	0.5	0.41 ± 0.12	52 ± 13	7.8 ± 0.3	1.7 ± 0.2
	2	1.93 ± 0.06	43 ± 1.3	8.7 ± 3.1	1.8 ± 0.3
	5	4.00 ± 0.38	52 ± 5.2	8.8 ± 2.4	1.8 ± 0.2
Male Dog (n=3 or 6)	0.5	2.17 ± 0.36	9.8 ± 1.7	3.4 ± 0.2	4.0 ± 0.3
	1.5	7.07 ± 1.37	8.9 ± 1.5	3.9 ± 0.8	4.4 ± 0.1
II. Oral					
Species	Dose[b] (mg/kg)	$AUC_{0-\infty}$ (μM-hr)	C_{max} (μM)	T_{max} (hr)	$t_{1/2}$ (hr)
Male Rat (n=3 or 4)	2	1.17 ± 0.14	0.21 ± 0.13	2.2 ± 1.8	2.6 ± 0.6
	20	14.8 ± 1.97	4.00 ± 0.38	0.9 ± 0.3	2.1 ± 0.2
	60	59.6 ± 6.25	17.1 ± 6.55	0.9 ± 0.3	2.3 ± 0.6
	180	214 ± 26.4	27.8 ± 6.71	1.4 ± 0.8	3.9 ± 0.2
Male Dog (n=3 or 6)	0.4	1.59 ± 0.44	0.24 ± 0.081	1.3 ± 0.6	3.8 ± 0.7
	1.6	7.08 ± 1.52	1.32 ± 0.24	0.8 ± 0.3	4.5 ± 0.7
	10	53.6 ± 13.3	10.3 ± 2.05	0.7 ± 0.3	4.7 ± 1.8
	30	155 ± 30.5	26.5 ± 6.25	0.8 ± 0.3	4.7 ± 0.3

[a] Male Sprague-Dawley rats and beagle dogs were dosed with a solution of the phosphate salt in saline.
[b] Doses are expressed in mg equivalents of freebase/kg. Mean ± SD values are listed.

Oral Pharmacokinetic Parameters for L-000224715 in Rats and Dogs[1]

Species	Dose[2] (mg/kg)	AUC (μM•hr)	C_{max} (μM)	T_{max} (hr)	$t_{1/2}$ (hr)
Rat	2	1.18±0.14	0.21±0.13	2.2±1.8	2.6±0.6
	20	14.9±1.97	4.00±0.38	0.9±0.2	2.1±0.2
	60	59.6±6.35	17.1±6.55	0.9±0.2	2.3±0.6
	180	214±26.4	27.8±6.71	1.4±0.7	3.9±0.2
Dog	0.4	1.59±0.44	0.24±0.08	1.3±0.6	3.8±0.7
	1.6	7.08±1.52	1.32±0.24	0.8±0.3	4.5±0.7
	10	53.6±13.3	10.3±2.05	0.7±0.3	4.7±1.8
	30	155±30.5	26.5±6.25	0.8±0.3	4.7±0.3
	50	399±50	2.3±16.0	1.3±0.6	5.0±0.3

[1] Male Sprague-Dawley rats and beagle dogs were dosed with an aqueous solution of the phosphate salt in saline. Mean ± standard deviation values are listed (N=3-6).
[2] Dose is expressed in mg of freebase/kg body weight.

2.6.4.4 Distribution

Tissue distribution of MK-0431 in rats: MK-0431 distributes to tissues rapidly and widely following intravenous dosing, partitioning equally between blood and plasma. MK-0431 distributes poorly to brain, but is present in most tissues at concentrations exceeding that in plasma (**Tables 2 and 3**). Within 4 hours post-dose, the highest drug concentrations are found in organs associated with excretion: gastrointestinal tract, kidneys, urinary bladder, and liver. High drug concentrations are also found 4 hours post-dose in non-excretory organs, including the lungs, thymus, spleen, and secretory organs (pancreas, pituitary, thyroid).

MK-0431 concentration in all organs is significantly lower at 24 hours than at 4 hours, indicating that drug is not retained in any organ evaluated.

Table 2:
Tissue Distribution of MK-0431 Relative to Plasma Concentration (radioactivity)

Tissue:Plasma Ratio	Tissues
< 1x	Brain
1x to 2x	Blood, bone, bone marrow, heart, mesenteric lymph nodes, skin, eyes, fat
> 2x	Adrenals, GI tract, epididymis, kidney, liver, lungs, pancreas, pituitary, prostate, skeletal muscle, spleen, testes, thymus, thyroid, urinary bladder

Table 3:
Tissue:Plasma concentration ratios of MK-0431-related radioactivity after a single 2 mg/kg intravenous dose.

Sample	Tissue:Plasma Ratio					
	5 Minutes (Group 1)		1 Hour (Group 2)		4 Hours (Group 3)	
	Mean	SD	Mean	SD	Mean	SD
Adrenal Glands	7.83	3.06	7.73	0.46	8.84	1.03
Blood	1.16	0.01	1.14	0.03	1.13	0.06
Bone (femur)	1.07	0.15	1.10	0.24	1.11	0.09
Bone Marrow	2.05	0.82	3.10	1.54	2.37	1.10
Brain	0.08	0.01	0.11	0.02	0.13	0.04
Cecum	2.49	0.21	5.17	0.61	70.91	18.07
Epididymis	0.80	0.22	3.14	0.27	7.34	0.59
Eyes	1.02	0.30	0.99	0.09	1.04	0.06
Fat (mesenteric)	0.62	0.20	0.66	0.21	0.97	0.21
Heart	3.30	0.38	2.55	0.37	2.75	0.26
Intestine, Large	3.10	0.36	5.17	0.08	33.54	16.32
Intestine, Small	5.84	0.64	23.90	2.84	48.49	10.61
Intestinal Contents	1.12	0.33	27.67	4.31	96.86	12.52
Kidney	13.41	1.81	13.88	0.88	27.53	3.87
Liver	11.74	1.62	15.64	3.16	35.04	1.12
Lungs	6.43	0.89	9.02	1.72	15.22	3.20
Lymph Nodes, Brachial	3.84	1.23	4.06	0.94	3.84	0.56
Lymph Nodes, Mesenteric	1.65	0.60	1.41	0.74	2.03	NA
Pancreas	6.63	1.71	7.81	1.95	6.45	0.45
Pituitary Gland	7.81	5.48	6.37	4.40	9.55	6.53
Prostate	3.33	0.51	5.84	3.55	6.08	3.06
Skeletal Muscle	1.68	0.74	4.20	0.41	3.36	0.06
Skin (subscapular)	1.29	0.15	2.43	0.22	2.32	0.14
Spleen	5.52	0.81	3.47	1.72	10.08	7.36
Stomach	3.88	0.34	6.18	0.32	4.39	0.65
Stomach Contents	0.26	0.31	0.46	0.24	0.27	0.14
Testes	0.23	0.05	1.53	0.16	5.03	0.97
Thymus	1.79	0.09	4.29	0.51	5.46	0.74
Thyroid	7.64	1.03	3.30	0.39	4.76	1.34
Urinary Bladder	6.14	2.21	21.96	15.51	22.63	9.80

NA = Not applicable
*Plasma radioactivity concentration equals 0.7, 0.2, and 0.06 µg*eq/g at 5min, 1hr, and 4hr post-dose, respectively.*

Recovery of drug-related radioactivity was ≥ 95% by 120 hours following an intravenous or oral dose. MK-0431 was recovered in the feces and urine in approximately equal quantities (**Table 4**).

Table 4: Total recovery of radioactivity 120 hours after a single intravenous or oral dose of MK-0431.

Sample	Time (hours)	Recovery (% of dose)			
		Intravenous (Group 5)		Oral (Group 6)	
		Mean	SD	Mean	SD
Urine	0-120	53.49	5.80	37.27	2.59
Feces	0-120	40.22	3.23	58.92	2.51
Cage Wash	120	0.52	0.25	0.24	0.05
Cage Wipe	120	0.00	0.00	0.38	0.66
Carcass	120	0.52	0.05	0.49	0.05
Total	**0-120**	**94.76**	**2.78**	**97.30**	**0.55**

Placental transfer of MK-0431: In rats and rabbits, MK-0431 readily crosses the placenta into the fetal circulation after dams receive oral doses from gestation days 6 to 20 (*see* Reproductive Toxicity section and **Table 5**).

Table 5: Fetal and maternal MK-0431 plasma concentration on gestation day 20

Species	Dose (mg/kg/day)	Time (hr)	Plasma Concentration (μM)		Ratio (Fetal/Maternal)
			Maternal	Fetal	
Rat	250	2	11.7 ± 3.23	5.52 ± 1.67	0.463 ± 0.0274
		24	1.33 ± 0.258	0.990 ± 0.106	0.807 ± 0.111
	1000	2	41.3 ± 6.35	17.9 ± 2.90	0.435 ± 0.0196
		24	7.44 ± 2.54	5.27 ± 1.15	0.787 ± 0.0779
Rabbit	125	2	31.9 ± 4.95	18.1 ± 3.66	0.663 ± 0.205
		24	0.847 ± 0.0674	0.247 ± 0.00531	0.296 ± 0.0195

Mean ± standard error of the mean (n=4 animals per time point) [Sec. 2.6.5.5]

Plasma protein binding and blood partitioning of MK-0431: Approximately 32% to 38% of tritiated MK-0431 binds to plasma proteins, as assessed by ——————, from several species including humans (**Table 6**). There was no difference among species and the percent binding was largely independent of drug concentration. MK-0431 preferentially bound albumin (64%) vs. α1-glycoprotein (25%).

Partitioning of MK-0431 (0.1, 1, 10μM) was equal between blood and plasma (blood:plasma concentration ratio = 1) in samples evaluated from rats, dogs, and humans. Thus, blood clearance of MK-0431 will approximate plasma clearance.

Table 6: In vitro plasma protein binding of tritiated MK-0431 in several species

Concentration (μM)	Percent Bound[a]				
	Mouse	Rat	Rabbit	Dog	Human
0.02	34 ± 1.1	n.d.[b]	42 ± 1.6	n.d.	46 ± 2.7
0.1	n.d.	36 ± 0.6	n.d.	32	41 ± 3.6
0.2	29 ± 2.2	n.d.	29 ± 1.1	n.d.	37
1.0	n.d.	32 ± 0.7	n.d.	37	37 ± 2.2
2.0	27 ± 0.2	n.d.	24 ± 0.6	n.d.	36 ± 3.3
5.0	34 ± 2.1	36 ± 1.3	n.d.	n.d.	36 ± 6.3
10	n.d.	32 ± 0.5	n.d.	31	34 ± 1.0
50	34 ± 1.1	34 ± 1.2	n.d.	n.d.	n.d.
200	33 ± 0.9	28 ± 1.6	n.d.	n.d.	n.d.
Mean ± SD	32 ± 3.1	33 ± 3.0	32 ± 9.3	33 ± 3.2	38 ± 4.1

[a] Numbers represent mean ± standard deviation for at least three determinations. When standard deviation values are not listed, the number was determined from single or duplicate determinations. The binding in human plasma (when associated with standard deviation values) represents the mean of 6 or 12 determinations using plasma from 3 or 4 individuals, except for 0.2 and 10 μM, which used 1 and 3 determinations from 1 individual, respectively.
[b] n.d. = Not determined.

MK-0431 is a substrate for murine P-glycoprotein: Following intravenous administration of MK-0431 to Mdr1a-deficient (-/-) and wild type (+/+) mice, brain concentrations of drug were higher in the (-/-) than in the (+/+). This resulted in higher brain-to-plasma concentration ratios for the (-/-) than the (+/+) mice (-0.2 to 1.5, and <0.2, respectively), indicating that MK-0431 is a substrate for the mouse P-glycoprotein which influences tissue concentrations of drug.

The transport of MK-0431 was further assessed in LLC-PK1 cells that overexpress murine (Mdr1a) or human (Mdr1) p-glycoprotein. As shown in **Table 7**, equal and bi-directional transport of MK-0431 in 'control' cells is converted to basolateral-apical uni-directional transport in cells expressing murine or human p-glycoprotein. Quantitatively, p-glycoprotein transport remains low (≤ 8% over 4 hours) in this assay.

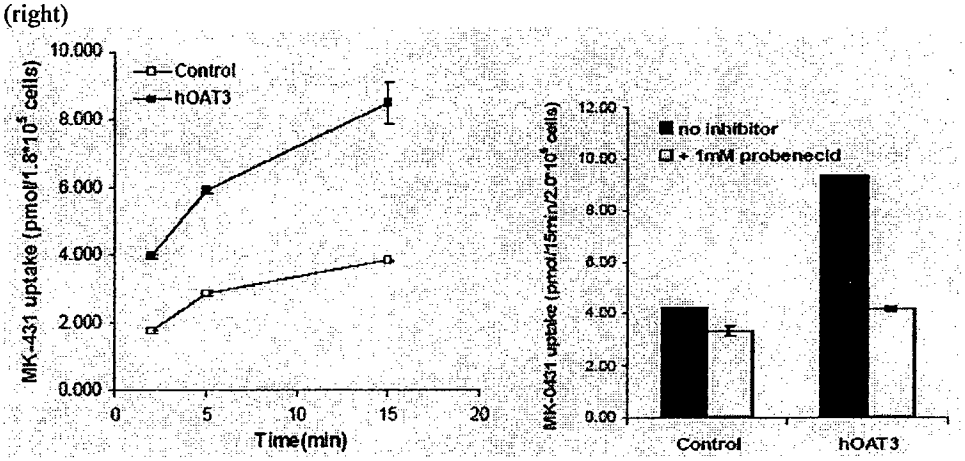
Table 7: Transport of MK-0431 in LLC-PK1, Mdr1, and Mdr1a cell lines

	% Transported			
Time (hr)	1	2	3	4
LLC-PK1 A→B	1.0	0.7	2.4	3.1
LLC-PK1 B→A	0.7	1.6	2.5	3.9
LLC-Mdr1a A→B	0.2	0.3	0.5	0.4
LLC-Mdr1a B→A	1.3	2.9	4.3	6.4
LLC-MDR1 A→B	0.6	0.6	0.7	0.9
LLC-MDR1 B→A	1.9	3.3	4.8	7.7
	B→A/A→B Ratio			
Time (hr)	1	2	3	4
LLC-PK1	0.7	2.3	1.0	1.3
LLC-Mdr1a	6.5	9.7	8.6	16.0
LLC-MDR1	3.2	5.5	6.9	8.6

MK-0431 is a substrate for human OAT3: As shown in Table 8, MK-0431 is taken up by cells expressing the human organic anion transporter-3 (hOAT3) more than by cells not expressing hOAT3. Uptake was saturable with a Km of 162μM. Such uptake is inhibited by probenicid, an inhibitor of hOAT-mediated transport, confirming that MK-0431 is a substrate for hOAT-3.

MK-0431 did not interact with hOAT1, hOAT4, hOCT2, or hPEPT1 *in vitro*.

Table 8: Uptake of ^{14}C-MK-0431 by hOAT3-transfected CHOK1 cells (left) and inhibition by probenicid (right)

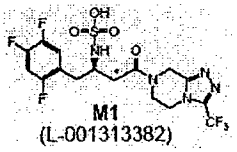
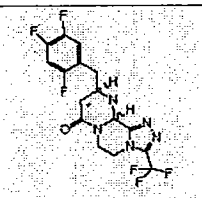
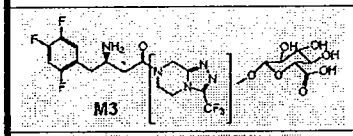
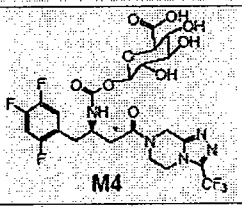
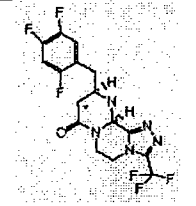
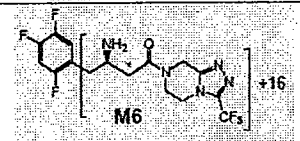
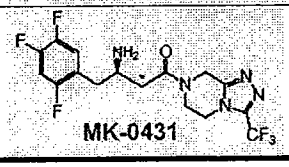


2.6.4.5 Metabolism

In vivo Metabolism: **Table 9** depicts six metabolites identified in plasma from humans and test species at one and eight hours following an oral dose. Relative quantities of metabolites shown in **Table 9** are similar when carried out to 12h and 18h post-dose in humans. All metabolites identified in human plasma were also present in either rat or dog plasma, except for M4 (present at < 1%). Parent MK-0431 accounts for 80% to 90% of total radioactivity in human, rat, and dog plasma, indicating minimal metabolism of parent. No metabolite in human plasma exceeded 7% of total radioactivity. DPP4 inhibitory activity of M1, M2, and M5 is 300 to 1000-fold less than the parent, contributing little to overall pharmacodynamic activity.

Thus, an oral dose of MK-0431 is minimally metabolized (~20%) to six plasma metabolites and several non-plasma glutathione conjugates. Human plasma metabolites are found in either dog or rat plasma in equal or greater amounts; potential toxicity of metabolites was therefore adequately assessed in non-clinical studies. No human plasma metabolite exceeded 7% of total radioactivity following an oral dose of parent, negating the need for separate genotoxicity/carcinogenicity testing of metabolites.

Table 9: Plasma Metabolites in Humans vs. Non-clinical Test Species

Structure	Present in Species	Percent Total Radioactivity in Plasma after Oral Dosing					
		1 hour			8 hour		
		Hu	Rat	Dog	Hu	Rat	Dog
 M1 (L-001313382)	Human Rat Rabbit	3%	3%	--	3%	--	--
 M2 *cis* (L-001029341)	Human Rat Rabbit Dog Mouse	1%	1%	--	1%	2%	8%
 M3	Human Rat Rabbit Mouse	< 1%	3%	--	< 1%	2%	--
 M4	Human Rabbit Mouse	< 1%	--	--	< 1%	--	--
 M5 *trans* (L-001029343)	Human Rat Rabbit Dog Mouse	4%	2%	4%	7%	5%	25%
 M6	Human Rat Rabbit Mouse	1%	--	--	2%	1%	--
 MK-0431	---	90%	92%	96%	78%	88%	67%

In Vitro Metabolism: Liver microsomes and hepatocytes from humans and test species minimally metabolized MK-0431 in 1- and 4-hour incubation assays (≤ 14%, **Table 10**). The low level of metabolism *in vitro* precluded definitive identification of metabolites, but trace amounts of M2, M3, M5, M6, and 2 glutathione adducts were detected.

Table 10: Total metabolism (%) of MK-0431 by liver microsomes and hepatocytes *in vitro*

Test system	Gender	Incubation Time	Total Metabolism (%)[a]					
			Mouse	Rat	Rabbit	Dog	Monkey	Human
Liver microsomes	Male	1 hr	10	1	1	1	9	2
Liver microsomes	Female	1 hr	10	1	3	2	13	2
Hepatocytes	Male	4 hr	n.d.[b]	14	n.d.	1	n.d.	13

MK-0431 is metabolized by CYP3A4 to the primary oxidative metabolites M2, M5, and M6. Metabolism by CYP2C8 contributes a minor degree to formation of M2 and M5, but not M6. This was confirmed by incubation of MK-0431 with recombinant human CYP enzymes and with liver microsomes containing anti-CYPs 3A4 or 2C8 antibodies (Table 11).

Table 11: Metabolism of MK-0431 to M2, M5, and M6 is blocked by anti-CYPs 3A4 and 2C8 antibodies.

Metabolite	Percent Inhibition of Formation	
	Anti-CYP3A4	Anti-CYP2C8
M2	96	54
M5	94	52
M6	94	0

Inhibition and Induction of CYP450 Enzymes: MK-0431 did not inhibit the in vitro CYP450 activity in pooled liver microsomes (IC50 >100μM, CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4). Pre-incubation of MK-0431 with liver microsomes, with or without NADPH regeneration, did not result in CYP450 inhibition.

MK-0431 (1 and 10μM) did not significantly induce mRNA nor increase activity of CYP3A4 in human primary hepatocytes from 3 organ donors. Rifampicin, as a positive control, significantly induced mRNA and activity of CYP3A4 in parallel incubations.

P-glycoprotein Functional Interactions: *In vivo* and *in vitro* studies identify MK-0431 as a substrate for p-glycoprotein. MK-0431 was further assessed for interference in p-glycoprotein-dependent transport of selected agents. Using LLC-PK1 cells that express either human p-glycoprotein (mdr1) or empty vector determined that MK-0431 (0.3-500μM) does not reduce transport of digoxin, verapamil, vinblastine, ritonavir, or quinidine. Cyclosporin, a potent inhibitor of p-glycoprotein, reduced transport of all these agents (**Table 12**).

Table 12: Effect of MK-0431 on p-glycoprotein-dependent transport of various agents in MDR-1-expressing LLC-PK1 cells.

MK-0431 (μM)	B-A/A-B Ratio of Test Compound at 4 hr									
	Digoxin (2 μM)		Verapamil (1 μM)		Vinblastine (5 μM)		Ritonavir (5 μM)		Quinidine (5 μM)	
	LLC-PK1	LLC-MDR1	LLC-PK1	LLC-MDR1	LLC-PK1	LLC-MDR1	LLC-PK1	LLC-MDR1	LLC-PK1	LLC-MDR1
0	2.5	6.3	1.0	4.3	2.2	11.0	2.2	7.7	0.8	8.2
0.3	2.1	6.3	1.2	4.4	2.3	11.3	2.4	12.7	1.0	10.0
1	3.1	6.2	1.0	4.9	2.4	14.0	2.0	12.9	0.9	7.1
5	n.d.[a]	6.8	n.d.	4.5	n.d.	12.7	n.d.	11.3	n.d.	9.2
20	n.d.	8.2	n.d.	4.2	n.d.	9.5	n.d.	12.1	n.d.	9.5
50	n.d.	5.7	n.d.	5.6	n.d.	13.1	n.d.	12.2	n.d.	9.5
100	2.4	5.7	1.0	4.9	2.2	10.4	2.0	12.4	n.d.	8.1
250	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	8.4
500	1.6	6.5	1.0	3.7	1.5	10.6	1.4	13.0	0.8	5.6
CsA[b]	0.7	1.0	1.2	1.0	0.7	1.2	0.9	2.1	0.7	0.9

[a] n.d. = Not determined
[b] CsA = cyclosporin A (10 μM)

2.6.4.6 Excretion

Excretion of a single intravenous or oral dose of ^{14}C-MK-0431, detected as drug-related radioactivity, was split nearly equally via urine and feces in the rat and primarily via urine in the dog (**Table 13**). Greater than 94% of an oral dose is recovered in the feces and urine collectively. Twenty percent of drug-related radioactivity was recovered in the bile of cannulated rats, lower than the 40-60% recovered in feces from intact rats.

Contrary to excretion in rats and dogs, excretion in humans was predominately urinary. An average of 87% of administered ^{14}C-MK-0431 was recovered in urine vs. 13% in the feces. The metabolite profile in urine and feces was similar to that in plasma, except for the absence of M3 and M4 (glucuronides) in feces.

Table 13: Excretion of MK-0431 after intravenous and oral dosing in rats and dogs.

RAT (0 to 120 hr)				
Dose (mg/kg)	Route	Percent of Administered Dose		
		Urine	Feces	Total
2	IV	53.5 ± 5.8	40.2 ± 3.2	94.8 ± 2.8
5	P.O.	37.3 ± 2.6	58.9 ± 2.5	97.3 ± 0.6
DOG (0 to 96 hr)				
0.5	IV	62.0 ± 14.2	9.8 ± 4.5	89.0 ± 5.6
2	P.O.	64.6 ± 6.4	17.3 ± 16.8	94.3 ± 17.4
Mean ± SD of n = 3 animals per route. Total recovery includes radioactivity in the cage wash and debris.				

HUMAN (0 to 168 hr) n=5				
80 mg	P.O.	87 ± 5.2	13.1 ± 5.3%	100.1 ± 4.1

MK-0431 is excreted in milk of rats: MK-0431 is excreted in the milk of lactating rats in a 4:1 concentration ratio to plasma (**Table 14**).

Table 14: Excretion of MK-0431 in milk of lactating rats

Species - Dose (mg/kg/day):	Rat -250	Rat -1000
Lactation day/ Time (hr):	Day 14/ 2 hr	Day 14/ 2 hr
Concentration (μM)[a]:		
Maternal plasma	14.8 ± 3.90	33.5 ± 6.20
Maternal milk	60.9 ± 24.7	136 ± 30.6
Ratio (Milk/Plasma)	3.85 ± 0.531	3.93 ± 0.522

2.6.4.7 Pharmacokinetic drug interactions

MK-0431 does not interfere in p-glycoprotein-mediated transport, despite itself being a substrate. MK-0431 does not appear to inhibit CYP450 enzymes nor induce CYP3A4. MK-0431 is a substrate for hOAT3 transport, but potential interference in transport of other organic ions appears low given the high Km for transport saturation (162μM). The results predict a low probability for pharmacokinetic drug interactions via the aforementioned pathways.

2.6.4.8 Other Pharmacokinetic Studies

Chiral Inversion: Merck collected plasma samples from individuals treated with 200mg of MK-0431 at 2 hr and 15 hr post-dose (sub#247, 8/22/05). Plasma concentrations were measured using a chiral separation method to identify the isomer, L-400224. The analysis found no peak at 1.4 min representing L-400244, suggesting that no chiral conversion had taken place.

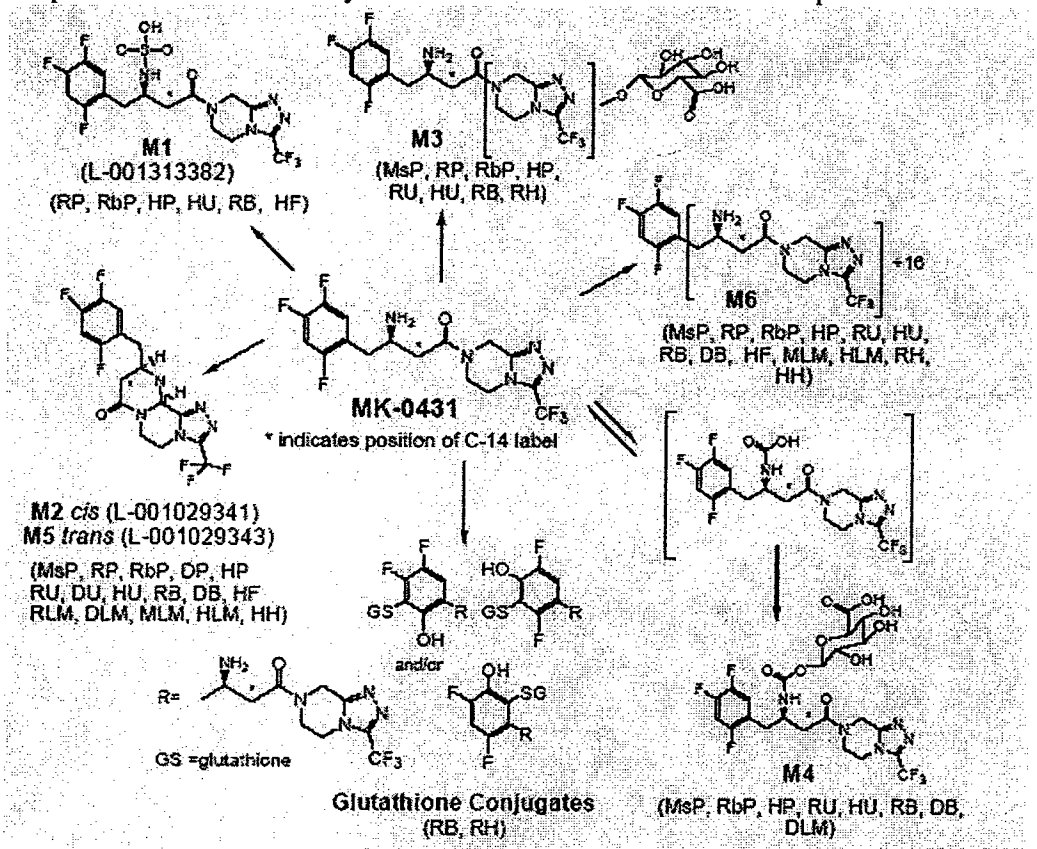
2.6.4.9 Discussion and Conclusions

Pharmacokinetics of an equivalent oral dose by body surface area in dogs and rats is similar to humans relative to AUC and Cmax exposure (**Table 15**). However, clearance is higher in dogs and rats resulting in substantially shorter plasma half-lives of 2-4 hours in dogs and rats and 13 hours in humans.

2.6.4.9 Tables and figures to include comparative TK summary

Figure 1

Proposed Metabolic Pathway of ^{14}C-MK-0431 in non-clinical test species and humans



(Parenthetical abbreviations refer to biological matrices in which MK-0431 metabolites have been detected: MsP = Mouse Plasma, RP = Rat Plasma, RU = Rat Urine, RB = Rat Bile, RbP = Rabbit Plasma, DP = Dog Plasma, DU = Dog Urine, DB = Dog Bile, HP = Human Plasma, HU = Human Urine, HF = Human Feces, RLM = Rat Liver Microsomes, DLM = Dog Liver Microsomes, MLM = Monkey Liver Microsomes, HLM = Human Liver Microsomes, RH = Rat Hepatocytes, HH = Human Hepatocytes)

Figure 2:
Comparative Toxicokinetic Summary Table

Daily Dose (mg/kg)	Steady-State AUC (μM·hr)							
	Mice		Rats		Dogs		Female Rabbits	Humans[a]
	M	F	M	F	M	F		
0.5								2.13
1								3.71
2					9.99	7.93		8.48
4								16.2
8								29.6
10					55.9	55.2		
12								
20			15.3	12.3				51.1, 27.6
			19.2	15.0				
50					248	216		
60			49.3	44.9				
			70.6	48.9				
125								
180			165	149			189	
			227	157				
250	233	240						
	211	227						
500	597	585	517	412				
	616	433	520	466				
750			885	815				
			626	635				
1000	1230	1390	1110	1130				
	1550	1040						
1500			1500	2050				
			1050	1040				
			1630	1440				
2000	2110	4420	1050	2310				
3000			1550	3040				
4000	ID	ID						

ID = Insufficient data
a Geometric least squares mean

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Tables: Multiple dose PK in humans and mean PK parameters after a single oral dose in Phase I studies

Preliminary PK after repeated oral doses (10 days) of L-224715 in young healthy male volunteers (protocol 04)

Multiple Dose Study	T_{max}, hr		C_{max}, nM		$AUC_{0-\infty}$, μM.hr		t½, hr
	D1	D10	D1	D10	D1	D10	D10
25 mg	4.4	2.6	140	176	1.75	2.17	13.5
50 mg	3.4	3	290	387	2.93	3.77	13.8
100 mg	2.8	3	937	987	7.65	8.75	13.5
200 mg	1.4	1.2	2372	2354	15.5	16.3	12.7
400 mg	2.2	1.6	3701	3848	28.4	30.2	11.6

The ratio of AUC on day 10 to day 1 was about 1.3 fold, suggesting slight accumulation of L-000224715 with repeated drug exposure.

Mean Pharmacokinetic Parameters Across Phase I Studies Following Multiple Oral 100-mg Doses of MK-0431 Administered Once Daily to Fasting Healthy Subjects

Study	Formulation	N	$AUC_{0-24\ hr}$ (μM•hr)[†]	C_{max} (nM)[†]	T_{max} (hr)[‡]	$t_{1/2}$ (hr)[§]	Cl_R (mL/min)[†]	$f_{e,0-\infty}$[‖]
P004	Capsule	8	8.48	941	3	14.4	363	0.758
RC715A111	Capsule	6	7.87	888	2	11.99	376	0.721

† Geometric Least-Squares Mean or Geometric Mean.
‡ Median.
§ Harmonic Mean for Apparent terminal $t_{1/2}$.
‖ Arithematic Least-Squares Mean.

TABLE 15

Comparative pharmacokinetics of repeated oral doses in humans, dogs, and rats

	Dose mg/m^2 (mg/kg)	AUC, μM*h	Cmax, μM	Tmax, h	$T_{1/2}$, h	Cl, ml/min/kg
*Human	53 (1.4)	10	1	3	13	5
Dog	53 (2.6)	11	2	0.8	4.5	9
Rat	53 (8.8)	7	2	0.9	2	50

Comparison based on body surface area-equivalent doses.
* Based on 70kg body weight

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

Single dose toxicity studies were conducted in CD-1 mice and Sprague-Dawley rats with two different formulations of MK-0431: anhydrous drug and the monophosphate salt (latter identical to clinical formulation). Drug was administered by oral gavage in all studies.

MK-0431 was lethal in mice at ≥ 2000 mg/kg and in rats at 3000 mg/kg, corresponding to exposures of ≥ 1500μM*hr AUC_{0-24}. The minimum lethal dose in rodents exceeds clinical exposure by ≥ 150-fold.

Clinical signs documented in rats at 3000 mg/kg included decreased activity, hunched posture, and labored breathing. Death of one rat occurred at this dose level.

Causes of death were not identified nor pursued by the investigators. Moreover, the toxicological assessment was limited to evaluating clinical signs within one day of dosing in rats and within 14 days of dosing in mice. Thus, these studies provide no toxicological information other than identifying acutely lethal doses in rats and mice.

Toxicokinetic data indicate that the anhydrous and monophosphate formulations of MK-0431 provide similar exposure as AUC_{0-24} in mice and rats, at least up to a 500mg/kg dose. The anhydrous formulation provides ~50% higher exposure than the monohydrate formulation at higher doses.

SINGLE DOSE TOXICITY STUDIES			
SPECIES	**NOAEL**	**MRHD MULTIPLE (100mg; 10μM*hr)**	**FINDINGS**
Mouse, CD-1 GLP study # TT026410 *Anhydrous drug*	**Minimum Lethal Dose** 2000 mg/kg	200-400x	Death from undetermined cause on drug day 1: 1 male, 1 female of 60 mice at 2000 mg/kg 18 males, 17 females of 60 mice at 4000 mg/kg *Doses:* 250, 500, 1000, 2000, 4000mg/kg *AUC_{0-24}:* 236, 591, 1310, 2110/4420 (m/f) μM*hr *(no AUC for 4000mg/kg)*
Mouse, CD-1 GLP study # TT030590 *monophosphate salt*	No Lethal Dose Found	n.a.	No deaths observed on drug day 1. (n=30/group) No necropsy or other toxicological assessment done. *Doses:* 250, 500, 1000mg/kg *AUC_{0-24}:* 219, 524, 1550/1040 (m/f) μM*hr
Rat, Sprague-Dawley GLP study # TT022566 *Anhydrous drug*	**Minimum Lethal Dose** > 2000 mg/kg	n.a.	No deaths observed in 3/3 female rats within 14 days after a single oral dose. Salivation noted with dosing. Body weight was unchanged. *Doses:* 2000 mg/kg; no exposure data
Rat, Sprague-Dawley GLP study # TT020900 *Anhydrous drug*	**Minimum Lethal Dose** 3000 mg/kg	150-300x	Death of 1/12 females from undetermined cause at 3000 mg/kg on drug day 1 . All high dose males/females (n=12/sex) showed ↓ activity, labored breathing, urine staining, reddish discharge from nose, cold to touch, hunched posture. Urine staining in low dose females (2/12) and some mid-dose males and females. *Doses:* 750, 1500, 3000 mg/kg *AUC_{0-24}:* 840, 1750, 1550/3040 (m/f) μM*hr
Rat, Sprague-Dawley GLP study # TT030600 *monophosphate salt*	No Lethal Dose Found	n.a.	No deaths observed in 9 males and females on drug day 1 after a single oral dose. No toxicological assessment was performed. *Doses :* 500, 750, 1500 mg/kg *AUC_{0-24} :* 464, 630, 1050 μM*hr

n.a., not applicable

2.6.6.3 Repeat-dose toxicity

General toxicity was assessed in Sprague-Dawley rats and in Beagle dogs in studies up to 6 months and 12 months duration, respectively. Exposure to Januvia ranged from 1x to 20x the 100mg MRHD in all repeat dose studies except for the 3 month rat study that evaluated exposures up to 200x MRHD. Additional toxicity studies of 1 and 3 months duration were conducted in CD-1 mice.

Note that all repeat dose toxicity studies used the anhydrous formulation, not the monohydrate formulation proposed for clinical use. This does not present a problem because both formulations provide similar AUC exposure at least in rodents up to 500 mg/kg.

The high dose 3-month study in rats was the only study that adequately identified target organs. No significant toxicities in rats were elicited in shorter term studies or the chronic 12-month study that evaluated Januvia at 1x to 20x multiples of the MRHD. Studies in dogs identified NOAEL doses based primarily on neurological clinical observations rather than target organ identification.

High-dose Target Organ Study in Rats

This 3 month study in rats evaluated toxicity of Januvia at 50x to 200x multiples of clinical exposure at the MRHD of 100mg. Clear target organs were identified at 1000, 1500, and 2000mg/kg:

Kidney: Renal tubule degeneration and necrosis occurred at 1500 and 2000mg/kg in males and females, respectively. Renal tubular necrosis was cited as the cause of death for males and females in both dose groups. Markers of toxicity included increased BUN and creatinine, increased urine volume, and increased proteinuria. Kidney toxicity was not observed in dogs.

Liver: Hepatocellular hypertrophy with increased liver weight was observed at 500mg/kg and higher, likely reflecting an adaptive response to drug. However, hepatocellular degeneration and necrosis occurred at 2000mg/kg and is considered adverse. Liver enzymes ALT and ALP increased a modest 2-fold at doses $\geq$1000 mg/kg. A similar increase in ALT was seen in high dose dogs in the 15-day study, but was not reproducible in longer-term studies.

Heart: Myocardial degeneration and necrosis was observed at 1500 and 2000mg/kg. Heart mineralization was also observed in one high-dose dog that subsequently died (probable intubation event) in the 6 month study and in another high-dose dog that survived 52 weeks of dosing. Merck did not measure CPK in any study despite FDA recommendations, so a more accurate assessment of muscle injury is not possible.

Teeth: Ameoblast and odontoblast degeneration observed at 1000mg/kg progressed to incisor thickening and occasional tooth breakage at higher doses. Other than teeth, no

remarkable findings in other bone were reported. However, it is possible that rapidly growing bone may be adversely effected by high concentrations of Januvia.

Bone Marrow: Minimal to moderate necrosis was observed in 1/15 males at 1500mg/kg and in 2/15 males at 2000mg/kg. No females were effected. Such effects were not seen in dogs except for one case of bone marrow depletion present in one female that died prematurely in the 6 month study.

Lymph nodes: Depletion of lymph nodes and the thymus occurred at 2000mg/kg and is likely secondary to physical stress.

Target Organ Study in Mice

A complete 3 month toxicity study in mice, conducted in support of carcinogenicity studies, identified the *kidney* as the primary target organ. Renal pelvis dilatation with substantial loss of parenchyma was present in males and females at 500 and 750mg/kg, respectively, along with increased BUN and increased kidney weight. At least one death (from 6) at 1000mg/kg is related to renal toxicity.
The LOAEL and NOAEL provide a $\geq$ 50x and $\geq$ 20x multiple of exposure at the MRHD of 100mg.

Clinical Signs in Dogs

Some consistent neurological clinical signs were present in all the dog studies, primarily at the high dose of 50 mg/kg. Reduced activity, hunched posture, ataxia, tremor, and sporadic emesis were most common. Recumbency and head tilt were less commonly observed. Respiratory distress was present in the 6 and 12 month studies, described as abnormal and audible respiration, labored breathing, and open-mouthed breathing. These signs typically started shortly after dosing (salivation pre-dosing) and lasted for several hours. Shorter term studies claim that these signs dissipated with time, but longer term studies note that the signs persisted to the end of the study. No consistent drug-related change in gross or histopathology was observed in these studies. Reasons for the relative intolerance of dogs to the 50 mg/kg dose are not known.

Non-Reproducible or Equivocal Toxicities

Skeletal Muscle Toxicity: Slight myofiber degeneration was found in 1/4 male and female dogs at the high dose in the 3 month study, and again in 1/4 high dose males in the 6 month study. Such findings were absent in the 52 week study. Note that CPK measurements were never done which may have identified some dogs with muscle injury not detected with histochemistry. These findings are of minimal clinical concern because the LOAEL is a 20x multiple of clinical exposure and the incidence and severity appears low.

Eyelid Swelling: Eyelid swelling with lymphatic distension was observed in 2/4 high dose males in the 15 day study. This finding was absent in all subsequent longer-term studies.

Footpad complications: Interdigit cysts and reddening of the footpads was common in the 27 week and 53 week studies in beagle dogs, requiring intervention with cage pads or soaking paws in Xenodyne. However, there is no clear relationship to drug treatment as the incidence is similar in the control groups. There is no evidence that the paw cysts progressed with time (as seen with DPP4 inhibitor-related skin lesions).

Lung Toxicity: Studies of 6 months duration reported that 5 rats and 1 dog in the high dose groups died due to intubation errors. This is clearly the case for 1 rat with an esophageal perforation; however, the cause is less certain for other early-deceased rats with multifocal hemorrhage and pleuritis of the lungs. The early-deceased dog showed respiratory distress prior to death and lung discoloration/necrosis upon necropsy. All other dogs in the high dose groups of the 6 and 12 month study showed abnormal respiration with bronchial sounds and some showed labored breathing, but none showed histological changes to the lungs that would explain the respiratory distress.

The drug-relatedness of these events is equivocal and would require toxicity studies of 6 months duration with higher doses to provide clarity. Such studies are not needed, however, because the LOAEL and NOAEL provide an adequate 20x and 10x multiple of clinical exposure from a 100mg dose.

TABLE: REPEAT DOSE TOXICITY STUDIES IN CD-1 MICE			
STUDY DURATION	NOAEL	MRHD MULTIPLE (100mg; 10μM*hr)	FINDINGS
1 Month GLP study #TT026420	**Minimum Lethal Dose** 1000 mg/kg	100-200x	**Death** at 1000 and 2000mg/kg : 1000mpk: 4/60 died by drug day 10 (m+f) 2000mpk: 23/60 died by drug day 6 (m+f) *Doses:* 125, 250, 500, 1000, 2000 mg/kg $AUC_{0\text{-}24}$: 96, 223, 576, 1130/2340 (m/f) μM*hr
3 Months GLP study #TT036010	250 mg/kg Males 500 mg/kg Females	*20x Males *50x Females **estimated*	**Death** at 1000mg/kg: *Females:* 5/15 died by drug day 5, unknown cause but 'treatment-related' *Males:* 1/15 died on drug day 43, dilated renal pelvis; another 4 males were found dead on days 3, 10, 16, & 75 due to 'intubation accidents'. **Renal toxicity**, Males 500mg/kg & Females 750mg/kg: ↑ BUN, ↑ kidney weight, renal pelvis dilatation with loss of parenchymal tissue **Liver toxicity**, Males 1000 mg/kg (females not examined) ↑ AST, ALT, centrilobular hypertrophy (very slight) No effect ≤ 250 mg/kg males, ≤ 500 mg/kg females *Doses* : 75, 250, 500, 750, 1000 mg/kg $AUC_{0\text{-}24}$: Not measured

TABLE: REPEAT DOSE TOXICITY STUDIES IN SD RATS			
STUDY DURATION	**NOAEL**	**MRHD MULTIPLE (100mg; 10μM*hr)**	**FINDINGS**
15 Days GLP Study #TT020320	**> 180mg/kg**	**> 20x**	No drug-related deaths No drug-related change in BW, ophthalmic, hematology, chemistry, or urinalysis. No drug-related change in organ weight, necropsy, histopathology. *Doses*: 20, 60, 180 mg/kg (+ placebo) $AUC_{0\text{-}24}$: 14, 47, 157 μM*hr
3 Months GLP Study #TT020580	**> 180mg/kg**	**> 20x**	No drug-related deaths Salivation in all high dose males and females No drug-related change in BW, ophthalmic, hematology, chemistry, or urinalysis. No drug-related change in organ weight, necropsy, histopathology *Doses:* 20, 60, 180 mg/kg (+ placebo) $AUC_{0\text{-}24}$: 17, 60, 192 μM*hr
High Dose 3 Months GLP Study #TT020860	**500 mg/kg**	**50x**	**Death** at 1500 and 2000mg/kg: *Males:* 1/15 dead at 1500mg/kg drug week 14; 6/15 dead at 2000mg/kg drug week 12, renal tubular necrosis *Females:* 1/15 dead at 2000mg/kg, renal tubular necrosis BW gain ↓ 14%, 25% at 1500, 2000mg/kg **Kidney toxicity:** Males ≥ 1500mg/kg, Females 2000mg/kg *Males*: ↑ BUN, creatinine (≤ 2x), ↑ urine volume and protein, tubular degeneration and necrosis *Females*: Tubular degeneration and necrosis **Liver toxicity:** Males 2000mg/kg Degeneration and necrosis at 2000mg/kg (m) Hypertrophy and ↑ weight ≥ 500mg/kg, dose-dependent, males and females ↑ ALT, ALP (≤ 2x) 1000mg/kg and higher **Heart toxicity:** Males ≥ 1500mg/kg Myocardial degeneration and necrosis **Teeth toxicity:** Males, Females ≥ 1000mg/kg

			Incisor thickening, ameoblast and odontoblast degeneration **Bone Marrow:** Necrosis in males ≥ 1500mg/kg **Lymphoid depletion** of thymus, lymph nodes *Doses:* 500, 1000, 1500, 2000 mg/kg (+ placebo) $AUC_{0\text{-}24}$: 493, 1120, 1540, 2310 (f) µM*hr
6 Months GLP Study # TT020810	**> 180mg/kg**	**> 20x**	No drug-related deaths. 5 deaths observed (2 HD m,f, 1 MD m) likely gavage-related Salivation around dosing times in most HD and some MD rats. No drug-related change in BW, ophthalmic, hematology, chemistry, or urinalysis. No drug-related change in organ weight, necropsy, histopathology. Lung findings noted in early-death rats (hemorrhage, pleuritis, esophogeal perforation). *Doses:* 20, 60, 180 mg/kg (+ placebo) $AUC_{0\text{-}24}$: No TK data collected; From 91 day study: 17, 60, 192 µM*hr

TABLE: REPEAT DOSE TOXICITY STUDIES IN BEAGLE DOGS			
STUDY DURATION	**NOAEL**	**MRHD MULTIPLE (100mg; 10μM*hr)**	**FINDINGS**
15 Days GLP Study #TT020330	**10 mg/kg**	**TK data not collected**	No drug-related deaths Salivation, ↓ activity, hunch posture, white emesis at 50mg/kg during week 1, dissipated week 2. Eyelid swelling with lymphatic distension in 2/4 high dose males. No change in BW, ophthamology, ECG, hematology, chemistry, urinalysis at drug day 9 **Liver toxicity**: ALT ↑ 2x in 2/4 high dose males; no histopathology change. No change in organ weight, necropsy, or other histopathology. *Doses:* 2, 10, 50 mg/kg (+ placebo) *$AUC_{0\text{-}24}$:* TK data not collected
3 Months GLP Study #TT026270	**10 mg/kg**	**5x**	No drug-related deaths. Salivation, ↓ activity, ataxia, recumbency, tremor at 50mg/kg, generally dissipated after first week. No change in BW, ophthamology, ECG, hematology, chemistry, urinalysis. No liver toxicity, eyelid swelling as seen previously. **Skeletal muslce toxicity**: Slight myofiber degeneration in 1/4 males and females at 50mg/kg. No change in organ weight, necropsy, or other histopathology. *Doses:* 2, 10, 50 mg/kg (+ placebo) *$AUC_{0\text{-}24}$:* 10, 50, 220 μM*h/ml
6 Months GLP Study #TT020790	**10 mg/kg**	**5x (based on 3 month TK)**	No drug-related deaths: Death of 1/4 high dose females ascribed to intubation accident; prior respiratory distress and lung discoloration/necrosis documented, also bone marrow depletion and heart mineralization. Trembling, ↓ activity, abnormal respiration and labored breathing, ataxia, emesis in all 50mg/kg males and females. Abnormal respiration in 2 10mg/kg males.

			No change in BW, ophthamology, ECG, hematology, chemistry, urinalysis. No liver toxicity, eyelid swelling as seen previously. **Skeletal muscle toxicity**: Slight myofiber degeneration in 1/4 males at 50mg/kg. No change in organ weight, necropsy, or other histopathology. *Doses:* 2, 10, 50 mg/kg (+ placebo) *$AUC_{0\text{-}24}$:* From 3 month study: 10, 50, 220 µM*h/ml
12 Months GLP Study #TT020710	**10 mg/kg**	**5x** **(based on 3 month TK)**	No drug-related deaths Salivation, ↓ activity, abnormal respiration and labored breathing, emesis, recumbency, trembling in high dose males and females within 3-5hrs of dosing; incidence persisted throughout study. BW decreased 7% to 10% in high dose males/females. No change in ophthamology, ECG, hematology, chemistry, urinalysis. No liver toxicity, eyelid swelling, or skeletal muscle toxicity as seen previously. No change in organ weight, necropsy, or other histopathology. Focal heart mineralization in 1/4 high dose females, focal ossification of valve in 1/4 high dose males. *Doses:* 2, 10, 50 mg/kg (+ placebo) *$AUC_{0\text{-}24}$:* From 3 month study: 10, 50, 220 µM*h/ml

2.6.6.4 Genetic toxicology

Genetic toxicity of Januvia was evaluated by three in vitro assays (Ames, hepatocyte alkaline elution, and chromosome aberration) and one in vivo assay (murine micronucleus induction).

Ames Assay: Januvia was tested using Salmonella and E.coli strains (with and without S9 mixture) up to a maximum concentration of 6000 µg/plate. The mean histidine and tryptophan revertant values observed in these assays were not 2 fold greater than the negative-controls. Under conditions of this assay, Januvia was not mutagenic.

Salmonella typhimurium or *Escherichia coli* Strains Revertants per Plate

his^+/trp^+

	TA100 20-Dec-2001				TA1535 20-Dec-2001				TA97a 20-Dec-2001			
	Without S-9		With S-9		Without S-9		With S-9		Without S-9		With S-9	
Conc./Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (±SD)	204.6	11.0	230.2	19.2	17.2	5.2	19.9	6.3	238.9	14.0	328.8	17.7
30 µg	201.3	30.0	220.7	10.8	14.3	4.0	18.0	4.0	236.3	4.6	320.3	22.0
100 µg	195.0	14.0	229.7	12.1	14.3	4.9	24.3	4.6	251.0	8.2	328.3	4.5
300 µg	196.3	8.3	226.3	14.4	16.7	3.1	24.7	5.1	239.3	21.1	321.7	25.5
1000 µg	191.3	18.0	232.3	13.6	17.3	7.8	21.7	8.0	227.0	19.3	339.3	16.3
3000 µg	204.0	17.7	204.7	4.6	16.3	2.1	27.0	5.2	228.0	20.7	338.3	20.0
6000 µg	195.0	13.5	221.0	4.0	19.0	8.2	23.7	9.2	159.7	53.2	266.7	33.3
DMSO(±SD)	195.9	20.1	206.0	16.1	17.8	4.2	19.3	4.5	226.6	20.2	327.3	22.0
2AA 1.0 µg	190.3	7.8	763.3	76.7	19.7	4.0	84.3	24.2	254.3	22.7	759.0	15.7
2AA 2.0 µg	205.0	36.3	1690.7	172.0	17.0	5.3	163.7	14.6	248.0	22.9	1562.0	67.8
Water 100 µL	204.0	11.0	230.2	19.2	17.2	5.2	19.9	6.3	238.9	14.0	328.8	17.7
Na azide 0.75 µg	499.3	5.7			532.3	32.6						
ICR-191 1.5 µg									1573.3	271.5		

	TA98 20-Dec-2001				NP2 uvrA pKM101 20-Dec-2001			
	Without S-9		With S-9		Without S-9		With S-9	
Conc./Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (±SD)	48.3	9.8	58.9	8.7	146.2	15.1	169.0	17.2
30 µg	37.7	4.7	51.3	11.3	140.3	19.7	157.3	3.2
100 µg	38.7	3.8	54.0	9.5	159.0	16.5	182.0	10.4
300 µg	47.0	8.2	52.0	5.6	136.3	11.2	176.7	11.3
1000 µg	46.7	4.6	55.0	1.6	131.0	5.2	159.3	4.0
3000 µg	32.7	2.3	43.3	6.0	160.3	4.0	167.7	11.0
6000 µg	36.0	3.5	52.3	12.7	147.7	13.6	169.3	21.5
DMSO(±SD)	43.6	4.3	59.2	4.3	145.6	14.0	174.2	17.3
2AA 1.0 µg	51.7	7.5	360.7	38.6				
2AA 2.0 µg	55.7	4.5	896.3	95.9	131.0	15.1	609.2	11.0
2AA 5.0 µg					139.3	17.6	1359.0	101.0
2NF 1.0 µg	461.7	55.9						
4NQO 1.0 µg					809.7	82.6		

Salmonella typhimurium/*E. coli* genotype:
TA100 his G46 (base subst) uvrB rfa pKM101 (R factor)
TA1535 his G46 (base substitution) uvrB rfa
TA97a his D6610 (frameshift) uvrB rfa pKM101 (R factor).
TA98 his D3052 (frameshift) uvrB rfa pKM101 (R factor).
NP2 uvrA pKM101 (trp-) uvrA pKM101 (R factor).

S-9 - Metabolic activation.
SD - Standard deviation.

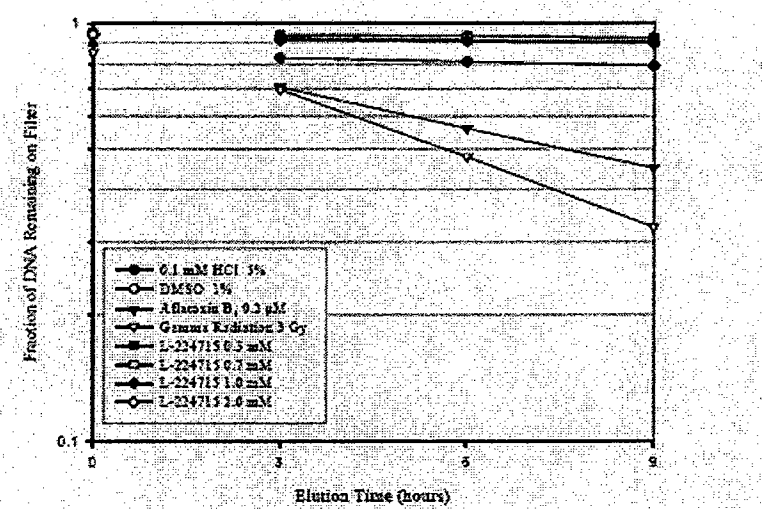
2AA - 2-Aminoanthracene. DMSO - Dimethyl sulfoxide. 2NF - 2-Nitrofluorene.
AF-2 - 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide. MMS - Methylmethane sulfonate. 4NQO - 4-Nitroquinoline oxide.

1 Maron DM, Ames BN. Mutation Res 1983;113:173-215.
2 Matsushima T et al. Progress Mutation Res 1985;1:307-95.

Hepatocyte Alkaline Elution Assay: Januvia was tested in uninduced rat hepatocytes and in hepatocytes from a rat induced with sodium phenobarbital (75 mg/kg/day, IP, for 4 days prior to hepatocyte harvest) and β-naphthoflavone (a single dose at 80 mg/kg, IP, 2 days prior to harvest).

Assessable concentrations ranged from 0.3 to 2 mM Januvia. There was no significant cytotoxicity and there were no increases in the induced elution slope of 0.020 or greater at any soluble dose of Januvia tested. The positive controls for the assay in an uninduced rat, aflatoxin B1 and gamma radiation, produced induced elution slopes of 0.093 and 0.163, respectively, with no significant cytotoxicity indicating that the assay was working as expected. Under the assay conditions, Januvia did not increase DNA breaks in rat hepatocytes suggesting that Januvia was not mutagenic in alkaline elution assay.

Summary of DNA Strand Breaks and Cytotoxicity in Rat Hepatocytes. TT #02-8484



Chromosome Aberration Assay: The test system for this assay was Chinese hamster ovary cells (CHO cells, subclone WBL). Assessable concentrations of Januvia ranged from ~2 to 5mM for 3 hour treatments with and without S9, and 1.5 to 2mM for the 20 hour treatment without S9. Higher concentrations were excessively cytotoxic in a preliminary assay. Cyclophosphamide and mitomycin C served as positive controls. There was no significant increase in chromosomal aberrations under any condition. There was a slight increase in endoreduplication (2.6%) following the 3-hr treatment with S9 compared to control (0%) but was within the historical control range (0 to 3.4%). Endoreduplication can occur in cells spontaneously and is thought to result from inhibition of normal DNA synthesis or a temporary cell cycle block in the G2 phase. Positive controls significantly increased chromosomal aberration in CHO cells except for low dose mitomycin C.

Summary of Cytotoxicity and Aberrations at 20 Hours

Treatment	Cell Growth (PD, % Control)	% Aberrant Cells	Frequency Abs per 100 Cells[a]	% Endoreduplication
With S-9 (3 hours)				
H_2O 5%[e]	100	1.0	1.0	0.0
H_2O 5%[e]	100	0.0	0.0	0.0
CP (5 µM)	75	16.0**	19.5	
CP (10 µM)[b]	72	30.0**	46.0	
L-224715 (mM)				
2.5	83	1.0	1.0	0.4
3.0	79	ns		
3.5	71	ns		
4.0	78	ns		
4.25	65	2.0	2.0	2.4
4.5	65	ns		
4.75	50	1.0	1.0	2.6
5.0	37	ns		
Without S-9 (3 hours)				
H_2O 5%[e]	100	2.5	2.5	
H_2O 5%[e]	100	0.0	0.0	
MMC (0.5 µM)	92	4.5*	4.5	
MMC (1.5 µM)[c]	63	64.0**	116.0	
L-224715 (mM)				
2.0	78	2.0	2.0	
2.5	74	ns		
3.0	65	ns		
3.5	63	1.0	1.0	
4.0	51	ns		
4.5	41	ns		
5.0	48	1.5	2.0	

Summary of Cytotoxicity and Aberrations at 20 Hours

Treatment	Cell Growth (PD, % Control)	% Aberrant Cells	Frequency Abs per 100 Cells[a]	% Endoreduplication
Without S-9 (20 hours)[d]				
H_2O 5%[e]	100	ns		
H_2O 5%[e]	100	ns		
L-224715 (mM)				
1.5	88	ns		
2.0	69	ns		
2.5	41	ns		
3.0	27	ns		
3.5	17	ns		
4.0	23	ns		
4.5	Dead: discarded	ns		

PD = Population doubling
ns = Not scored
Cyclophosphamide (CP) and Mitomycin C (MMC) are positive controls
Endoreduplication (scored when an increase is noted on scanning slide): Percentage of endoreduplicated cells based on 500 mitotic cells.
[a] The total number of aberrations per 100 cells, since a cell may have more than one aberration
200 cells scored per point except where noted.
[b] 50 cells scored.
[c] 25 cells scored.
[d] Series not scored because appropriate cytotoxicity levels were not obtained.
[e] Acidified water was used (pH adjusted to approximately 4.0 with 0.1 N HCl).
* $p \leq 0.05$, ** $p \leq 0.01$ compared to the relevant control group using a one-sided Fisher's Exact Test. Since several comparisons with a common control were made, an adjustment procedure of Dunnett was used to assess the overall significance of each comparison for doses of test compound.

Summary of Cytotoxicity and Aberrations at 20 Hours

Treatment	Cell Growth (PD, % Control)	% Aberrant Cells	Frequency Abs per 100 Cells[a]
Without S-9 (20 hours)			
H_2O 5%[d]	100	2.5	2.5
H_2O 5%[d]	100	2.0	2.0
MMC (0.5 µM)[c]	83	3.5	3.5
MMC (1.5 µM)[b,c]	52	72.0*	116.0
L-224715 (mM)			
1.0	95	ns	
1.25	90	ns	
1.5	81	3.0	2.0
1.75	75	2.5	4.5
2.0	56	3.5	3.5
2.25	40	ns	
2.5	11	ns	
2.75	23	ns	
3.0	8	ns	

PD = Population doubling
ns = Not scored.
Mitomycin C (MMC) is a positive control.
Endoreduplication (scored when an increase is noted on scanning slides): Percentage of endoreduplicated cells based on 500 mitotic cells.
[a] The total number of aberrations per 100 cells, since a cell may have more than one aberration
200 cells scored per point except where noted.
[b] 25 cells scored.
[c] Positive controls were treated for 3 hours.
[d] Acidified water was used (pH adjusted to approximately 4.0 with 0.1 N HCl).
* $p \leq 0.01$ compared to the relevant control group using a one-sided Fisher's Exact Test. Since several comparisons with a common control were made, an adjustment procedure of Dunnett was used to assess the overall significance of each comparison for doses of test compound.

Note: The first 20-hour assay (*left panel*) was not scored due to excessive toxicity. A repeat study with an optimized dose range (*right panel*) provided acceptable cytotoxicity and yielded no increase in chromosome aberrations.

2.6.6.5 Carcinogenicity

Brief Summary

Carcinogenic potential of MK-0431 was evaluated in 2 year studies in mice and rats. The Executive Carcinogenesis Assessment Committee (Exec CAC) in a meeting held 08 Aug 2006 concluded that both studies adequately assessed carcinogenesis. MK-0431 significantly increased the incidence of combined liver adenoma/carcinoma in male and female rats, and increased liver carcinomas in female rats at 500mg/kg (62x MRHD). Non-genotoxic, chronic hepatotoxicity is the suggested etiological event but this is based on weak evidence of liver toxicity. MK-0431 did not produce any drug-related tumors in CD-1 mice up to 500mg/kg (72x MRHD).

MK-0431 poses a minimal carcinogenic risk to humans.

Carcinogenesis in Sprague-Dawley Rats: 2 year study

Summary:

Sprague Dawley rats were administered daily gavage doses of placebo (2 control groups) or Januvia at 50, 150, and 500mg/kg for 106 weeks.

The Exec CAC agreed that the study was adequate, noting prior Exec CAC concurrence with the doses (filed in DFS, 02 June 2003). Selection of the high dose was based on the maximum tolerated dose (MTD) from a 14 week toxicity study, defined as ameoblast/odontoblast degeneration at 1000mg/kg and thickening/missing incisors at 1500mg/kg.

The Committee found that the study was positive for combined liver adenomas and carcinomas in males and females and for liver carcinomas in females at the 500mg/kg dose.

Evaluation of tumor findings:

Liver tumors: Hepatocellular carcinoma increased in high dose males and females, and adenoma increased in high dose males. The intermediate doses did not increase tumor incidence. Although the high dose produced liver tumors, the response is not robust: the incidence in males just exceeds the historical high, tumor latency is similar across groups, and the mid-dose did not produce pre-neoplastic hyperplasia or hypertrophy of hepatocytes. An exception is the incidence of liver carcinoma in high dose females, which exceeded the historical high (12% vs. 2%) but was similar to the incidence in high dose males (12% vs. 14%).

Liver Tumor Incidence in MALE SD Rats n=50/group					
Liver	Control 1	Control 2	50mg/kg	150mg/kg	500mg/kg
Hepatocellular *Adenoma	1 (2%)	1 (2%)	0	1 (2%)	5 (10%)
*†Carcinoma	1 (2%)	3 (6%)	6 (12%)	1 (2%)	7 (14%)
*†Adenoma + Carcinoma	2	4	6	2	12

*Statistical significance (Positive trend test)
† Statistical significance (hi/lo pairwise comparison)

Liver Tumor Incidence in FEMALE SD Rats n=50/group					
Liver	Control 1	Control 2	50mg/kg	150mg/kg	500mg/kg
Hepatocellular Adenoma	1 (2%)	0	2 (4%)	3 (6%)	3 (6%)
*Carcinoma	0	1 (1%)	1 (2%)	1 (2%)	6 (12%)
*Adenoma + Carcinoma	1	1	3	4	9

* Statistical significance (Positive trend test and hi/lo pairwise comparison)

Historical Control Data for Liver Tumors in SD Rats				
	Males		Females	
[1]Sponsor	Adenoma	Carcinoma	Adenoma	Carcinoma
Rarity of tumor	2%	4%	2%	0.8%
Min/Max Incidence in positive studies	2%-8%	2%-12%	2%-8%	2%
[2] Rarity of tumor	2%	3.5%	2%	0.7%
Min/Max Incidence in positive studies	0.8%-8%	0.8%-12%	1%-2%	0.8%-2.5%

Rarity of tumor = (# tumors/total # organs)*100
[1]Sponsor data from 14 carcinogenicity studies (1993-2005) conducted at Merck;1677 males and 1684 females.
[2] historical data, March 1998 Report.

Notes: Merck's historical data comes from diet-restricted studies. Four of the 14 studies were initiated 5 years prior to starting the study with MK-0431. [redacted] historical data also comes from diet-restricted rats from 26 studies.

Mechanism of Action for Liver Tumors: On the origin of induced liver tumors, the Sponsor suggests that hepatic toxicity preceded tumor development. No mechanistic studies are offered, but hepatotoxicity in a prior 13 week study is cited as predictive evidence of liver tumorigenesis in the 2 year study. Hepatocellular injury in the 2 year study is slight, consisting of basophilic/eosinophilic cellular alterations (both sexes) or cystic degeneration (males only). However, the severity of hepatic findings did not correlate with the presence of tumors. Overall, it is plausible that slight but chronic hepatocellular injury at 500mg/kg was the seminal event in tumorigenesis, but the evidence is weak.

MK-0431 tested negative in the standard genotoxicity battery, supporting the conclusion that hepatocellular injury and tumor development occurred by a non-genotoxic mechanism.

Summary Incidence Non-neoplastic Liver Histopathology in SD Rats

Organ	Finding	Control 1	Control 2	50mg/kg	150mg/kg	500mg/kg
FEMALES, n=50 per group						
Liver	Basophilic cellular alteration	11	8	11	8	16
	Eosinophilic cellular alteration	12	7	7	16	20
MALES, n=50 per group						
Liver	Basophilic cellular alteration	6	2	1	2	8
	Eosinophilic cellular alteration	25	18	19	25	31
	Cystic degeneration	6	8	8	13	24
	Bile duct hyperplasia	0	0	1	2	0

Safety Margins: Toxicokinetic data was not collected in the 2 year study. Based on TK data from a 14-week and a parallel 27-week study, exposure at the tumorigenic dose of 500mg/kg is 62x the MRHD of 100mg/kg. Exposure at 150mg/kg (NOEL for liver tumors) is 19x the MRHD. The Sponsor makes an argument that safety margins based on body weight is the more relevant comparison, because MK-0431 has a shorter half-life in rats than in humans (4hrs vs. 13hrs); such a comparison provides a 75-fold safety margin.

Toxicokinetics			
Species	Dose, mg/kg/d	AUC, μM·h	Safety margins based on AUC_{0-24} (animal/human)
SD Rat	50	50	6x
	150	150	19x
	500	495	62x

Relevance to Human Risk: Liver tumors induced by MK-0431 in rats is considered a negligible risk to humans, based on 1) a 19-fold safety margin between the NOEL (150mg/kg) and the MRHD based on AUC, and 2) there is no evidence of liver injury, based on liver function tests, in patients administered 100mg for up to 1 year.

Carcinogenesis in CD-1 Mice: 2 year study

Summary

CD-1 mice were administered daily gavage doses of placebo (2 control groups) or Januvia at 50, 125, 250, or 500mg/kg for 106 weeks.

The Exec CAC agreed that the study was adequate, noting that the MTD was reached based on renal toxicity at the high dose.

The Committee found that the study was negative for statistically significant drug induced tumors.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Prior Exec CAC concurrence with dose selection was not obtained for the study in mice. Thus, adequacy of doses used in the study is emphasized in this review.

The carcinogenic assessment in mice was adequate based on sufficient survival to allow meaningful statistical comparisons and its achieving the maximum tolerated dose (MTD) in males and females.

Survival and MTD

Statistical analysis by Merck and the FDA indicates no significant difference in survival to termination for males and females. Moderate to severe renal hydronephrosis was present in some mice at 500mg/kg, and was cited as the cause of death for 1 female and 4 males. Thus, renal hydronephrosis appears to be the dose-limiting toxicity (i.e., MTD) in this study.

Table 1

Summary Incidence Hydronephrosis in CD-1 Mice FEMALES, n=50 per group							
Organ	Finding	Control 1	Control 2	50mg/kg	125mg/kg	250mg/kg	500mg/kg
Kidney	Hydronephrosis (mod/severe)	0	0	0	0	0	3
MALES, n=50 per group							
Kidney	Hydronephrosis (mod/severe)	0	0	0	0	1	9

Table 2

Total Survival and Mortality in Mice (Incidence, n=50/group)				
	Males		**Females**	
Dose Group	*Survival*	*Mortality*	*Survival*	*Mortality*
Control 1	33 (66%)	17 (34%)	35 (70%)	15 (30%)
Control 2	25 (50%)	25 (50%)	32 (64%)	18 (36%)
50 mg/kg	29 (58%)	21 (42%)	29 (58%)	21 (42%)
125 mg/kg	30 (60%)	20 (40%)	33 (66%)	17 (34%)
250 mg/kg	30 (60%)	20 (40%)	29 (58%)	21 (42%)
500 mg/kg	29 (58%)	21 (42%)	26 (52%)	24 (48%)

Evaluation of tumor findings:
The Exec CAC found that the study was negative for statistically significant drug induced tumors.

Toxicokinetics: TK data was not obtained in the 2 yr carcinogenicity study. Based on TK data from a 5-week and a parallel 27-week study, exposure at the low and high dose is, respectively, 6x and 72x the MRHD of 100mg/kg.

Species	Dose, mg/kg/d	AUC, μM.h	Safety margins based on AUC_{0-24} (animal/human)
CD-1 Mice (5 wk TK study)	50	46 (F)	6x
	125	96	12x
	250	223	28x
	500	576	72x

2.6.6.6 Reproductive and developmental toxicology

Summary

Merck evaluated MK-0431 in fertility, embryo-fetal, and pre/post-natal development studies in SD rats. Embryo-fetal development was also assessed in NZ white rabbits. The primary reviews follow this summary.

Exposure to MK-0431 in the definitive studies ranged from ~12 to 90x MRHD in the rat and ~6 to 50x MRHD in the rabbit.

Fertility Studies

Doses of 125, 250, and 1000mg/kg MK-0431 were evaluated in male and female rats. Resorptions and post-implantation losses increased in females dosed 250 and 1000mg/kg. There was no effect on male fertility or sperm motility/number. The high dose of 1000mg/kg was associated with urine-stained fur in males and females, and the 250mg/kg dose caused a 15% decrease in body weight in males but did not effect females. The low dose of 125mg/kg did not effect fertility of males or females.

Embryonic Development Studies

Rats: The maternally toxic dose appears to be 1000mg/kg, though the toxicity (urine-stained fur) is certainly not dose-limiting. Skeletal findings (vertebral and rib malformations/variations) clearly increased at 1000mg/kg and may be considered secondary to maternal toxicity.

The 250mg/kg dose was not toxic to rat dams. At this dose, a single fetus showed multiple skeletal vertebral findings, including a malformation (unspecified cervical vertebra), incomplete ossification of thoracic vertebra and sternebra, sacral vertebra variation, and supernumerary rib. The incidence of incomplete ossification of thoracic vertebrae at 250mg/kg was increased relative to the concurrent control, but was well within the historical control range.

Rabbit: The maternally toxic dose was 500mg/kg, defined by weight loss, reduced food intake, and several deaths. No fetuses from this dose group were evaluated.

Doses of 62.5 and 125mg/kg were not toxic to rabbit dams. At 125mg/kg, one pup had multiple cardiovascular malformations, and the incidence of absent caudate lobe of lung slightly increased to 2.9% from 2.0% in control. A tail malformation was also reported at 125mg/kg in a prior dose-ranging study (clubbed foot malformation also present in control group). Hemorrhagic ovary was present in 2.7% of pups at 62.5mg/kg. These findings are either a single occurrence, similar in incidence to the control group, or not dose-dependent. A relation to drug treatment is not clear.

Post-natal Development Studies

Maternally toxic doses appear to be 250 and 1000mg/kg, defined by a transient 15% to 18% reduction in weight gain at both doses from GD6 to GD12 concomitant with an 8% reduction in food intake at GD8, and urine-stained fur at 1000mg/kg. Findings at these maternally toxic doses include a possible increase in pup mortality from LD3 to LD21 at both doses, and 10% reduced body weight at 1000mg/kg that persisted to the post-weaning stage. Notably, skeletal and visceral examinations were not done in F1 pups, only external examinations.

The 125mg/kg dose was not toxic to dams. At this dose, the number of F1 litters with dead pups by LD3 increased to 6 versus 4 in control concomitant with more pup deaths (9 vs. 4 in control). However, the number of F1 litters with dead pups at higher doses was equal to the control group (i.e., no dose-dependence). One F1 pup at 125mg/kg had a polydactyly external malformation, though no malformations were reported at higher doses. Mating and reproduction of the F1 generation was not effected by drug treatment.

The F2 generation did not show differences vs. control on clinical signs, body weight, viability, or external malformations/variations.

Toxicokinetic Studies

Toxicokinetic studies in pregnant/lactating rats and rabbits indicate that MK-0431 crosses the placenta (44-80% in rats, 30-66% in rabbits), and is excreted in maternal milk (rats, 4:1 milk:plasma ratio). Thus, rodent fetuses are exposed to MK-0431 during gestation and weaning.

FERTILITY AND EARLY EMBRYONIC DEVELOPMENT

Oral Fertility study in Female Rats
125, 250, 1000 mg/kg

Key study findings:

- Drug-related urine staining of the fur seen in most females at 1000mg/kg.
- Doses of 125 and 250mg/kg were not toxic to females.
- The number of females with resorptions increased at 250 and 1000mg/kg, with 1 to 2 individuals showing higher-than-average resorptions. Late resorptions were also higher at 1000mg/kg.
- Post-implantation loss increased at 250 and 1000mg/kg, with 3 females in each dose group showing higher-than-average losses.

SD Rats: Female Fertility Adverse Effect	NOAEL (AUC*)	Multiple of MRHD (10 uM*h AUC)
General Toxicity Urine-stained fur	250 mg/kg	No TK
Fertility ↑ females with resorptions at 250/1000mpk ↑ late resorptions 1000mpk	250 mg/kg	No TK

Study no.: 02-728-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 05 Sept 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431, lot L-000224715-006F016, purity —— HPLC

Methods

Doses:	125, 250, 1000 mg/kg and placebo
Species/source	SD rats, [redacted]
Number/sex/group: (main study)	24 females per dose group
Route, formulation, dose volume	Oral gavage of drug in 0.5% MC/5 mM HCl; 5ml/kg dose volume Dosed 14 days prior to mating to GD7; C-section on GD 15-17
Toxicokinetic groups	Not done

Parameters and endpoints evaluated:

IN-LIFE OBSERVATIONS	FREQUENCY
Mortality & cageside observations	Daily checks
Clinical examination	Physical signs only
Body weight	Every 2-4 days during dosing, through to GD15
Food consumption	Over 4 day intervals: pre-mating 1-5 and 8-12; GD1-5 and 8-12
Estrous cycle determination	Not done
Breeding procedure	20 night co-habitation of 1 male and 1 female; sperm in lavage or seminal plug defined GD0
POST-MORTEM EVALUATIONS	
Macroscopic	Thoracic and abdomimal visceral necropsy done on all F0 females (not extensive)
Organ weights	Not done
Uterine & Ovarian exams	All females; implants classified as live, dead, or resorbed.
Sperm analysis	Not done

Results

Mortality: No drug-related mortalities.
Female 02-7780 in the 250mg/kg group was found dead on GD6 (end of dosing period). A necropsy uncovered a hemothorax and discolored lung, consistent with an intubation accident. The reviewer agrees with this diagnosis.

Clinical signs: Urine-stained fur was observed at 1000mg/kg during the mating and gestational periods. This is related to drug and was observed in most females at this dose.

Body weight: Body weights were unaffected by treatment.

Food consumption: Food consumption was unaffected by treatment.

Toxicokinetics: not collected

Necropsy:
Macroscopic observations: No drug-related findings were reported. A tan mass on the kidney consistent with nephroblastoma was reported in one low-dose female; the occurrence of this renal tumor is stated to be consistent with Merck's historical data for a control rat population (data not shown).

Organ weights: Organ weights not collected.

Estrous Cycle Effects: Not evaluated

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Fertility: All mated females were impregnated with no apparent drug-related change in the time to mating (Table 4).

TABLE 4. L-000224715: Oral Fertility Study in Female Rats. TT #02-728-0
Summary of Reproductive Performance

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
FEMALES COHABITED	24	24	24	24
MALES COHABITED	0	0	0	0
FEMALES DISCONTINUED DURING COHABITATION	0	0	0	0
MATED FEMALES	24	24	24	24
PREGNANT FEMALES	24	24	23	24
FOUND DEAD DURING GESTATION	0	0	1	0
SACRIFICED DURING GESTATION	0	0	0	0
CESAREAN SECTIONED	24	24	22	24
NOT PREGNANT FEMALES	0	0	1	0
LIVE	0	0	1	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
MATINGS PER 4-DAY PERIODS OF COHABITATION				
DAYS 1 to 4	22[a]	14[b]	19[a]	22[a]
DAYS 5 to 8	0	1	1	0
DAYS 9 to 12	1	0	1	0
DAYS 13 to 16	0	6	3	2[a]
DAYS 17 to 20	1	3	0	0
TIME TO MATING (4-DAY PERIODS) ± S.D.	1.25±0.90	2.29±1.65	1.50±1.06	1.25±0.85
MATING INDEX, %	100	100	100	100
FECUNDITY INDEX, %	100	100	96	100
FERTILITY INDEX, %	100	100	96	100

MATING INDEX = MATED FEMALES/FEMALES COHABITED EXCLUDING FEMALES DISCONTINUED DURING COHABITATION
FECUNDITY INDEX = PREGNANT FEMALES/MATED FEMALES EXCLUDING FEMALES WITH AN UNDETERMINED PREGNANCY STATUS
FERTILITY INDEX = PREGNANT FEMALES/FEMALES COHABITED EXCLUDING FEMALES DISCONTINUED DURING COHABITATION OR WITH AN UNDETERMINED PREGNANCY STATUS
[a] NUMBER INCLUDES ONE FEMALE WITH AN ESTIMATED BREEDING DATE.
[b] NUMBER INCLUDES TWO FEMALES WITH ESTIMATED BREEDING DATES.

Uterine Examination: One to two females per group were not evaluated because only estimates were available for GD0. The number of corpora lutea and implants were not affected by treatment.

The number of females with resorptions increased at 250 and 1000mg/kg compared to control. One female at 250mg/kg and two females at 1000mg/kg had atypically high numbers of resorptions compared to the group mean (Table 5a).

Post-implantation loss increased at 250 and 1000mg/kg due to higher-than-average losses in 3 females in each dose group.

TABLE 5. L-000224715: Oral Fertility Study in Female Rats. TT #02-728-0
Summary of Laparotomy Data

	Control[a]	125 mg/kg/day[a]	250 mg/kg/day[a]	1000 mg/kg/day[a]
MATED FEMALES	24	24	24	24
PREGNANT	24	24	23	24
ESTIMATED BREEDING DATE	1	2	1	2
EXAMINED LIVE LITTER	23	22	21	22
RESORBED OR DEAD LITTER	0	0	0	0
FOUND DEAD	0	0	1	0
SACRIFICED	0	0	0	0
NOT PREGNANT	0	0	1	0
LIVE	0	0	1	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
CORPORA LUTEA	363	356	341	373
CORPORA LUTEA/PREGNANT FEMALE	15.8	16.2	16.2	17.0
%PERI-IMPLANTATION LOSS (LITTER MEAN)	4.3	5.2	3.4	2.1
IMPLANTS	347	340	329	365
IMPLANTS/PREGNANT FEMALE	15.1	15.5	15.7	16.6
RESORPTIONS	10	14	30	25
%RESORPTIONS/IMPLANTS (LITTER MEAN)	2.9	3.8	9.1	6.8
DEAD FETUSES	0	0	0	0
%DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.0	0.0	0.0	0.0
%POSTIMPLANTATION LOSS (LITTER MEAN)	2.9	3.8	9.1	6.8
LIVE FETUSES	337	326	299	340
UNEXAMINED SEX	337	326	299	340
LIVE FETUSES/PREGNANT FEMALE	14.7	14.8	14.2	15.5

Table 5a
Summary of resorption data in female rats

Dose, mg/kg	# females with resorptions	# resporptions per female
0	9	≤ 2
125	10	≤ 2
250	12	≤ 2 one w/ 7
1000	14	≤ 3 two w/ 5-6

Oral Fertility study in Male Rats
125, 250, 1000 mg/kg

Key study findings:

- Urine-stained fur in most 1000mg/kg males.
- Body weight decreased dose-dependently at all doses, with a 15% and 20% decrease in 250mg/kg and 1000mg/kg males by drug week 8 compared to control males.
- There was no drug-related effect on male fertility or sperm motility/head count.
- Results from uterine examinations of females mated with dosed males were unremarkable.

SD Rats: Male Fertility Adverse Effect	NOAEL (AUC*)	Multiple of MRHD (10 µM*h AUC)
General Urine-stained fur BW ↓ 15-20% at 250 and 1000mg/kg	125 mg/kg	No TK
Fertility No Findings	> 1000 mg/kg	No TK

Study no.: 03-715-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 02 April 2003
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431, lot L-000224715-006F020, purity [redacted]

Methods

Doses:	125, 250, 1000 mg/kg and placebo
Species/source	SD rats, from [redacted]
Number/sex/group: (main study)	24 males per dose group
Route, formulation, dose volume	oral gavage of drug in 0.5% MC/5 mM HCl , 5 ml/kg dose volume Males dosed for 29 days prior to cohabitation, during cohabitation, and until 1 day prior to sacrifice. Approx. 8 weeks total dosing period.
Toxicokinetic groups	Not done

Parameters and endpoints evaluated:

IN-LIFE OBSERVATIONS	FREQUENCY
Mortality & cageside observations	Daily checks
Clinical examination	Physical signs only
Body weight	Twice weekly for males Premating day 1 and GD 0, 7, 15 for females
Food consumption	Twice weekly, looked at feeder cups
Breeding procedure	After 4 weeks of drug treatment, males were cohabitated with females in a 1:1 ratio. Cohabitation was limited to 10 nights, and the female was replaced on the 5th night if there was no evidence of breeding.
POST-MORTEM EVALUATIONS	
Macroscopic	Thoracic/visceral necropsy of all males
Organ weights	• Testes • Left cauda epididymis for normalizing sperm count by grams of tissue
Uterine & Ovarian exams	Females sacrificed on GD 15/16 for uterine evaluation
Sperm analysis	Samples from 16 males/group; at least 150 cells per male were analyzed for motility. Head counts were also done on bisbenzimide-stained samples. Placebo and high dose groups evaluated

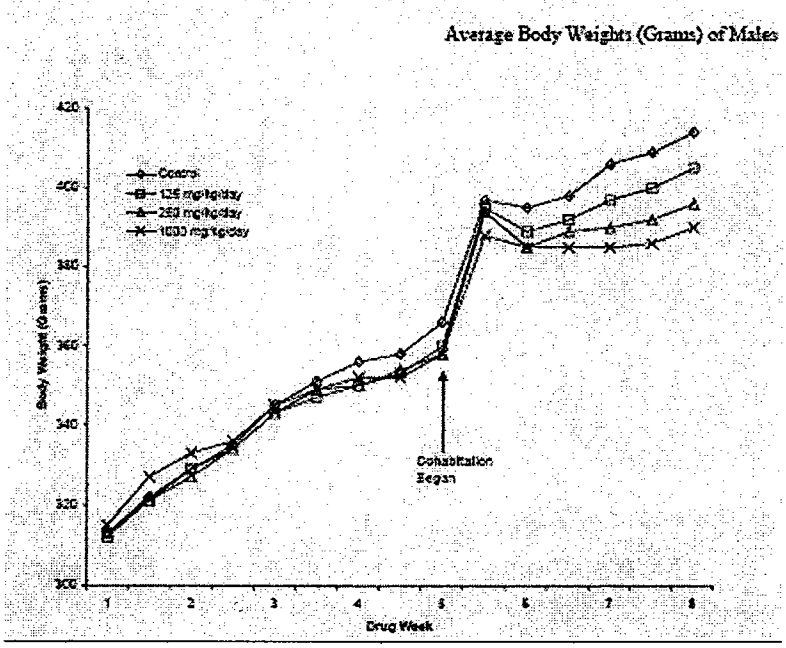
Results

Mortality: None

Clinical signs: Salivation in some 250mg/kg males and most 1000mg/kg males likely related to poor drug palatability; no emesis reported. Urine-stained fur was observed in most 1000mg/kg males within 2-10 days of treatment.

Body weight: Body weight dose-dependently decreased at all doses compared to control males. By drug week 8, body weight was ~15% and 20% lower at 250mg/kg and 1000mg/kg compared to control.

Figure A-1. L-000224715: Oral Fertility Study in Male Rats. TT #03-715-0



Food consumption: Food consumption was not effected by treatment despite reduced weight gain.

Toxicokinetics: Not collected.

Necropsy:
Macroscopic observations: There were no drug-related gross changes.

Organ weights: There was no drug-related change in testicular weight.

Testes and the right epididymis from control and 1000mg/kg males showed unremarkable histomorphology.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Fertility: There was no drug-related effect on mating, fecundity, or fertility indices (Table 4).

TABLE A-4. L-000224715: Oral Fertility Study in Male Rats, TT #03-715-0
Summary of Reproductive Performance of Males

TREATMENT GROUP	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
FEMALES COHABITED[a]	24 (3)	24 (2)	24 (1)	24 (2)
MALES COHABITED	24	24	24	24
FEMALES DISCONTINUED DURING COHABITATION	0	0	0	0
MATED FEMALES	23	24	24	24
PREGNANT FEMALES	22	24	22	21
FOUND DEAD DURING GESTATION	0	0	0	0
SACRIFICED DURING GESTATION	0	0	0	0
CESAREAN SECTIONED	22	24	22	21
NOT PREGNANT FEMALES	1	0	2	3
LIVE	1	0	2	3
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
NOT BRED	1	0	0	0
LIVE	1	0	0	0
MATING INDEX, %[b]	96	100	100	100
FECUNDITY INDEX, %	96	100	92	88
FERTILITY INDEX, %[b]	92	100	92	88

MATING INDEX = MATED FEMALES/FEMALES COHABITED EXCLUDING FEMALES DISCONTINUED DURING COHABITATION
FECUNDITY INDEX = PREGNANT FEMALES/MATED FEMALES EXCLUDING FEMALES WITH AN UNDETERMINED PREGNANCY STATUS
FERTILITY INDEX = PREGNANT FEMALES/FEMALES COHABITED EXCLUDING FEMALES DISCONTINUED DURING COHABITATION OR WITH AN UNDETERMINED PREGNANCY STATUS

[a] NUMBER IN PARENTHESIS INDICATES FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION AND THAT WERE REMOVED AND REPLACED FOR THE LAST 5 NIGHTS.
[b] CALCULATION EXCLUDES FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION.

Uterine Examination: There was no drug-related effect on the number of corpora lutea, implants, or resorptions.

TABLE A-5. L-000224715: Oral Fertility Study in Male Rats, TT #03-715-0
Summary of Laparotomy Data

TREATMENT GROUP	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
MATED FEMALES	23	24	24	24
PREGNANT	22	24	22	21
EXAMINED LIVE LITTER	22	24	22	21
RESORBED OR DEAD LITTER	0	0	0	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
NOT PREGNANT	1	0	2	3
LIVE	1	0	2	3
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
CORPORA LUTEA	358	395	357	313
CORPORA LUTEA/PREGNANT FEMALE± S.D.	16.3± 2.0	16.5± 2.6	16.2± 2.4	14.9± 2.4
%PERI-IMPLANTATION LOSS (LITTER MEAN)± S.D.	4.4± 9.1	4.1± 5.8	1.9± 3.3	3.7± 5.8
IMPLANTS	344	380	350	303
IMPLANTS/PREGNANT FEMALE± S.D.	15.6± 2.7	15.8± 2.9	15.9± 2.3	14.4± 2.7
RESORPTIONS	25	35	11	11
%RESORPTIONS/IMPLANTS (LITTER MEAN)± S.D.	7.1± 9.3	6.7± 8.9	3.0± 4.0	3.6± 4.2
%DEAD FETUSES/IMPLANTS (LITTER MEAN)± S.D.	0.0	0.0	0.0	0.0
%POSTIMPLANTATION LOSS (LITTER MEAN)± S.D.	7.1± 9.3	6.7± 8.9	3.0± 4.0	3.6± 4.2
LIVE FETUSES	319	355	339	292
SEX NOT EXAMINED	319	355	339	292
LIVE FETUSES/PREGNANT FEMALE± S.D.	14.5± 2.9	14.8± 3.2	15.4± 2.3	13.9± 2.6

Sperm Evaluation: There was no drug-related effect on sperm count and motility.

TABLE A-6. L-000224715: Oral Fertility Study in Male Rats. TT #03-715-0
Summary of Sperm Counts and Vas Deferens Sperm Motility

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
SPERM COUNT / CAUDA EPIDIDYMIS (x 10^6) ± S.D.	268.0 ± 60.6 (24)	NE	NE	270.1 ± 38.8 (24)
SPERM COUNT / GRAM CAUDA EPIDIDYMIS (x 10^6) ± S.D.	792.9 ± 129.2 (24)	NE	NE	763.9 ± 75.2 (24)
% SPERM MOTILITY ± S.D.	90.8 ± 3.0 (16)	91.3 ± 4.3 (16)	91.6 ± 4.1 (16)	89.5 ± 3.1 (16)

VALUES ARE MEANS ± S.D.
(N) = GROUP SIZE. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.
NE = NOT EVALUATED

Embryofetal development

Oral dose range-finding reproduction study in pregnant female rats

SPECIES **DOSES AND ADMINISTRATION** **# ANIMALS**	• No dose-limiting toxicities observed • Maternal toxicity consisted of urine-stained fur at 1000mg/kg • Pup toxicity consisted of less BW gain at 500 and 1000mg/kg • Merck chose doses up to 1000mg/kg for definitive studies
Study TT 02-721-5 (non-GLP) SD Rats, pregnant Dosed from GD6 to LD20 125, 250, 500, 1000 mg/kg n=10 females per dose group	Laparatomy data not obtained (e.g., pre/post-implant losses)

Procedures Performed	Yes	No	Procedures Performed	Yes	No
Physical examinations	✓		Ophthalmology		✓
Body weights	✓		Electrocardiogram		✓
Food consumption	✓		Organ weights		✓
Water consumption		✓	Necropsy		✓
Hematology	✓		Histology		✓
Clinical chemistry	✓		Toxicokinetics		✓
Urinalysis		✓			

Results for Dams

Mortality: No drug-related deaths were reported.
One control female died during orbital bleeding, and one 250mg/kg female was sacrificed due to abnormal respiratory sounds and decreased activity. Similar findings were not seen at 500 or 1000mg/kg, and are thus considered unrelated to treatment.

Clinical Signs: Urine-stained fur was observed in 4/10 females at 1000mg/kg, possibly secondary to renal dysfunction. Excessive salivation was also noted at 500 and 1000mg/kg that likely reflects poor drug palatability.

Body Weight: There was no drug-related change in body weight or food consumption.

Hematology: There was no drug-related change in RBC or WBC parameters.

Clinical Chemistry: There was no drug-related change in renal markers, LFT, serum protein, electrolytes, or serum lipids.

Reproductive Performance: All surviving females gave birth to a similar number of live pups; there was no drug-related increase in the number of dead pups at delivery or at postpartum day 21.

Results for F_1 Pups

Mortality: There were no drug-related deaths at delivery or at LD21.

Clinical Signs: There were no drug-related clinical signs reported.

External Exam: No external malformations or variations were reported (i.e., Merck recorded '0' for both measures in all dose groups from a total of 667 pups examined.

Body Weight: Pup body weight at 500 and 1000mg/kg lagged behind lower dose groups and the control groups despite equal body weight at birth. The lag appears dose-dependent, about 5% and 9% below control at 500 and 1000mg/kg, respectively.

	CONTROL	125 MG/KG/DAY	250 MG/KG/DAY	500 MG/KG/DAY	1000 MG/KG/DAY
LIVE FEMALE PUP WEIGHT (GM) (L.M.) ±S.D.					
POSTNATAL DAY 0	6.1 ± 0.5	6.0 ± 0.5	6.2 ± 0.2	6.1 ± 0.5	5.9 ± 0.4
POSTNATAL DAY 7	15.7 ± 1.8	14.9 ± 1.1	15.7 ± 1.4	14.8 ± 1.6	14.2 ± 1.5
POSTNATAL DAY 14	32.3 ± 2.9	31.8 ± 1.6	32.1 ± 2.6	30.7 ± 2.2	30.0 ± 1.8
POSTNATAL DAY 21	52.9 ± 4.5	52.0 ± 2.8	51.5 ± 3.1	50.7 ± 3.9	48.4 ± 2.6
LIVE MALE PUP WEIGHT (GM) (L.M.) ±S.D.					
POSTNATAL DAY 0	6.5 ± 0.5	6.4 ± 0.5	6.5 ± 0.2	6.5 ± 0.5	6.2 ± 0.5
POSTNATAL DAY 7	16.5 ± 1.8	15.7 ± 1.4	16.2 ± 1.1	15.4 ± 1.7	14.7 ± 1.5
POSTNATAL DAY 14	33.5 ± 2.4	32.7 ± 1.8	32.9 ± 2.3	31.5 ± 2.2	31.1 ± 1.5
POSTNATAL DAY 21	55.6 ± 3.9	53.6 ± 4.0	54.0 ± 3.0	52.0 ± 3.7	50.2 ± 3.2

Pup Body Weight Changes
(Percent Difference in Litter Mean Values from Concurrent Control)

	L-000224715	
	500 mg/kg/day	1000 mg/kg/day
Females		
PND 7	-5.7	-9.6
PND 14	-5.0	-7.1
PND 21	-4.2	-8.5
Males		
PND 7	-6.7	-10.9
PND 14	-6.0	-7.2
PND 21	-6.5	-9.7

Oral developmental toxicity study in rats
125, 250, 1000mg/kg

Key study findings:
Dams

- Maternal toxicity present at 1000mg/kg, defined by urine-stained fur.

Fetuses

- The number of live fetuses, the sex ratio, and fetal body weight did not differ with treatment from controls.

- The number of litters and fetuses with skeletal malformations increased at 1000mg/kg. Findings include a malformed cervical vertebra, absent vertebra, absent rib, and hypoplastic rib. The incidence is in the upper end of the historical range.

- The incidence of wavy ribs, a skeletal variation, also increased at 1000mg/kg. The incidence of 1.5% exceeds the historical range of up to 0.27%.

- External and visceral findings are conspicuously low in this study.

NOAEL Determination
The maternally toxic dose appears to be 1000mg/kg, though the toxicity (urine-stained fur) is certainly not dose-limiting. Skeletal findings (malformations/variations) clearly increased at 1000mg/kg and may be considered secondary to maternal toxicity.

The 250mg/kg dose was not toxic to dams. At this dose, a single fetus showed multiple skeletal vertebral findings, including a malformation (cervical vertebra), incomplete ossification of thoracic vertebra and sternebra, sacral vertebra variation, and supernumerary rib, but their incidence was within the historical control range. The incidence of incomplete ossification of thoracic vertebrae at 250mg/kg was increased relative to the concurrent control, but was well within the historical control range.

SD Rats, Segment 2 Adverse Effect	NOAEL (AUC_{0-24}*)	Multiple of MRHD (10 µM/h)
Dams Urine-stained fur suggestive of renal impairment	250mg/kg (*276µM*h)	25x
Fetuses ↑ skeletal malformations/variations at 1000mg/kg vs. concurrent control group	250 mg/kg (*121-220µM*h)	12-22x

*AUC from study # TT 03-717-0 done in pregnant SD rats. Fetal exposure based on 44% to 88% of exposure in dams (from same study).

Study no.: TT 02-721-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 05 Sept 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431 anhydrous phosphate salt; lot L-000224715-006F016; purity [redacted]

Methods

Doses:	125, 250, 1000 mg/kg and placebo
Species/source	SD rats, from [redacted]
Number/sex/group: (main study)	22 females per group
Route, formulation, dose volume	Oral gavage of drug in 0.5% MC/5mM HCl; 5 ml/kg dose volume. Females dosed from GD6 to GD20
Toxicokinetic groups	TK data not collected

IN-LIFE OBSERVATIONS	FREQUENCY
Cageside observations & clinical exams	Physical signs daily from GD6 to GD21
Body weight & food consumption	BW every other day from GD6 to GD21 (dosing period) and on GD0; food consumption recorded over 2 day intervals during dosing.
POST-MORTEM EVALUATIONS	
Maternal necropsy	Thoracic and abdominal viscera examined from all females on **GD21**
Ovarian/Uterine examinations	Pregnancy status and # corpora lutea determined
Placental examination	Gross examination
Teratologic (Fetal) examination	All fetuses weighed and sexed All fetuses given external examination Half of fetuses given visceral exam Half of fetuses given coronal head exam All fetuses given skeletal exam

Results

Mortality (dams): None

Clinical signs (dams): Urine staining of fur in 50% of 1000mg/kg females during the dosing period starting at GD7. No other drug-related findings recorded.

Body weight (dams): Female body weight was unaffected by treatment.

TABLE 2. L-000224715: Oral Developmental Toxicity Study in Rats: TT #02-721-0
Average Maternal Body Weight Changes (Grams ±S.D.) of F0 Females

TREATMENT GROUP	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
GESTATION PERIOD				
DAYS 0-6	33 ± 6(23)	34 ± 7(23)	35 ± 6(22)	34 ± 6(22)
DAYS 6-21	142 ± 17	141 ± 16	142 ± 16	138 ± 19
DAYS 0-21	175 ± 20	175 ± 19	177 ± 20	173 ± 22

(N)=GROUP SIZE. APPEARS ONLY IF DIFFERENT FROM PREVIOUS N.

Food consumption (dams): Food consumption was unaffected by treatment.

Necropsy of Dams: There were no treatment-related gross necropsy changes in the study

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

No differences are observed from the control group. The slightly higher incidence of resorptions at 1000mg/kg would have occurred prior to initiating dose administration at GD6, and are therefore not related to drug.

Disposition of Dams during Gestation				
	Control	125 mg/kg	250 mg/kg	1000 mg/kg
# on study	22	22	22	22
# pregnant	22	22	22	22
# died pregnant	0	0	0	0
# early delivery	0	0	0	0
# with total resorption	0	0	0	0
# females with resorptions	4	5	3	9
# with viable fetuses at GD29	22	22	22	22

Uterine/Ovarian Exam: See Table 4 below
Pre-implantation loss was similar across all dose groups; no change in the number of implants or corpora lutea.

The incidence of females with resorptions increased from 4 in control to 9 in the 1000mg/kg group, but without an increase in the average number of resorptions per female (≤ 3). Two late resorptions were observed in the 1000mg/kg groups compared to none for the lower dose groups.

The number of live fetuses, the sex ratio, and fetal body weight did not differ with treatment from controls.

Placental Morphology: The only placental abnormality from ~320 placentas/groups was identified in one placenta from a late resorption in the 1000 mg/kg group. The nature of the abnormality was not described.

TABLE 4. L-000224715: Oral Developmental Toxicity Study in Rats. TT #02-721-0
Summary of Laparotomy Data from F0 Females

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
MATED FEMALES	22	22	22	22
PREGNANT	22	22	22	22
EXAMINED LIVE LITTER	22	22	22	22
RESORBED OR DEAD LITTER	0	0	0	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
NOT PREGNANT	0	0	0	0
LIVE	0	0	0	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	0
CORPORA LUTEA	352	379	362	365
CORPORA LUTEA/PREGNANT FEMALE± S.D.	16.0± 1.7	17.2± 3.4	16.5± 2.1	16.6± 1.3
%PERI-IMPLANTATION LOSS (LITTER MEAN)± S.D.	5.2± 5.7	9.0±14.6	4.3± 5.5	1.3± 3.1
IMPLANTS	333	337	346	360
IMPLANTS/PREGNANT FEMALE± S.D.	15.1± 1.6	15.3± 2.2	15.7± 2.0	16.4± 1.4
RESORPTIONS	7	5	4	17
%RESORPTIONS/IMPLANTS (LITTER MEAN)± S.D.	2.1± 4.7	1.4± 2.7	1.0± 2.9	4.6± 6.3
DEAD FETUSES	0	0	0	0
%DEAD FETUSES/IMPLANTS (LITTER MEAN)± S.D.	0.0	0.0	0.0	0.0
%POSTIMPLANTATION LOSS (LITTER MEAN)± S.D.	2.1± 4.7	1.4± 2.7	1.0± 2.9	4.6± 6.3
LIVE FETUSES	326	332	342	343
FEMALES	163	164	164	163
MALES	163	168	178	180
SEX RATIO (LITTER MEAN)± S.D.	0.50± 0.11	0.50± 0.12	0.48± 0.13	0.47± 0.09
LIVE FETUSES/PREGNANT FEMALE± S.D.	14.8± 1.6	15.1± 2.1	15.5± 1.9	15.6± 1.4
LIVE FETAL WEIGHT (GM, LITTER MEAN)± S.D.				
FEMALES	4.75± 0.23	4.61± 0.30	4.71± 0.25	4.67± 0.19
MALES	5.01± 0.25	4.86± 0.26	5.03± 0.24	4.92± 0.23

Offspring (malformations, variations, etc.):

External malformations and variations: No external malformations or variations were found in any group (Table 5).

TABLE 5. L-000224715: Oral Developmental Toxicity Study in Rats. TT #02-721-0
Summary of Fetal Exams

SUMMARY OF EXTERNAL EXAMINATIONS

TREATMENT GROUP:	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
TOTAL LITTERS EXAMINED	22	22	22	22
LIVE FETUSES/LITTERS EXAMINED	326/ 22	332/ 22	342/ 22	343/ 22
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)	0	0	0	0
LITTERS WITH MALFORMATIONS (%)	0	0	0	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)	0	0	0	0
LITTERS WITH VARIATIONS (%)	0	0	0	0
PLACENTAL MORPHOLOGY				
NO. ABNORMAL PLACENTAS/TOTAL EXAMINED[A]	0/326	0/332	0/342	0(1)/343 (21

[A] NUMBERS IN PARENTHESES REPRESENT PLACENTAS FROM LATE RESORPTIONS.

Visceral malformations and variations: No drug-related visceral malformations or variations were reported. The overall incidence of visceral findings is remarkably low. (Table 6)

TABLE 6. L-000224715: Oral Developmental Toxicity Study in Rats. TT #02-721-0
Summary of Fetal Exams

SUMMARY OF VISCERAL EXAMINATIONS

TREATMENT GROUP:		Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
THORACIC AND ABDOMINAL EXAMINATION					
TOTAL LITTERS EXAMINED		22	22	22	22
LIVE FETUSES/LITTERS EXAMINED		169/ 22	172/ 22	177/ 22	177/ 22
LITTERS WITH MALFORMATIONS (%)		0	1(4.5)	0	1(4.5)
LITTERS WITH VARIATIONS (%)		0	0	1(4.5)	0
TYPE AND NUMBER OF FETAL ALTERATIONS (%,L.M.±S.D.)	CLASS				
Abnormal Origin Subc. Art.	(M)	0	0	0	1(0.57± 2.7)
Absent Kidney	(M)	0	1(0.57± 2.7)	0	0
Ureter Variation	(V)	0	0	1(0.57± 2.7)	0

Coronal Examination: No malformations/variations were found in coronal sections of the head. (Table 7)

TABLE 7. L-000224715: Oral Developmental Toxicity Study in Rats. TT #02-721-0
Summary of Fetal Exams

SUMMARY OF CORONAL EXAMINATIONS

TREATMENT GROUP:	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
TOTAL LITTERS EXAMINED	22	22	22	22
LIVE FETUSES/LITTERS EXAMINED	169/ 22	172/ 22	176/ 22	177/ 22
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)	0	0	0	0
LITTERS WITH MALFORMATIONS (%)	0	0	0	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)	0	0	0	0
LITTERS WITH VARIATIONS (%)	0	0	0	0

Skeletal malformations and variations:

Malformations: There is an apparent dose-dependent increase in the number of fetuses and litters with skeletal malformations at 250 and 1000mg/kg. The increase is most apparent at 1000mg/kg with 4 malformations present compared to none in the controls. The incidence is in the upper end of the historical range (see Sponsor's table below).

In the 250mg/kg group, a single pup (#07) from female 02-7663 had multiple skeletal findings, including the malformation (cervical vertebra), incomplete ossification of thoracic vertebra and sternebra, sacral vertebra variation, and supernumerary rib.

Variations: The incidence of wavy ribs increased at 1000mg/kg (5 incidents, 1.5%) compared to none in controls, and exceeds the historical high of 0.27%.

Ossification: Incomplete ossification of the thoracic vertebra was more frequent at 250 and 1000mg/kg; however, the incidence is well within the historical control range.

Skeletal Malformations				
	Control n=326	125mg/kg n=331	250mg/kg n=342	1000mg/kg n=343
# fetuses with malformations	0	0	1 (0.3%)	8 (2.4%)
# litters with malformations	0	0	1 (4.5%)	4 (18%)
Cervical vertebra (malformation not specified)	0	0	1 (0.3%)	1 (0.3%)
Absent vertebra	0	0	0	2 (0.6%)
Absent rib	0	0	0	1 (0.3%)
Hypoplastic rib	0	0	0	5 (1.4%)

Skeletal Variations				
	Control n=326	125mg/kg n=331	250mg/kg n=342	1000mg/kg n=343
# fetuses with variations	56	33	43	51
# litters with variations	14	16	14	19
Wavy rib	0	0	0	5 (1.5%)

Skeletal Ossification				
	Control n=326	125mg/kg n=331	250mg/kg n=342	1000mg/kg n=343
# fetuses with incomplete ossification	9	17	6	11
# litters with incomplete ossification	8	6	5	8
thoracic vertebra	0	0	3 (0.8%)	2 (0.6%)
hyoid	0	0	0	1 (0.6%)

Historical Control data from Sponsor, spanning 1998-2002.

Historical Control Data for Skeletal Examinations[b]

Fetal Alterations	% Litter Mean	Historical Control Range % Litter Mean
Cervical Vertebra Malformation	0.061	[0 to 0.455]
Absent Vertebra	0.106	[0 to 0.649]
Absent Rib	0.113	[0 to 0.606]
Hypoplastic Rib	0.295	[0 to 1.240]
Sacral Vertebra Variation	0.069	[0 to 0.587]
Vertebral Count Variation	0.207	[0 to 1.136]
Wavy Rib	0.012	[0 to 0.267]
Cervical Rib	2.093	[0 to 3.970]
Supernumerary Rib	7.483	[2.604 to 13.545]

[b] Includes historical control data from a total of 23 studies representing 500 Litters (7354 Fetuses)

Historical Control Data for Ossification Data[c]

Fetal Alterations	% Litter Mean	Historical Control Range % Litter Mean
Incomp. Oss. Cervical Vertebra	0.125	[0 to 0.699]
Incomp. Oss. Thoracic Vertebra	0.671	[0 to 1.694]
Incomp. Oss. Lumbar Vertebra	0.284	[0 to 1.053]
Incomp. Oss. Sternebra	2.519	[0.350 to 4.527]
Incomp. Oss. Hyoid	0.418	[0 to 6.066]

[c] Includes historical control data from a total of 23 studies representing 500 Litters (7354 Fetuses)

<table>
<tr><th colspan="2">Oral range-finding study in pregnant rabbits</th></tr>
<tr><td>SPECIES
DOSES AND ADMINISTRATION
ANIMALS</td><td>• One drug-related death at 500mg/kg ; no other findings in surviving females or in lower dose groups

• Merck chose 62.5, 125, 500mg/kg for definitive study. A high dose of 250mg/kg would be more appropriate.</td></tr>
<tr><td>Study TT 02-722-5 (non-GLP)

NZ White Rabbits

62.5, 125, 250, 500mg/kg
Dosed GD7 to GD20

n=10 females per dose</td><td>
<table>
<tr><th>Procedures Performed</th><th>Yes</th><th>No</th><th>Procedures Performed</th><th>Yes</th><th>No</th></tr>
<tr><td>Physical examinations</td><td>✓</td><td></td><td>Necropsy</td><td></td><td>✓</td></tr>
<tr><td>Body weights</td><td>✓</td><td></td><td>Toxicokinetics</td><td></td><td>✓</td></tr>
<tr><td>Food consumption</td><td>✓</td><td></td><td>External fetal examination</td><td>✓</td><td></td></tr>
<tr><td>Water consumption</td><td></td><td>✓</td><td>Visceral fetal examination</td><td></td><td>✓</td></tr>
<tr><td>Hematology</td><td>✓</td><td></td><td>Skeletal fetal examination</td><td></td><td>✓</td></tr>
<tr><td>Clinical chemistry</td><td>✓</td><td></td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td colspan="2"><u>Mortality:</u> One 500mg/kg female sacrificed on GD25 following a spontaneous abortion. This female lost 15% body weight and food intake was reduced to nearly zero. Death is considered treatment-related although the cause was not determined.</td></tr>
<tr><td colspan="2"><u>Clinical Signs</u>: No drug-related physical signs were reported.</td></tr>
<tr><td colspan="2"><u>Body Weight</u>: Excluding the sacrificed female, weight gain at 500mg/kg (+159g) was similar to the control group (+163g). Lower dose groups were also comparable to control.</td></tr>
<tr><td colspan="2"><u>Food Cons.:</u> Food intake decreased 6 to 16% in 500mg/kg females (excluding the sacrificed female) during the dosing phase. Food intake rebounded after dosing ended, indicating a drug-related cause. Lower dose groups were comparable to control.</td></tr>
<tr><td colspan="2"><u>Hematology</u>: There was no drug-related change in RBC or WBC parameters</td></tr>
<tr><td colspan="2"><u>Clinical Chem</u>: There was no drug-related change in LFT, renal markers, electrolytes, or serum proteins. Serum triglycerides and cholesterol tended to increase at 500mg/kg.</td></tr>
<tr><td colspan="2"><u>Placental Morphology:</u> Gross examination revealed one abnormal placenta from 66 evaluated.</td></tr>
<tr><td colspan="2"><u>Laparotomy Data</u>: No drug-related effect on pre- or post-implantation loss, resorptions, the number of live fetuses, or fetal body weight.</td></tr>
</table>

External Fetal Exam: A tail malformation in one fetus is reported at 125mg/kg, and a clubbed hindfoot in one control fetus.

TABLE 6. L-000224715: ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS. IT# 02-723-5
SUMMARY OF EXTERNAL EXAMINATION OF FETUSES

TREATMENT GROUP:		CONTROL	62.5 MG/KG/DAY	125 MG/KG/DAY	250 MG/KG/DAY	500 MG/KG/DAY
LIVE FETUSES/LITTERS EXAMINED		67/ 8	65/ 9	66/ 9	79/ 8	71/ 9
DEAD FETUSES/LITTERS EXAMINED		0	2/ 2	2/ 1	0	0
FETUSES WITH MALFORMATIONS		1	0	1	0	0
% LM		1.2	0.00	1.1	0.00	0.00
LITTERS WITH MALFORMATIONS (%)		1 (12)	0	1 (11)	0	0
FETUSES WITH VARIATIONS		0	0	0	0	0
% LM		0.00	0.00	0.00	0.00	0.00
LITTERS WITH VARIATIONS (%)		0	0	0	0	0
PLACENTAL MORPHOLOGY						
NO. ABNORMAL PLACENTAS/TOTAL EXAMINED [a]		0/67	0 (5)/65 (5)	1 (6)/66 (6)	0/79	0 (2)/71 (2)
TYPE AND NUMBER OF FETAL ALTERATIONS & LM	CLASS					
TAIL MALFORMATION	(M)	0	0	1 (1.1)	0	0
CLUBBED HINDFOOT	(M)	1 (1.2)	0	0	0	0

(LM) = LITTER MEAN (M) = MALFORMATION (V) = VARIATION

[a] NUMBERS IN PARENTHESIS REPRESENT PLACENTAS FROM DEAD FETUSES OR LATE RESORPTIONS.

Appears This Way
On Original

<table>
<tr><th colspan="2">Oral range-finding study in non-pregnant rabbits</th></tr>
<tr><td>SPECIES
DOSES AND ADMINISTRATION
ANIMALS</td><td>NOAEL: 225mg/kg

Death in 1/6 females, weight loss, ↓ food intake at 675 mg/kg</td></tr>
<tr><td>Study TT 02-722-6 (non-GLP)

NZ White Rabbits

Oral gavage for 14 days
25, 75, 225, 675 mg/kg

n=6 females per dose group</td><td>
<table>
<tr><th>Procedures Performed</th><th>Yes</th><th>No</th><th>Procedures Performed</th><th>Yes</th><th>No</th></tr>
<tr><td>Physical examinations</td><td>✓</td><td></td><td>Ophthalmology</td><td></td><td>✓</td></tr>
<tr><td>Body weights</td><td>✓</td><td></td><td>Electrocardiogram</td><td></td><td>✓</td></tr>
<tr><td>Food consumption</td><td>✓</td><td></td><td>Organ weights</td><td></td><td>✓</td></tr>
<tr><td>Water consumption</td><td></td><td>✓</td><td>Necropsy</td><td></td><td>✓</td></tr>
<tr><td>Hematology</td><td>✓</td><td></td><td>Histology</td><td></td><td>✓</td></tr>
<tr><td>Clinical chemistry</td><td>✓</td><td></td><td>Toxicokinetics</td><td></td><td>✓</td></tr>
<tr><td>Urinalysis</td><td></td><td>✓</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td colspan="2"><u>Mortality:</u> One female at 675 mg/kg was found dead on drug day 9; the dose group was terminated on drug day 10 due to body weight loss and decreased food consumption.</td></tr>
<tr><td colspan="2"><u>Clinical Signs</u>: No clinical signs were reported at ≤ 225mg/kg.</td></tr>
<tr><td colspan="2"><u>Body Weight</u>: Body weight decreased at 675 mg/kg starting on drug day 3, dropping 249g (~7%) by drug day 9. Lower dose groups were comparable to control with an average ~50g weight gain.</td></tr>
<tr><td colspan="2"><u>Food Consump.</u> Food intake decreased at 675mg/kg starting on drug days 2-3, dropping nearly 50% by drug day 5. Lower dose groups were comparable to control at 125g/day (diet-restricted).</td></tr>
<tr><td colspan="2"><u>Hematology</u>: There was no significant change in RBC or WBC parameters at ≤ 225mg/kg. (675mg/kg group not evaluated)</td></tr>
<tr><td colspan="2"><u>Clinical Chemistry</u>: There was no significant change in LFT, renal markers, electrolytes, or serum proteins at ≤ 225mg/kg. (675mg/kg group not evaluated)</td></tr>
<tr><td colspan="2"><u>No necropsy, histology, or TK data obtained.</u></td></tr>
</table>

Oral developmental toxicity study in rabbits
62.5, 125, 500mg/kg

Key study findings:
Dams

- The 500mg/kg group was terminated on GD18 due to deaths, weight loss, and reduced food intake.
- Laparatomy and fetal data was not collected for the 500mg/kg group.
- The next highest dose of 125mg/kg did not produce any sign of maternal toxicity.

Fetuses

- Findings include diffuse hemorrhagic ovary in 2.7% of pups at 62.5mg/kg, multiple cardiovascular malformations in 1 pup at 125mg/kg, and absent caudate lobe of lung at slightly increased incidence at 125mg/kg (2.9% vs. 2.0% in control).

NOAEL Determination

The dose-ranging study in pregnant females predicted deaths in the current study, but Merck nonetheless terminated the dose group after the first 2 deaths late in the study. Terminating the 500mg/kg group was not necessary, as the surviving 500mg/kg females showed no physical signs other than reduced body weight and food consumption.

The few fetal findings at maternally non-toxic doses are either not dose-dependent (hemorrhagic ovary), a single occurrence (cardiovascular malformations), or similar in incidence to the control group (lung findings). None are considered related to drug treatment.

NZ Rabbits, Segment 2 Adverse Effect	NOAEL (AUC_{0-24}*)	Multiple of MRHD (10 uM*h)
Dams Death at 500mg/kg No effects at 125mg/kg	125mg/kg (*189 µM*h)	19x
Fetuses None examined at 500mg/kg No drug-related effects at 125mg/kg	> 125mg/kg (*57-125 µM*h)	6-12x

*AUC from study # TT 03-716-0 done in pregnant SD rats. Fetal exposure based on 30% to 66% of exposure in dams (from same study).

Study no.: TT 02-722-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 23 Oct 2002
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431 anhydrous phosphate salt; lot L-000224715-006F020; purity [redacted]

Methods

Doses:	62.5, 125, 500 mg/kg and placebo
Species/source	Rabbit, New Zealand White from [redacted]
Number/sex/group: (main study)	18 females per dose group
Route, formulation, dose volum	oral gavage of drug in 0.5% MC/ 5 mM HCl, 5ml/kg dose volume Dosed from GD7 to GD 20
Toxicokinetic groups	TK data not collected

IN-LIFE OBSERVATIONS	FREQUENCY
Cageside observations & clinical exams	Daily during gestation
Body weight & food consumption	BW on GD0, then every other day starting at GD7 FC measured over 2 day intervals during gestation
POST-MORTEM EVALUATIONS	
Maternal necropsy	Necropsy on GD28 (8 days after end of dosing)
Ovarian/Uterine examinations	Pregnancy status/corpora lutea number recorded
Placental examination	Placenta from 1 female in the control and 125mg/kg group was examined microscopically. Other placentas were given a gross examination.
Teratologic (Fetal) examination	External, visceral, skeletal, coronal examinations done on all fetuses.

Results

Note: The 500mg/kg group was terminated early (GD18) due to early death, reduced body weight and food consumption, and the lack of feces in some individuals. Laparatomy and fetal data were not collected for this dose group.

Mortality (dams): Three 500mg/kg females were found dead, one on GD9 and two on GD16. One death was caused by a intubation accident, the other two are considered treatment-related.

Clinical signs (dams): In the 500mg/kg group, no feces were noted in 4/17 females between GD13 and GD18. No physical signs were reported in lower dose groups.

Body weight (dams): Body weight increased approx. 120g in control, 62.5, and 125mg/kg females by GD28. Body weight decreased 108g by GD15 in 500mg/kg females. (Table 2)

TABLE 2. L-000224715: Oral Developmental Toxicity Study in Rabbits. TT #02-722-0
Average Maternal Body Weight Changes (Grams ±S.D.) of F0 Females

TREATMENT GROUP	Control	62.5 mg/kg/day	125 mg/kg/day	500 mg/kg/day
GESTATION PERIOD				
DAYS 0-7	3 ± 67(17)	29 ± 78(17)	34 ± 61(18)	64 ± 61(17)
DAYS 7-15	120 ± 61	113 ± 72	118 ± 81	-108 ± 195(16)
DAYS 7-21	142 ± 77	145 ± 75	144 ± 100	
DAYS 21-28	146 ± 90	134 ± 53	146 ± 56	

(N) = GROUP SIZE. APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

Food consumption (dams): Food intake remained relatively constant at 120g/day in doses up to 125mg/kg; however, food intake decreased 50% in 500mg/kg females by GD10.

Necropsy of Dams: No changes were seen in dams up to 125mg/kg (high dose females not examined). Necrotic placental sites in the left uterine horn of one female at 125mg/kg (#02-02830) was considered unrelated to treatment and consistent with spontaneous resorptions. The right horn of this animal contained normal placental sites. Implantation data for this animal is consistent with other females in the dose group.

Placental morphology was unchanged, except for the case described above. Histological examination of 1 placenta from control and 125mg/kg group showed no change.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

There was no change in pre- or post-implantation losses, the number of live fetuses, the sex ratio, or fetal body weight. (Table 4)

TABLE 4. L-000224715: Oral Developmental Toxicity Study in Rabbits. TT #02-722-0
Summary of Laparotomy Data

	Control	62.5 mg/kg/day	125 mg/kg/day	500 mg/kg/day[a]
MATED FEMALES	18	18	18	19
PREGNANT	17	17	16	17
EXAMINED LIVE LITTER	17	17	16	0
RESORBED OR DEAD LITTER	0	0	0	0
FOUND DEAD	0	0	0	3
SACRIFICED	0	0	0	14
NOT PREGNANT	1	1	0	2
LIVE	1	1	0	0
FOUND DEAD	0	0	0	0
SACRIFICED	0	0	0	2
CORPORA LUTEA	179	176	172	
CORPORA LUTEA/PREGNANT FEMALE± S.D.	10.5± 2.1	10.4± 2.0	9.6± 1.5	
%PERI-IMPLANTATION LOSS (LITTER MEAN)± S.D.	7.0± 6.0	10.7±10.6	8.4±16.4	
IMPLANTS	166	157	157	
IMPLANTS/PREGNANT FEMALE± S.D.	9.8± 2.0	9.2± 2.0	8.7± 2.1	
RESORPTIONS	14	3	5	
%RESORPTIONS/IMPLANTS (LITTER MEAN)± S.D.	7.9±19.5	1.7± 3.9	3.2± 5.4	
DEAD FETUSES	1	0	0	
%DEAD FETUSES/IMPLANTS (LITTER MEAN)± S.D.	0.6± 2.4	0.0	0.0	
%POSTIMPLANTATION LOSS (LITTER MEAN)± S.D.	8.5±19.7	1.7± 3.9	3.2± 5.4	
LIVE FETUSES	147	154	152	
FEMALES	79	83	69	
MALES	68	71	83	
SEX RATIO (LITTER MEAN)± S.D.	0.54± 0.16	0.52± 0.18	0.45± 0.20	
LIVE FETUSES/PREGNANT FEMALE± S.D.	8.6± 1.5	9.1± 1.9	8.4± 2.1	
LIVE FETAL WEIGHT (GM, LITTER MEAN)± S.D.				
FEMALES	36.4 ± 3.4	36.7 ± 2.7	36.6 ± 4.8	
MALES	37.1 ± 4.3	37.7 ± 3.5	38.6 ± 3.7	

%PERI-IMPLANTATION LOSS = ((NO. CORPORA LUTEA - NO. IMPLANTS) / NO. CORPORA LUTEA) X 100
%POSTIMPLANTATION LOSS = ((NO. RESORPTIONS + NO. DEAD FETUSES) / NO. IMPLANTS) X 100
SEX RATIO = (TOTAL NO. LIVE FEMALE FETUSES / TOTAL NO. LIVE FETUSES)
[a] DOSE GROUP TERMINATED ON GESTATION DAYS 15 TO 18 DUE TO EXCESSIVE MATERNAL TOXICITY.

Offspring (malformations, variations, etc.):
External malformations and variations: Omphalocele was reported in 1 fetus at 62.5 mg/kg. (from female 02-0805)

SUMMARY OF EXTERNAL EXAMINATIONS

TREATMENT GROUP:		Control	62.5 mg/kg/day	125 mg/kg/da
TOTAL LITTERS EXAMINED		17	17	16
LIVE FETUSES/LITTERS EXAMINED		147/ 17	154/ 17	152/ 16
DEAD FETUSES/LITTERS WITH DEAD FETUSES		1/ 1	0/0	0/0
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)		2(1.8 ± 5.4)	1(0.74± 3.0)	0
LITTERS WITH MALFORMATIONS (%)		2(12)	1(5.9)	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)		0	0	0
LITTERS WITH VARIATIONS (%)		0	0	0
PLACENTAL MORPHOLOGY				
NO. ABNORMAL PLACENTAS/TOTAL EXAMINED[a]		0(11)/147(11)	0/154	1(2)/152(3)
TYPE AND NUMBER OF FETAL ALTERATIONS (%,L.M.±S.D.)	CLASS			
Agnathia	(M)	1(1.2 ± 4.9)	0	0
Omphalocele	(M)	0	1(0.74± 3.0)	0
Tail Malformation	(M)	1(0.65± 2.7)	0	0

(L.M.) = LITTER MEAN (M) = Malformation
[a] NUMBERS IN PARENTHESES REPRESENT PLACENTAS FROM DEAD FETUSES OR LATE RESORPTIONS

Visceral malformations and variations: See Table 6

1. Diffusely hemorrhagic ovary is reported for 4 pups from 2 females at 62.5mg/kg. Hemorrhage is reported in some organs for some DPP4 inhibitors.

2. Multiple cardiovascular malformations are reported for a single fetus in the 125mg/kg group.

3. The incidence of absent caudate lobe of lung increased at 125mg/kg (2.9% vs 2.0% in control).

TABLE 6. L-000224715: Oral Developmental Toxicity Study in Rabbits. TI #02-722-0

SUMMARY OF VISCERAL EXAMINATIONS

TREATMENT GROUP:		Control	62.5 mg/kg/day	125 mg/kg/day
THORACIC AND ABDOMINAL EXAMINATION				
TOTAL LITTERS EXAMINED		17	17	18
LIVE FETUSES/LITTERS EXAMINED		147/ 17	154/ 17	152/ 18
DEAD FETUSES/LITTERS WITH DEAD FETUSES		1/ 1	0/0	0/0
LITTERS WITH MALFORMATIONS (%)		3(18)	6(35)	2(11)
LITTERS WITH VARIATIONS (%)		9(53)	9(53)	6(33)
TYPE AND NUMBER OF FETAL ALTERATIONS (%,L.M.±S.D.)	CLASS			
Persistent Atriovent. Canal	(M)	0	0	1(0.79± 3.4)[a]
Persistent Truncus Arteriosus	(M)	0	0	1(0.79± 3.4)[a]
Abnormal Origin Subc. Art.	(M)	0	0	1(0.79± 3.4)[a]
Absent Azygos Vein	(M)	0	1(0.65± 2.7)	0
Vena Cava Malformation	(M)	0	1(0.59± 2.4)	0
Retrocaval Ureter	(M)	4(2.5 ± 5.6)	4(2.5 ± 4.6)	2(0.85± 3.6)
Absent Gallbaldder	(M)	0	1(0.65± 2.7)	0
Carotid Branching Variation	(V)	5(3.9 ± 6.5)	1(0.74± 3.0)	4(2.7 ± 7.0)
Diffusely Hemorrhagic Kidney	(V)	1(1.2 ± 4.9)	0	0
Diffusely Hemorrhagic Ovary	(V)	0	4(2.7 ± 8.1)	0
Small Gallbladder	(V)	0	4(2.3 ± 4.3)	0
Absent Caudate Lobe of Lung	(V)	3(2.0 ± 4.4)	3(1.9 ± 6.2)	5(2.9 ± 6.9)
Discolored Liver	(V)	1(1.2 ± 4.9)	0	0
Splenic Variation	(V)	1(0.59± 2.4)	0	0

(L.M.) = LITTER MEAN (M) = Malformation (V) = Variation

[a] MULTIPLE MALFORMATIONS OBSERVED IN THE SAME FETUS.

Skeletal malformations and variations: See Table 8

The incidence of pelvic bone variation is increased at 62.5 and 125mg/kg (1.5% and 1.3% respectively) compared to 0% in the control group.

TABLE 8. L-000224715: Oral Developmental Toxicity Study in Rabbits. TT #02-722-0

SUMMARY OF SKELETAL EXAMINATIONS

TREATMENT GROUP:		Control	62.5 mg/kg/day	125 mg/kg/day
TORSO AND LIMB EXAMINATION				
TOTAL LITTERS EXAMINED		17	17	18
LIVE FETUSES/LITTERS EXAMINED		147/ 17	154/ 17	152/ 18
DEAD FETUSES/LITTERS WITH DEAD FETUSES		1/ 1	0/0	0/0
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)		4(2.6 ± 6.2)	0	0
LITTERS WITH MALFORMATIONS (%)		1(18)	0	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)		25(17 ±15.5)	21(14 ±15.0)	25(17 ±20.0)
LITTERS WITH VARIATIONS (%)		12(71)	9(53)	11(61)
HEAD EXAMINATION				
TOTAL LITTERS EXAMINED		17	17	18
LIVE FETUSES/LITTERS EXAMINED		147/ 17	154/ 17	152/ 18
DEAD FETUSES/LITTERS WITH DEAD FETUSES		1/ 1		
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)		1(1.2 ± 4.9)	0	0
LITTERS WITH MALFORMATIONS (%)		1(5.9)	0	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)		0	0	0
LITTERS WITH VARIATIONS (%)		0	0	0
TYPE AND NUMBER OF FETAL ALTERATIONS (%,L.M.±S.D.)	CLASS			
Cervical Vertebra Malformation	(M)	1(0.65± 2.7)	0	0
Thoracic Vertebra Malformation	(M)	4(2.6 ± 6.2)	0	0
Lumbar Vertebra Malformation	(M)	1(0.65± 2.7)	0	0
Absent Rib	(M)	1(0.65± 2.7)	0	0
Branched Rib	(M)	1(0.65± 2.7)	0	0
Hyoid Bone Malformation	(M)	1(1.2 ± 4.9)	0	0
Cervical Rib	(V)	2(1.4 ± 3.9)	0	0
Short 13th Rib	(V)	23(15 ±15.4)	19(12 ±14.4)	22(15 ±20.1)
Sternebral Variation	(V)	1(0.65± 2.7)	0	1(0.56± 2.4)
Pelvic Bone Variation	(V)	0	2(1.5 ± 4.2)	2(1.3 ± 4.0)

(L.M.) = LITTER MEAN (M) = Malformation (V) = Variation

Coronal malformations and variations: See Table 7

Hydrocephalus was reported for 1 fetus at 62.5mg/kg (from female 02-0804).

TABLE 7. L-000224715: Oral Developmental Toxicity Study in Rabbits. TT #02-722-0

SUMMARY OF CORONAL EXAMINATIONS

TREATMENT GROUP:		Control	62.5 mg/kg/day	125 mg/kg
TOTAL LITTERS EXAMINED		17	17	18
LIVE FETUSES/LITTERS EXAMINED		146[a]/17	153[a]/17	151[a]/18
FETUSES WITH MALFORMATIONS (%,L.M.±S.D.)		0	1(0.59± 2.4)	0
LITTERS WITH MALFORMATIONS (%)		0	1(5.9)	0
FETUSES WITH VARIATIONS (%,L.M.±S.D.)		0	0	0
LITTERS WITH VARIATIONS (%)		0	0	0
TYPE AND NUMBER OF FETAL ALTERATIONS (%,L.M.±S.D.)	CLASS			
Hydrocephalus	(M)	0	1(0.59± 2.4)	0

(L.M.) = LITTER MEAN (M) = Malformation

[a] SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

Prenatal and postnatal development

Oral postnatal developmental toxicity study in rats
125, 250, 1000mg/kg

Key study findings:

F0 Generation

Maternally toxic doses are 250 and 1000mg/kg; the maternally non-toxic dose is 125mg/kg.

Maternal toxicity is evidenced by:

1. Urine-stained fur at 1000mg/kg
2. Transient (GD6-12) 15% to 18% decrease in body weight gain at 250 and 1000mg/kg.
3. Transient (GD8) 8% to 12% decrease in food intake at 250 and 1000mg/kg.

F1 Generation

Effects at 250/1000mg/kg (maternally toxic dose):

1. Death of 4 pups from 2 dams at 250mg/kg and 8 pups from1 dam at 1000mg/kg on LD11-21.

2. Reduced body weight (10%) at 1000mg/kg persisting to the post-weaning stage.

3. Longer passive avoidance test scores at 250/1000mg/kg during session 1 but not session 2 assays.

Effects at 125mg/kg (maternally non-toxic dose):

1. The number of litters with dead pups by LD3 increased to 6 versus 4 in control, concomitant with more pup deaths at 125mg/kg (9 vs. 4 in control).

2. One pup had a polydactyly external malformation (none reported at 250/1000mg/kg).

F2 Generation

There were no differences from control on clinical signs, body weight, viability, or external malformations/variation in F2 newborns, which were subsequently sacked on LD0.

Other comments:
Visceral and skeletal malformations/variations were not assessed in the F1 generation.

The study text and pathologist's report differ markedly in describing the timing and type of deaths observed in this study. The relation of these deaths to drug treatment is therefore unclear, despite Merck's dismissal of the deaths as unrelated to drug.

1. F0 Female #3764 (1000mg/kg) was either euthanized on LD13 after losing 8 pups or was found dead on post-natal day 30. The study report differs from the pathologist's report on this animal. Either case does not exclude a relation to drug treatment.

2. F0 Female #3766 of the 1000mg/kg group was 'inadvertently' killed on LD20 without explanation. This female gave birth to the fewest newborns of the dose group (9 vs. 14.5 group average).

3. F1 female #4593 from the 1000mg/kg group was found dead either during post-weaning week 3 or on gestational day 13. Again, accounts differ in the text and pathologist's report. A relation to drug treatment is uncertain though unlikely.

SD Rats, Segment 3 **Adverse Effect**	**NOAEL** **($AUC_{0\text{-}24}$*)**	**Multiple of MRHD** **(10 ug*h/ml)**
Dams ↓ BW gain at 250/1000mg/kg Urine stained fur, possible death at 1000mg/kg	125 mg/kg (est. 125 µM*h)	No TK (est. 12x)
F1 Generation ↓ BW at 1000mg/kg ↑ pup deaths 250/1000mg/kg by LD21	125 mg/kg	F1 exposure to drug unknown
F2 Generation No Findings	> 1000 mg/kg	not applicable

Study no.: TT 03-714-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 03 April 2003
GLP compliance: Yes
QA reports: yes (x) no ( )

Drug, lot #, and % purity: MK-0431 anhydrous phosphate salt; lot L-000224715-006F020; [redacted] purity

Methods

Doses:	125, 250, 1000mg/kg and placebo Dosed GD6 to LD20
Species/source	SD rats from [redacted]
Number/sex/group: (main study)	22 females per dose group
Route, formulation, dose volume	Oral gavage of drug in 0.5% MC/5 mM HCl; 5ml/kg dose volume
Toxicokinetic groups	None

Experimental Design:

Evaluation of F0 Dams:

- Physical exam
- Body weight and food consumption
- Ophthalmic exam
- Parturition and gestation length
- Necropsy of all dams on LD21/22

Evaluation of F1 Generation:

- On LD0, all pups were counted, sexed, weighed, and examined for external anomalies only.

- On LD3, litters reduced to 4 males, 4 females
  Physical signs and body weights monitored to LD21

- On LD22, 2 pups per sex were re-housed
  Physical signs and body weights monitored
  Developmental signs
  Ophthalmologic exam
  Behavioral assessment (1 male, 1 female per litter)
  Mated on post-natal week 11/12
  Parturition monitored

Evaluation of F2 Generation:

- On LD0, pups were counted, weighed, sexed, examined for external anomalies, and mortality was recorded. All pups were sacrificed on LD0.

Results

F_0 Generation

F_0 in-life:

Mortality: Merck states that there was no drug-related mortality. One 1000mg/kg female (#3764) was euthanized on LD13 because there were no surviving pups in that litter by LD14, but this is contradicted by the pathologist's report (see below). Another high dose female (#3766) was inadvertently killed on LD20.

Note that the pathologist's report lists female #3764 as having been found dead on post-natal day 30. This conflicts with the summary report and with the protocol stating that all F0 females were sacrificed on LD21/22. The pathologist states that 'the cause of death of found dead animals (incl. #3764) was not determined'. This animal was reportedly sacrificed by CO_2 asphyxiation on LD13. This discrepancy clearly demonstrates an internal problem with tracking of rats in this study.

Clinical Signs: Urine-stained fur was observed in 50% of females at 1000mg/kg, possibly related to renal dysfunction though not verified. Salivation was noted at 125mg/kg and above, related to the poor palatability of the drug formulation.

Body Weight (Table 2): Body weight gain decreased 15% and 18% in the 250 and 1000 mg/kg groups during the first week of dosing, GD6 to GD12. Thereafter, body weight gain was similar to or greater than the control group for the remaining dosing period, to LD21.

TABLE 2. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0.
Average Maternal Body Weight Changes (Grams ±S.D.) of F0 Females

TREATMENT GROUP	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
GESTATION PERIOD				
DAYS 0-6	32 ± 8(22)	33 ± 8(22)	35 ± 4(22)	35 ± 7(22)
DAYS 6-12	34 ± 8	34 ± 6	29 ± 6	28 ± 9
DAYS 12-21	89 ± 12	96 ± 19	99 ± 14	90 ± 16
DAYS 6-21	123 ± 16	130 ± 22	129 ± 17	118 ± 18
LACTATION PERIOD				
DAYS 0-7	27 ± 13(22)	25 ± 14(22)	30 ± 11(20)	24 ± 12(22)
DAYS 7-14	27 ± 9	28 ± 9	27 ± 9(22)	24 ± 9(21)
DAYS 14-21	-18 ± 14	-24 ± 10	-13 ± 12	-11 ± 10(20)
DAYS 0-21	36 ± 13	29 ± 15	44 ± 14(20)	36 ± 19

(N)=GROUP SIZE. APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

Food Consumption: Food consumption decreased slightly (8-12%) at 250 and 1000mg/kg on day 8 of gestation. Thereafter, food intake did not differ from the control group up through the dosing period to LD21.

F_0 necropsy: Gross examination of the thoracic and abdominal viscera showed no effect of treatment compared to the control group. Necropsy done on LD21/22.

F_0 Reproductive Performance: The length of gestation is similar in all groups (22.2 to 22.3 days). The number of live/dead newborns is not changed by treatment. (below and Table 5)

Reproductive Performance				
	Control n=22	125 mg/kg n=22	250 mg/kg n=22	1000 mg/kg n=22
Length of Gestation (days)	22.2	22.2	22.2	22.3
# Metrial glands/female	15.5	15.9	15.8	15.5
# live newborns	300	314	334	318
# dead newborns	5	6	2	1

Table 5

TABLE 5. L-0002247157; Oral Postnatal Developmental Toxicity Study in Rats. TI #03-714-0
Summary of Status of F1 Generation Prior to Weaning

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
PARENTAL FEMALES	22	22	22	22
METRIAL GLANDS PER FEMALE ± S.D.	15.5± 2.3	15.9± 2.4	15.8± 2.1	15.5± 1.6
% POSTIMPLANTATION SURVIVAL (L.M.) ± S.D.	89.3±10.6	90.0±16.4	96.3± 4.6	92.5± 8.0
FEMALES WITH LIVE PUPS DAY 0 POSTPARTUM	22	22	22	22
FEMALES WITH LIVE PUPS DAY 21 POSTPARTUM	22	22	22	21[a]
TOTAL PUPS DELIVERED				
LIVE PUPS(SEX RATIO, L.M.)	300(0.47)	314(0.48)	334(0.49)	318(0.56)
DEAD PUPS[N] (%, L.M ± S.D.)	5[4](1.4±3.2)	6[4](2.6±8.1)	2[2](0.8±2.5)	1[1](0.3±1.5)
% LIVE PUPS DELIVERED (L.M. ± S.D.)	98.6± 3.2	97.4± 8.1	99.2± 2.5	99.7± 1.5

[a] Female #3766 inadvertently killed on LD20 excluded from analysis

F_1 Weaning Period

F_1 Viability to LD21: Newborn deaths by LD3 is slightly higher in all dose groups compared to control (below and Table 5a). The number of litters effected is higher at 125mg/kg but equal to control at 250 and 1000mg/kg. The average number of live pups per litter up to post-natal day 7 is comparable across groups.

Additional deaths are reported at 250 and 1000mg/kg during LD4 to LD21 with an uncertain relation to drug treatment. Also, the average number of live pups per litter slightly decreased at 250 and 1000mg/kg by post-natal day 21 (7.8 and 7.6 versus 8.0 pups/litter in control).

At 250mg/kg, 3 of 4 deaths were sacrifices of moribund pups from a single dam on LD21. No details are given as to the nature of the moribund condition; these pups had no external anomaly on LD0.

At 1000mg/kg, 8 pups were found dead or missing (2) from a single dam on LD11-13. The dam (#3764) was presumably sacrificed on LD13. Merck dismisses the finding because only 1 of 22 dams lost these 8 pups, a somewhat unconvincing argument. The reason for the deaths was not discussed. An uncertain relationship to drug treatment is a more accurate interpretation.

F_1 Viability to LD21					
		Control	125 mg/kg	250 mg/kg	1000 mg/kg
LD1 to LD3	# pup deaths	4	9	6	8
	# litters effected	4	6	4	4
LD4 to LD21	# pup deaths	0	0	4	8
	# litters effected	0	0	2	1

Table 5a

TABLE 5. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #93-714-0
Summary of Status of F1 Generation Prior to Weaning

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
PARENTAL FEMALES	22	22	22	22
LIVE PUPS PER LITTER ± S.D.				
POSTNATAL DAY 0	13.6± 1.6	14.3± 3.1	15.2± 2.1	14.5± 1.1
POSTNATAL DAY 3	13.5± 1.6	13.9± 3.4	14.9± 2.3	14.1± 2.2
POSTNATAL DAY 3	8.0± 0.0	8.0± 0.0	8.0± 0.2	8.0± 0.0
POSTNATAL DAY 7	8.0± 0.0	8.0± 0.0	8.0± 0.2	8.0± 0.0
POSTNATAL DAY 14	8.0± 0.0	8.0± 0.0	8.0± 0.2	7.6± 1.7
POSTNATAL DAY 21	8.0± 0.0	8.0± 0.0	7.8± 0.7	7.6± 1.7[a]
PUP DEATHS[N] (% L.M. ± S.D.)				
POSTNATAL DAYS 1 - 3	4[4](1.3±2.8)	9[6](3.6±6.9)	6[4](1.9±4.5)	8[4](2.4±6.3)
POSTNATAL DAYS 4 - 7	0	0	1[1](0.6±2.7)	0
POSTNATAL DAYS 8 - 14	0	0	0	8[1](4.9± 21)
POSTNATAL DAYS 15 - 21	0	0	3[1](1.7±8.0)	0
POSTNATAL DAYS 4 - 21	0	0	4[2](2.3±8.3)	8[1](4.5± 21)

[a] Female #3766 inadvertently killed on LD20 excluded from analysis
[N] = number of litters

F_1 Body Weight: See Table 6.

At LD0, body weight was ~7% lower in 1000mg/kg newborns compared to control newborns, both male and female.

By LD21, body weight was ~10% lower in 1000mg/kg pups compared to control pups, both male and female, and body weight gain was similarly reduced by 5% to 10%.

Lower dose groups were comparable to the control group.

TABLE 6. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of Body Weights of F1 Pups Prior to Weaning

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
LIVE FEMALE PUP WEIGHT (GM) (L.M.) ± S.D.				
POSTNATAL DAY 0	6.16± 0.43	5.95± 0.46	5.90± 0.34	5.72± 0.41
POSTNATAL DAY 7	15.8 ± 1.6	15.1 ± 1.6	15.3 ± 1.0	14.2 ± 1.2
POSTNATAL DAY 14	32.8 ± 2.8	31.1 ± 3.6	32.0 ± 2.1	29.0 ± 2.5
POSTNATAL DAY 21	52.9 ± 4.6	50.3 ± 6.3	52.4 ± 3.6	47.2 ± 3.9[a]
LIVE MALE PUP WEIGHT (GM) (L.M.) ± S.D.				
POSTNATAL DAY 0	6.49± 0.46	6.32± 0.47	6.28± 0.34	6.06± 0.42
POSTNATAL DAY 7	16.5 ± 1.5	15.9 ± 1.6	16.0 ± 1.2	15.1 ± 1.4
POSTNATAL DAY 14	33.5 ± 2.7	32.5 ± 3.3	33.0 ± 1.2	30.7 ± 2.6
POSTNATAL DAY 21	54.9 ± 4.7	53.4 ± 6.5	53.3 ± 8.9	50.0 ± 3.8[a]

F_1 Physical Examination: There were no drug-related physical signs reported.

F_1 External Examination (Table 7): One control newborn had two external malformations (atresia ani and tail malformation) and one newborn at 125mg/kg had a polydactyly malformation. No anomalies were found at 250 and 1000mg/kg.

There was no F1 subset evaluated for visceral or skeletal malformations/variations.

TABLE 7. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of External Examinations of F1 Pups

SUMMARY OF EXTERNAL EXAMINATIONS POSTNATAL DAY 0

TREATMENT GROUP:		Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
TOTAL LITTERS EXAMINED		22	22	22	22
DELIVERED PUPS(LIVE/DEAD)/LITTERS EXAMINED		(300/ 5)/ 22	(314/ 6)/ 22	(334/ 2)/ 22	(318/ 1)/ 22
INTRAUTERINE PUPS(LIVE/DEAD)/LITTERS EXAMINED		(0/ 0)/ 0	(0/ 0)/ 0	(0/ 0)/ 0	(0/ 0)/ 0
PUPS WITH MALFORMATIONS (%,L.M.)		1(0.30)	1(0.27)	0	0
LITTERS WITH MALFORMATIONS (%)		1(4.5)	1(4.5)	0	0
PUPS WITH VARIATIONS (%,L.M.)		0	0	0	0
LITTERS WITH VARIATIONS (%)		0	0	0	0
TYPE AND NUMBER OF PUP ALTERATIONS (%,L.M.)	CLASS				
Atresia Ani	(M)	1(0.30)	0	0	0
Tail Malformation	(M)	1(0.30)	0	0	0
Polydactyly	(M)	0	1(0.27)	0	0

F_1 Post-weaning Period

Post-weaning Viability: One female in the 1000mg/kg group (#4593) was found dead in post-weaning week 3; no cause of death was found. The pathologist's report states that this female was found dead at GD18, a clear discrepancy.

Post-weaning physical exam: There were no drug-related physical signs reported.

Post-weaning body weight: Body weight at post-weaning week 1 was ~8% less at 1000mg/kg compared to the control group. The 8% decrement in body weight persisted in 1000mg/kg males and females to post-weaning week 12. Lower dose groups were reasonably comparable to the control group.

Post-weaning Body Weight				
Post weaning week	Control	125 mg/kg	250 mg/kg	1000 mg/kg
Females Week 1	79.9	78.2	79.5	73.5
Week 12	284	274	277	265
Males Week 1	86.9	85.6	56.4	80.1
Week 12	545	544	538	507

Reproductive Development post-weaning (n= 44 per group)

Females: The average time to vaginal opening in post-weaning females was 32.6 days for all dose groups including control; drug treatment had no apparent effect. All females showed signs of vaginal opening by postnatal day 36.

Males: The average time to preputial separation in post-weaning males was 43.7 days for all dose groups including control; drug treatment had no apparent effect. All males showed signs of preputial separation by postnatal day 50.

Behavioral Development post-weaning (n= 21-22 per group)

Passive Avoidance Test (Table 14): Tests were performed in 2 sessions.
Session 1: The mean number of trials required to reach assay criteria increased in males at 250 and 1000mg/kg (6.1 and 6.9 vs. 5.5 in control). The increase appears due to longer times for some individuals rather than a group shift. No difference was seen in females. A few animals never reached assay criteria but a dose-relation is not evident (two control males, one male/female at 125mg/kg, one female at 1000mg/kg).

Session 2: There was no difference in treated vs. control groups.

TABLE 14. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of Passive Avoidance Testing of F1 Generation

Female

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 35 TO 36 POSTNATAL - Session 1				
NO. ANIMALS TESTED	21[a]	22	22	21
Trials to criterion ± S.D.	6.0 ± 2.6	6.2 ± 1.5	5.3 ± 1.7	6.3 ± 2.4
NO. NOT ACHIEVING CRITERION	0	1	0	1
DAYS 42 TO 43 POSTNATAL - Session 2				
NO. ANIMALS TESTED	21	21	22	19
Trials to criterion ± S.D.	4.0 ± 1.2	4.0 ± 1.1	4.0 ± 1.2	3.8 ± 1.0
NO. NOT ACHIEVING CRITERION	0	0	0	0

[a] See individual table for exclusions

Male

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 35 TO 36 POSTNATAL - Session 1				
NO. ANIMALS TESTED	22	21	21	21
Trials to criterion ± S.D.	5.5 ± 1.0	5.2 ± 1.2	4.1 ± 1.6	6.9 ± 2.5
NO. NOT ACHIEVING CRITERION	2	1	0	0
DAYS 42 TO 43 POSTNATAL - Session 2				
NO. ANIMALS TESTED	20	20	21	21
Trials to criterion ± S.D.	4.7 ± 1.5	3.9 ± 0.9	3.9 ± 1.2	4.1 ± 1.4
NO. NOT ACHIEVING CRITERION	0	0	0	0

Auditory Startle Habituation (Table 15): There is no significant effect of drug treatment compared to the control groups.

TABLE 15. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of Auditory Startle Habituation of F1 Generation

Female

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 62 TO 64 POSTNATAL				
NO. ANIMALS TESTED	22	22	22	20
MEAN V max				
Trials 1-5 ± S.D.	788 ± 377	697 ± 344	596 ± 256	571 ± 263
Trials 6-10 ± S.D.	528 ± 349	431 ± 217	395 ± 180	391 ± 211
Trials 11-15 ± S.D.	446 ± 376	306 ± 179	348 ± 174	326 ± 168
Trials 16-20 ± S.D.	351 ± 226	295 ± 179	274 ± 133	324 ± 197
Trials 21-25 ± S.D.	304 ± 213	259 ± 152	240 ± 97	281 ± 182
Trials 26-30 ± S.D.	318 ± 226	263 ± 173	240 ± 129	304 ± 232
Trials 31-35 ± S.D.	304 ± 290	304 ± 216	240 ± 128	222 ± 143
Trials 36-40 ± S.D.	317 ± 393	234 ± 127	255 ± 133	233 ± 139
Trials 41-45 ± S.D.	332 ± 380	259 ± 162	213 ± 112	217 ± 132
Trials 46-50 ± S.D.	292 ± 198	253 ± 140	237 ± 112	249 ± 169
TRIALS 1-50 ± S.D.	397 ± 253	329 ± 127	304 ± 101	312 ± 147

Male

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 62 TO 64 POSTNATAL				
NO. ANIMALS TESTED	22	21	21	21
MEAN V max				
Trials 1-5 ± S.D.	776 ± 271	833 ± 367	845 ± 487	864 ± 357
Trials 6-10 ± S.D.	529 ± 336	512 ± 332	459 ± 341	633 ± 312
Trials 11-15 ± S.D.	417 ± 209	451 ± 248	328 ± 249	405 ± 239
Trials 16-20 ± S.D.	408 ± 211	335 ± 201	273 ± 181	374 ± 206
Trials 21-25 ± S.D.	358 ± 197	321 ± 208	196 ± 115	340 ± 182
Trials 26-30 ± S.D.	360 ± 195	329 ± 176	210 ± 180	363 ± 201
Trials 31-35 ± S.D.	327 ± 176	356 ± 231	175 ± 106	331 ± 182
Trials 36-40 ± S.D.	322 ± 233	312 ± 194	197 ± 105	395 ± 262
Trials 41-45 ± S.D.	171 ± 228	413 ± 232	219 ± 137	359 ± 218
Trials 46-50 ± S.D.	345 ± 170	344 ± 218	235 ± 192	398 ± 270
TRIALS 1-50 ± S.D.	420 ± 171	421 ± 193	314 ± 150	456 ± 197

Open-field Testing (Table 16): There is no significant effect of drug treatment compared to the control groups.

TABLE 16. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #01-714-5
Summary of Open-Field Testing of F1 Generation

Female

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 69 TO 71 POSTNATAL				
NO. ANIMALS TESTED	23	22	22	21
MEAN Horizontal activity				
0-10 min ± S.D.	5297 ± 903	4803 ± 989	5113 ± 1148	5216 ± 1118
10-20 min ± S.D.	3105 ± 823	2867 ± 709	3305 ± 944	3150 ± 867
20-30 min ± S.D.	2262 ± 1015	1991 ± 905	2394 ± 979	2483 ± 1085
30-40 min ± S.D.	1558 ± 1174	1631 ± 1074	1535 ± 1143	1869 ± 1042
40-50 min ± S.D.	1277 ± 1168	1493 ± 973	1411 ± 1109	1473 ± 956
50-60 min ± S.D.	1462 ± 998	1274 ± 999	1053 ± 839	1407 ± 925
0-60 MIN ± S.D.	2493 ± 724	2336 ± 461	2469 ± 709	2603 ± 688

Male

	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
DAYS 69 TO 71 POSTNATAL				
NO. ANIMALS TESTED	22	22	20[a]	21
MEAN Horizontal activity				
0-10 min ± S.D.	4063 ± 718	3909 ± 793	4056 ± 679	4139 ± 822
10-20 min ± S.D.	2829 ± 822	2615 ± 773	2518 ± 588	2708 ± 762
20-30 min ± S.D.	2113 ± 733	1869 ± 656	1713 ± 521	2019 ± 679
30-40 min ± S.D.	1635 ± 715	1439 ± 654	1125 ± 674	1697 ± 688
40-50 min ± S.D.	1235 ± 892	924 ± 724	1137 ± 645	1270 ± 837
50-60 min ± S.D.	1144 ± 968	885 ± 650	880 ± 664	1100 ± 849
0-60 MIN ± S.D.	2170 ± 629	1940 ± 463	1907 ± 427	2164 ± 611

[a] See individual table for exclusions.

Ophthalmolgic Exam: No drug-related effects were reported (data not shown).

F_1 Mating to Sacrifice Period

Mortality: None, according to the study report. However, the pathologist's report lists female #4593 from the 1000mg/kg group as having been found dead on GD13.

Physical Exam: There were no drug-related clinical signs during this period.

Body weight: Weight gain in F1 females during gestation was comparable across dose groups, despite slightly lower body weight at 1000mg/kg at the start of gestation. (Table 17)

TABLE 17. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Average Maternal Body Weights (Grams ±S.D.) of F1 Females

TREATMENT GROUP	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
GESTATION PERIOD				
DAY 0	272 ± 30(21)	268 ± 27(20)	263 ± 22(22)	261 ± 19(21)
DAY 7	312 ± 32	309 ± 28	302 ± 22	304 ± 24
DAY 14	352 ± 33	348 ± 31(21)	344 ± 27	345 ± 29
DAY 20	428 ± 33	422 ± 28	420 ± 35	423 ± 34
LACTATION PERIOD				
DAY 0	328 ± 30(21)	324 ± 25(21)	321 ± 26(22)	319 ± 28(21)

(N)=GROUP SIZE. APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

Reproductive Performance: Twenty-two F1 females were cohabitated with F1 males, resulting in 21-22 pregnancies per dose group. The time-to-pregnancy averaged 1 to 1.4 days and the length of gestation ranged from 22.2 to 22.3 days for all dose groups including control.

All females gave birth to a similar number of live newborns. The number of stillborns was similar or decreased in dosed groups compared to the control group. (Table 19)

TABLE 19. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of Status of F2 Generation at Parturition

TREATMENT GROUP:	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
PARENTAL FEMALES	21	21	22	21
METRIAL GLANDS PER FEMALE ± S.D.	16.7± 1.5	17.3± 1.6	17.3± 1.9	17.1± 2.0
% POSTIMPLANTATION SURVIVAL (L.M.) ± S.D.	89.5±16.1	90.9±15.7	93.2± 8.2	94.9± 4.2
FEMALES WITH LIVE PUPS DAY 0 POSTPARTUM	21	21	22	21
TOTAL PUPS DELIVERED				
LIVE PUPS(SEX RATIO, L.M.)	313(0.49)	329(0.45)	354(0.47)	340(0.49)
DEAD PUPS[N] (%, L.M. ± S.D.)	9[4](3.0±7.0)	6[4](1.9±4.4)	6[5](1.8±3.8)	2[2](0.5±1.6)
% LIVE PUPS DELIVERED (L.M. ± S.D.)	97.0± 7.0	98.1± 4.4	98.2± 3.8	99.5± 1.6
LIVE PUPS PER LITTER ± S.D.				
POSTNATAL DAY 0	14.9± 3.0	15.7± 3.1	16.1± 2.3	16.2± 1.7
PUP DEATHS[N] (%, L.M. ± S.D.)				
POSTNATAL DAYS 0 - 0	9[4](3.0±7.0)	6[4](1.9±4.4)	6[5](1.8±3.8)	2[2](0.5±1.6)

F_2 findings:

Clinical signs: All F2 newborns were sacrificed on LD0, so no observations were possible.

Body weight: There was no drug-related change in body weight.

TABLE 20. L-000224718: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of Body Weights of F2 Pups at Parturition

TREATMENT GROUP:	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
LIVE FEMALE PUP WEIGHT (GM) (L.M.) ± S.D.				
POSTNATAL DAY 0	6.03± 0.64	5.90± 0.31	5.94± 0.41	6.17± 0.52
LIVE MALE PUP WEIGHT (GM) (L.M.) ± S.D.				
POSTNATAL DAY 0	6.45± 0.61	6.31± 0.43	6.32± 0.46	6.53± 0.50

Viability: Viability of F2 was similar across groups on the day of birth, but short term (e.g., ≤ 3 day) viability was not possible because all F2 were sacrificed on LD0.

Gross Pathology: There were no drug-related external malformations or variations in F2 pups. Only 1 malformation was found among 1,336 pups. (Table 21).

TABLE 21. L-000224715: Oral Postnatal Developmental Toxicity Study in Rats. TT #03-714-0
Summary of External Examinations of F2 pups

SUMMARY OF EXTERNAL EXAMINATIONS POSTNATAL DAY 0

TREATMENT GROUP:	Control	125 mg/kg/day	250 mg/kg/day	1000 mg/kg/day
TOTAL LITTERS EXAMINED	21	21	22	21
DELIVERED PUPS(LIVE/DEAD)/LITTERS EXAMINED	(313/ 9)/ 21	(329/ 6)/ 21	(354/ 6)/ 22	(340/ 2)/ 21
INTRAUTERINE PUPS(LIVE/DEAD)/LITTERS EXAMINED	(0/ 0)/ 0	(0/ 0)/ 0	(0/ 0)/ 0	(0/ 0)/ 0
PUPS WITH MALFORMATIONS (%,L.M.)	0	1(0.30)	0	0
LITTERS WITH MALFORMATIONS (%)	0	1(4.8)	0	0
PUPS WITH VARIATIONS (%,L.M.)	0	0	0	0
LITTERS WITH VARIATIONS (%)	0	0	0	0
TYPE AND NUMBER OF PUP ALTERATIONS (%,L.M.)	CLASS			
Tail Malformation	(M) 0	1(0.30)	0	0

TABLE: REPRODUCTIVE TOXICITY STUDIES			
STUDY DURATION	**NOAEL**	**MRHD MULTIPLE (100mg; 10μM*hr)**	**FINDINGS**
Male Rat Fertility #TT 037150 GLP	**General: 125mg/kg** **Fertility: > 1000mg/kg**	**No TK**	*General* • Urine-stained fur • BW ↓ 15-20% at 250 and 1000mg/kg *Fertility* • No Findings *Doses:* 125, 250, 1000 mg/kg (+ placebo)
Female Rat Fertility #TT027280 GLP	**General: 250mg/kg** **Fertility: 250mg/kg**	**No TK**	*General* • Urine-stained fur *Fertility* • ↑ incidence of females with resorptions at 250 and 1000mg/kg; 1-2 females with ↑ resorptions; ↑ late resorptions • No other findings *Doses:* 125, 250, 1000 mg/kg (+ placebo)
Dose-Ranging Rat Embryonic Development #TT027215 Non-GLP	**No dose-limiting toxicities identified**		*General* • Urine-stained fur, salivation at 500, 1000mg/kg • No change in BW, clinical signs *Fetuses* • No laparatomy data obtained • No change in clinical signs, external exam • BW gain at 500, 1000mg/kg lagged 5% and 9% behind control pups despite equal birth weight *Doses:* 125, 250, 500, 1000 mg/kg (+ placebo)
Rat Embryonic Development #TT027210 GLP	**Dams: 250mg/kg** **Fetuses: 250mg/kg**	**General: 25x** **Fetuses: 12-25x**	*General* • Urine-stained fur at 1000 mg/kg • No change in BW, clinical signs *Fetuses* • ↑ skeletal malformations/variations at 1000mg/kg vs. concurrent control group • Placental transfer is 44-81% of maternal exposure • One fetus at 250mg/kg had multiple skeletal vertebral findings, including malformed cervical vertebra and incompletely ossified verterbra *Doses:* 125, 250, 1000 mg/kg (+ placebo) AUC: 276, 862 μM*hr at 250, 1000 mg/kg respectively (study TT 03-717-0)

Dose-Ranging Rabbit Embryonic Development #TT027225 Non-GLP			*General* • Death of 1/10 females at 500mg/kg on GD25 after spontaneous abortion. BW ↓ 15%, FC absent before death. • No findings in lower dose groups *Fetuses* • No effect on pre/post-implantation • No change in # live fetuses or fetal BW • No change in external exam *Doses:* 62.5, 125, 250, 500 mg/kg (+ placebo)
Rabbit Embryonic Development #TT027220 GLP	**Dams: 125mg/kg** **Fetuses: > 125 mg/kg**	**General: est. 19x** **Fetuses: 6-12x**	*General* • Death of 3/18 females at 500mg/kg; group stopped before parturition with ↓ BW, ↓ food intake, ↓ feces *Fetuses* • No fetuses examined in 500mg/kg group • No drug-related findings at 125mg/kg • Placental transfer is 30-66% maternal exposure *Doses:* 62.5, 125, 500 mg/kg (+ placebo) AUC: 189 µM*hr at 125 mg/kg
Rat Post-Natal Development #TT037140 GLP	**F0 Dams: 125mg/kg** **F1 progeny: 125mg/kg** **F2 progeny: > 1000mg/kg**	**F0 Dams: est. 12x** **F1 progeny: est. 12x**	*F0 Dams* • ↓ BW gain at 250/1000mg/kg (15-18%) GD6-12 • ↓ FC at 250/1000mg/kg (8-12%) GD8 • Urine-stained fur at 1000mg/kg • Death of 1 female at 1000mg/kg from unknown cause • Maternal toxic doses are 250/1000 mg/kg *F1 Progeny* • ↓ BW at 1000mg/kg persisting through post-weaning • Increased pup death at 250/1000mg/kg LD4-21 *F2 Progeny* • No findings *Doses:* 125, 250, 1000 mg/kg (+ placebo) *AUC:* Estimated 125, 250, 1000 µM*hr

Oral Toxicokinetic Study in Pregnant and Lactating Rats

Key Findings:

- MK-0431 crosses the placenta and is excreted in maternal milk.
- Rat fetuses will be exposed to MK-0431 during gestation and weaning.

Study no.: TT 03-717-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 08 April 2003
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431 anhydrous phosphate salt; lot L-000224715-006F020; —— purity

Purpose: To evaluate transfer of MK-0431 via the placenta and lactation in rats.

Methods: Pregnant female SD rats (26/group) were given oral gavage doses of MK-0431 at 250 and 1000mg/kg from GD6 to GD20 or LD14. Maternal and fetal plasma concentrations of MK-0431 were measured at 2 and 24 hours after the last dose on GD20, and in the maternal milk on LD14.

Results: Placental transfer of MK-0431 ranged from 44% to 80% of maternal exposure (Table B-1). Maternal milk contained ~4-fold higher concentration of MK-0431 compared to maternal plasma.

Conclusions: MK-0431 can cross the placenta and is excreted in maternal milk, indicating that fetuses will be exposed to drug during development and weaning.

Table B-1. L-000224715: Oral Toxicokinetic Study in Pregnant and Lactating Rats. TT #03-717-0

Mean Maternal Plasma L-000224715 Toxicokinetic Parameters and Placental Transfer - Gestation Day 20

	L-000224715 (mg/kg/day)	
	250	1000
Maternal Toxicokinetic Parameters		
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	276 ± 20.0	862 ± 47.7
C_{max} (μM)[b]	26.5 ± 5.80	56.9 ± 17.1
T_{max} (hr)[c]	1.0	1.0
Maternal Plasma Conc. (μM)[d]		
2 hours	11.7 ± 3.23	41.3 ± 6.35
24 hours	1.33 ± 0.258	7.44 ± 2.54
Fetal Plasma Conc. (μM)[d]		
2 hours	5.52 ± 1.67	17.9 ± 2.90
24 hours	0.990 ± 0.106	5.27 ± 1.15
Fetal/Maternal Plasma Ratio[e]		
2 hours	0.463 ± 0.0274	0.435 ± 0.0196
24 hours	0.807 ± 0.111	0.787 ± 0.0779

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Maximum mean plasma concentration ± SEM.
[c] Time at which C_{max} occurred.
[d] Values are the Mean ± SEM.
[e] Values are the Mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.

Table B-2. L-000224715: Oral Toxicokinetic Study in Pregnant and Lactating Rats. TT #03-717-0

Mean Maternal Plasma L-000224715 Toxicokinetic Parameters and Lactational Transfer - Lactation Day 14

	L-000224715 (mg/kg/day)	
	250	1000
Maternal Plasma Conc. (μM)[a]		
2 hours	14.8 ± 3.90	33.5 ± 6.20
Maternal Milk Conc. (μM)[a]		
2 hours	60.9 ± 24.7	136 ± 30.6
Milk/Maternal Plasma Ratio[b]		
2 hours	3.85 ± 0.531	3.93 ± 0.522

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Values are the Mean ± SEM of the milk concentration divided by the corresponding maternal plasma concentration.

Oral Toxicokinetic Study in Pregnant Rabbits

Key Findings:

- MK-0431 crosses the placenta in pregnant rabbits.
- Rabbit fetuses will be exposed to MK-0431 during gestation.

Study no.: TT 03-716-0
Volume #, and page #: NDA eCTD
Conducting laboratory and location: MRL, West Point, PA
Date of study initiation: 08 April 2003
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MK-0431 anhydrous phosphate salt; lot L-000224715-006F020; —— purity

Purpose: To evaluate MK-0431 exposure in fetuses and pregnant rabbits.

Methods: Fifteen female New Zealand White rabbits were given oral gavage doses of 125mg/kg MK-0431 from GD7 to GD20. Maternal and fetal blood concentrations of MK-0431 were measured at 2 and 24hours after the last dose on GD20.

Results: Fetal exposure to MK-0431 was 30% to 66% of maternal exposure, indicating substantial placental transfer of drug (Table B-1).

Conclusions: MK-0431 crosses the placenta and exposes fetuses to drug during gestation.

Table B-1. L-000224715: Oral Toxicokinetic Study in Pregnant Rabbits. TT #03-716-0

Mean Maternal and Fetal Plasma L-000224715 Toxicokinetic Parameters and Placental Transfer - Gestation Day 20

	L-000224715 (mg/kg/day)
	125
Maternal Toxicokinetic Parameters	
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	189 ± 10.4
C_{max} (μM)[b]	54.8 ± 4.08
T_{max} (hr)[c]	0.5
Maternal Plasma Conc. (μM)[d]	
2 hours	31.9 ± 4.95
24 hours	0.847 ± 0.0674
Fetal Plasma Conc. (μM)[d]	
2 hours	18.1 ± 3.66
24 hours	0.247 ± 0.00531
Fetal/Maternal Plasma Ratio[e]	
2 hours	0.663 ± 0.205
24 hours	0.296 ± 0.0195

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Maximum mean plasma concentration ± SEM.
[c] Time at which C_{max} occurred.
[d] Values are the mean ± SEM.
[e] Values are the mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.

2.6.6.8 Special toxicology studies

Skin lesion assessment of sitagliptin in a 14-week oral toxicity study in monkeys

Key study findings:

- No visible skin lesions were found after 14 week administration of 10, 30, or 100 mg/kg sitagliptin.
- Exposure to sitagliptin achieved a 2x, 4x, and 20x multiple of MRHD (10μM*h).
- Exposure to sitaliptin was confirmed by toxicokinetics and plasma DPP4 inhibition.
- No change in kidney weight or gross/micro histopathology was found.

Reviewer Comments
This study was performed at the request of the FDA based on findings of necrotizing skin lesions with other DPP4 inhibitors, with monkey being the most sensitive species. As of July 2006, the FDA considers the skin lesions a result of off-target inhibition of DPP8 or 9. The maximum drug concentration achieved in the study with sitagliptin is 41μM which is equivalent to the IC_{50} for inhibiting DPP8 (48μM) but well below the IC_{50} for inhibiting DPP9 (100μM). This was achieved at a large multiple (20x) of the clinical dose. The absence of skin lesions by 3 months at this exposure supports the current understanding of the mechanism of skin lesion formation by other less selective DPP4 inhibitors.

Note also that Rhesus monkeys were tested rather than Cynomolgus, the strain where skin lesions have been observed. The impact of choosing Rhesus on the study results is unknown; it is feasible that the Rhesus strain is less sensitive than Cynomolgus relative to skin lesions.

Study no.: TT 06-1005
Volume #, and page #: eCTD
Conducting laboratory and location: Merck Research Labs, PA
Date of study initiation: 09 January 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: MK-0431, lot L-000224715-010X036, [redacted] purity

Methods

Doses:	0, 10, 30, 100 mg/kg per day
Species/source	Rhesus monkeys, [redacted]
Age:	1-2 years
Weight:	2.3-2.8 kg females; 2.5-3.2 males
Number/sex/group: (main study)	3 per sex per group
Toxicokinetic groups	Collected from all animals on drug day 1 and drug week 12.
Recovery groups:	none
Route, formulation, dose volume	Oral nasogastric gavage. Vehicle was deionized water with 0.1N HCl.

Observation and Times:

Clinical Findings:	Daily checks
Body weights:	Pre-test, then weekly
Food consumption:	Estimated daily.
Photographs:	Photos of all control and high dose animals, incl. face, inguinal area, genitals, dorsal and ventral body profile, taken on drug week 13.
DPP4 Activity:	DPP4 enzyme activity was measured in blood samples from all dose groups in drug week 14.
Hematology:	not collected
Clinical chemistry:	not collected
Gross pathology:	Full necropsies of all animals at scheduled sacrifices.
Organ weights:	Brain and kidney weight collected. Skin samples from 'numerous sites' were collected and fixed.
Histopathology:	Sections of kidney were evaluated from all dose groups. Skin histopathology was done only in cases of visible gross lesions.

Results:

Mortality: None.

Clinical signs: No drug-related findings from physical exams. Photographs were taken but not submitted with this study report.

Body weights: No drug-related effect on body weight. (0.1-0.4 kg gain, no dose-dependence)

Food consumption: No drug-related effect on food consumption.

Gross pathology: There were no gross changes observed in the skin or the kidneys.

Organ weights: Kidney weight did not change from the control group (~0.5% of body weight).

Histopathology: There were no histological changes to the kidneys compared to the control group.

As there were no gross skin lesions, histopathology of skin was not evaluated. Random biopsies of skin from various regions were collected and fixed.

TABLE B-8. MK-0431: Fourteen-Week Oral Toxicity Study in Monkeys. TT #06-1005
Summary of Histomorphology

Group Number	Female 1	Female 2	Female 3	Female 4	Male 1	Male 2	Male 3	Male 4
NUMBER NECROPSIED	3	3	3	3	3	3	3	3
Kidney								
NO. EXAMINED MICROSCOPICALLY	3	3	3	3	3	3	3	3
Not Remarkable	3	3	3	3	3	2	3	3
Ectopic adrenal	-	-	-	-	-	1	-	-

KEY: GROUP 1 = Control
GROUP 2 = 10 mg/kg/day
GROUP 3 = 30 mg/kg/day
GROUP 4 = 100 mg/kg/day
- = NOT PRESENT.

Plasma DPP4 Activity: Plasma DPP4 activity at the nadir of plasma drug concentration was negligible (post-dose '0 hr' in Table) and remained negligible at 2 and 4 hours post-dose in all dose groups. Thus, plasma DPP4 is essentially completely inhibited for 24 hours at all doses.

Treatment-Related Inhibition of Plasma DPP-4 Enzyme
(Mean Values, Sexes Combined)

Parameter	Hours (Post-Dose)	MK-0431 (mg/kg/day)		
		10	30	100
Plasma DPP-4 Enzyme Inhibition (% Inhibition)	0	88.7	92.8	96.1
	2	97.3	98.1	98.2
	4	97.2	97.6	98.3

14-Week Oral Toxicity Study in Monkeys.

Toxicokinetics: Exposure was dose-proportional and there was no substantial sex difference in exposure. The Cmax at 100mg/kg was 40.9μM which is substantially below the IC_{50} for in vitro inhibition of DPP9 and equivalent to the IC_{50} for DPP8.

Relative to maximum human exposure (~10μM*h), exposure in this study achieved 2x, 5x, and 20x MRHD at 10, 30, and 100 mg/kg.

Table C-1. MK-0431: Fourteen-Week Oral Toxicity Study in Monkeys. TT #06-1005

Mean Plasma MK-0431 Toxicokinetic Parameters - Drug Day 1

	MK-0431 (mg/kg/day)		
	Females		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	14.0 ± 1.10	45.4 ± 4.06	208 ± 19.8
C_{max} (μM)	3.29 ± 0.595	10.8 ± 0.999	37.6 ± 5.28
T_{max} (hr)	1.2 ± 0.44	2.0 ± 0	2.0 ± 0
	Males		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	12.4 ± 1.54	45.2 ± 2.43	171 ± 17.1
C_{max} (μM)	3.76 ± 0.245	11.6 ± 1.71	37.6 ± 4.72
T_{max} (hr)	0.50 ± 0	1.0 ± 0	2.7 ± 0.33
	Sexes Combined		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	13.2 ± 0.919	45.3 ± 2.12	189 ± 14.3
C_{max} (μM)	3.52 ± 0.306	11.2 ± 0.904	37.6 ± 3.17
T_{max} (hr)	0.83 ± 0.25	1.5 ± 0.22	2.3 ± 0.21

Values are the mean ± SEM.

Mean Plasma MK-0431 Toxicokinetic Parameters - Drug Week 12

	MK-0431 (mg/kg/day)		
	Females		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	17.4 ± 1.05	49.2 ± 2.96	226 ± 15.3
C_{max} (μM)	3.05 ± 0.115	9.20 ± 1.43	39.4 ± 6.01
T_{max} (hr)	1.8 ± 0.73	1.3 ± 0.33	2.0 ± 0.58
	Males		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	15.0 ± 1.57	46.0 ± 0.820	246 ± 15.3
C_{max} (μM)	3.57 ± 0.307	13.6 ± 1.34	42.5 ± 1.31
T_{max} (hr)	1.0 ± 0	0.67 ± 0.17	2.0 ± 0.58
	Sexes Combined		
	10	30	100
$AUC_{0\text{-}24\ hr}$ (μM•hr)	16.2 ± 1.00	47.6 ± 1.55	236 ± 10.6
C_{max} (μM)	3.31 ± 0.188	11.4 ± 1.32	40.9 ± 2.84
T_{max} (hr)	1.4 ± 0.37	1.0 ± 0.22	2.0 ± 0.37

Values are the mean ± SEM.

Skin lesion assessment of L-000000826, a non-selective DPP4 inhibitor, in a 12-week oral toxicity study in monkeys
Study TT06-1025

Key study findings:

- Redness and swelling of limbs, facial area, abdomen, and genital area were observed at all doses of L-826 within 2-4 weeks of treatment.
- Death/early sacrifice done in 1/6, 4/6, and 2/6 monkeys at 50, 150, and 450mg/kg respectively, for poor physical condition.
- Two deaths (1 each at 50 and 450mg/kg) were due to focally extensive hemorrhages in the brain with necrosis of nervous tissue (strokes).
- Renal toxicity was present at all doses (↑ BUN, creatinine, urinary protein, microscopic tubule degeneration).

Reviewer Comments
Skin lesions in the Rhesus are milder than those seen in the Cynomolgus monkeys (internal data). This could be a difference in strain or a characteristic of L-826. Regardless, the signs and sites effected are the same in both strains. This data supports the hypothesis that skin lesions produced by other DPP4 inhibitors is due to off-target inhibition of DPP8/9 or other enzymes rather than DPP4.

Note that fatal brain hemorrhage in two monkeys is clearly drug-related in this study. It is reasonable that the vascular damage responsible for skin lesions was also responsible for the strokes.

DPP Selectivity of L-826 Test Article
Table 1 indicates that L-826 non-selectively inhibits DPPs 4, 8, and 9 compared to high DPP4 selectivity of sitagliptin.

Table 1

DPP selectivity of L-826 vs. sitgliptin			
	In vitro IC_{50}, μM		
	DPP4	DPP8	DPP9
L-826	0.43	1.2	1.2
Sitagliptin	0.015	48	> 100

Mortality and Skin Lesions in response to L-826
Seven deaths were reported: 1/6 at 50mg/kg, 4/6 at 150mg/kg, and 2/6 at 450mg/kg. Poor clinical condition prompted early sacrifices in 6/7 deaths; one monkey was found dead. Antecedent findings include swelling and purple discoloration of the genital and facial area, hunched appearance, decreased activity, unresponsiveness, and decreased skin turgor (dehydration).

One male at 450mg/kg and one female at 50mg/kg had focally extensive areas of hemorrhage in their brains, associated with extensive degeneration and necrosis of nervous tissue. The description suggests multi-focal large strokes.

Physical signs in surviving monkeys were apparent within 2-4 weeks of treatment and were similar in all dose groups. Redness and swelling was found in the facial area, abdomen, inguinal area, genital area, and fore and hind limbs. Swelling was also noted in the tail in some animals. One animal at 450mg/kg had scabs on the face and fore/hind limbs. Excessive scratching was noted at 450mg/kg.

Note that fulminant necrotizing ulcerative lesions, more typical with some DPP4 inhibitors in Cynomolgus monkeys, were not reported with L-826 in Rhesus monkeys. However, the redness and swelling observed is typically followed by lesion eruption, and the effected sites are consistent with studies using Cynomolgus monkeys (e.g., face, limbs, genital area). The results indicate that the skin 'lesions' are less severe in Rhesus than in Cynomolgus.

Toxicokinetics
L-826 inhibits DPP4, 8, and 9 activity with an in vitro IC_{50} of 0.5 to 1.2μM. Plasma drug concentrations ranged from 100 to 1000 μM at 50, 150, and 450mg/kg, indicating that activity of all three enzymes were largely inhibited. Substantial inhibition (≥ 95%) of DPP4 activity was confirmed in plasma samples from all dose groups.

Skin lesion assessment of L-000233357, a DPP8/9 selective inhibitor, in a 14-week oral toxicity study in monkeys
Study TT06-1056: Interim Report

This interim report describes findings in Rhesus monkeys administered daily nasogastric gavage doses of L-233357, a DPP8/9 selective inhibitor, after approximately 9 weeks of dosing.

There were no treatment-related physical signs after 4 weeks administration of 1, 3, or 10mg/kg. Thus, the high dose was increased to 30mg/kg in drug week 5. One male dosed 30mg/kg was sacrificed in drug week 7 (or 2 weeks at 30mg/kg) due to physical signs that included swelling in the abdominal, inguinal, and hind limb areas, decreased activity, and hunched appearance. A necropsy identified edema of the skin the inguinal and scrotal

regions. Other males administered 30mg/kg showed physical signs that included swelling in the abdominal, inguinal, and hind limb areas.

DPP selectivity of L-233357 vs. sitgliptin			
	In vitro IC_{50}, μM		
	DPP4	DPP8	DPP9
L-233357	30	0.038	0.055
Sitagliptin	0.015	48	> 100

Plasma concentration of L-233357 ranged from 1 to 15μM at 1 to 10mg/kg. This concentration should inhibit DPPs 8 and 9 but not DPP4. Exposure at 30mg/kg is not available, but DPP4 activity is inhibited ~65% two hours after dosing and is comparable to control at 4 hours after dosing. Thus, signs of vascular/skin toxicity are detected at 30mg/kg after ≥ 2 weeks of dosing.

The data suggest that inhibiting several DPP enzymes, including DPP4, may be required for producing vascular/skin toxicities. Other unidentified targets may also contribute. Of most value, the data persuasively indicates that inhibiting DPP4 alone is insufficient to cause this toxicity.

Dermal Toxicity of MK-0431

Dermal Sensitization in Mice: Study #04-5512
MK-0431 was topically applied to the ears of CBA/JHsd mice (5/group) at 1%, 10%, or 100% formulations in methyl ethyl ketone vehicle for 3 consecutive days. Tritiated thymidine was injected on test day 5 and cell proliferation was assessed in the draining auricular lymph nodes. The stimulation index from treatment groups was not different from the vehicle control. The positive control (hexylcinnamaldehyde in acetone:olive oil) significantly increased cell proliferation in the lymph node, validating the assay.

MK-0431 is not a dermal sensitizer under the conditions of this assay.

TABLE 4

STIMULATION INDEX (SI) DATA

GROUP	MATERIAL TESTED	n	GROUP MEAN DPM	SI
II	0% Vehicle Control	5	197.15	N/A
IV	1%	5	339.75	1.72
VI	10%	5	215.95	1.10
VIII	100%	5	238.95	1.21
X	25% Positive Control[a]	5	1962.55	5.05
XII	0% Positive Control Vehicle[a]	5	388.75	N/A

a Data were not included in the statistical analysis of the test substance groups.

There were no statistically significant increases in dpm data from vehicle control at $p < 0.01$.

Dermal Irritation in Rabbits: Studies #03-2591 and #04-5513

In two studies (exploratory and definitive), 500mg of MK-0431 was topically applied to the shaved back of 3 rabbits with using wetted occulusive dressing for 24 hours. Dermal irritation was assessed by Draize scoring up to 72 hours after the 24 hour exposure. Neither study observed skin irritation. Under the conditions of this assay, MK-0431 is not a dermal irritant.

Dermal Irritation in human ——— cultures in vitro: Study #05-5508

——— cultures are composed of a monolayer of human epithelial cells on agarose that form a functional stratum corneum. MK-0431 (25mg) was applied to the cultures for 15 minutes, allowed to recover for 42 hours, then viability was assessed by conversion of MTT (metabolic dye). MK-0431 did not decrease cell viability and was considered a non-irritant.

Ocular Toxicity of MK-0431

In vitro studies utilizing freshly isolated bovine corneas identified MK-0431 as a mild ocular irritant that increased corneal opacity and permeability (Table 1). MK-0431 was further tested in 3 rabbits by placing a 20% formulation (100mg MK-0431) into the left conjunctival sac. Findings included moderate conjunctival redness with discharge, chemosis, and slight corneal opacity. Slight conjunctival redness persisted in one rabbit for 8 days after treatment. All eyes were normal by day 15 post-dosing. Merck concludes that MK-0431 is a moderate ocular irritant in vivo.

Table 1
BCOP Results of the Test Article

Assay Date	IIVS Test Article Number	Sponsor's Designation	Conc. (w/v)	Exposure Time	Mean Opacity Value	Mean OD_{490} Value	*In Vitro* Score	pH
12/7/04	04AI40	L-000224715-006F029	20%	4 hours	2.9	0.127	4.8	DpH

DpH- Discolored pH Paper, the pH value could not be determined because the test article caused a color change on the pH paper that could not be identified on the pH scale.

MK-0431 Toxicity after Intravenous Administration in Rat and Dogs

Rats (Study #03-0560): MK-0431 (5 and 20mg/kg) was administered to male and female SD rats (15/group) via tail vein for 16 consecutive days. MK-0431 had no toxicological effect in this study; the NOAEL is greater than 20mg/kg intravenously (Table).

Table: 16 day intravenous MK-0431 in SD rats	
Mortality	None
Clinical Signs	None
Body weight/Food consumption	No effect
Ophthalamic exam	No effect
Hematology/Clinical Chemistry	No effect
Urinalysis	No effect
Gross Pathology	No findings
Histopathology	No findings

Dogs (Study #03-6140): MK-0431 (5 and 20mg/kg) was administered to male and female beagle dogs (4/group) intravenously for 14 consecutive days.

Toxicological findings were limited to drug-related physical signs. Redness of the ears and the whole body was observed in most dogs during the study. Skin redness in dogs occurs with other DPP4 inhibitors but was not observed with oral administration of MK-0431. Other findings at 20 mg/kg include swollen muzzle/eyelids in 2/4 females, decreased activity/recumbency in 2/4 females, and conjunctival redness/lacrimation in all males and females. The physical signs were not associated with changes in body weight, clinical chemistries, or gross/histological findings.

Table: 16 day intravenous MK-0431 in Dogs	
Mortality	None
Clinical Signs	20 mg/kg Dose *Ear and whole body redness*: dose-related incidence, all drug days *Swollen muzzle/eyelids*: Two females, drug days 5-14 *Decreased activity/recumbency:* Two females, drug days 1-2 *Conjunctiva redness:* All males/females, all drug days 5 mg/kg Dose *Ear redness*: 2/4 males, all females, drug days 2-14 *Conjunctiva redness:* 1 male/female, sporadic incidence
Body weight/Food consumption	No effect
Ophthalamic exam	No effect
ECG exam	No effect
Hematology/Clinical Chemistry	No effect
Urinalysis	No effect
Gross Pathology	No findings
Histopathology	No findings

MK-0431 + Metformin: Combination Toxicity Studies in Dogs:

Summary
A series of studies evaluated the potential toxicity of MK-0431 administered in combination with metformin to dogs. The combination of MK-0431 and high-dose metformin (50 mg/kg) in dogs may have resulted in more numerous and earlier deaths than observed with metformin alone (Study #TT 06-6000). The combination of MK-0431 and a lower dose of metformin (20 mg/kg) that better approximates human exposure resulted in no deaths and yielded no evidence of exacerbated toxicity (Study #TT 06-6017). Convincing evidence is provided that the deaths at 50 mg/kg is due to metformin toxicity and not to the combination (Study #TT 06-6018) . Nevertheless, there is a slight possibility of exacerbated toxicity in the setting of high exposure to metformin (≥ 400μM*h AUC) and clinical exposure to MK-0431 (≥ 10μM*h AUC).

MK-0431 + Metformin: 14 week oral toxicity study in dogs
50 mg/kg Metformin and 2, 10, 50 mg/kg MK-0431

Key study findings:

- Mortality occurred in females of all combination groups and the metformin-alone group. Deaths with the combination appear more numerous and occur earlier than with the metformin-alone group.
- Mortality occurred in females only; all males survived to termination.
- The metformin-alone death may be due to lactic acidosis, indicated by high plasma lactate and low bicarbonate.
- Earlier deaths with the combination are not adequately explained.
- Three deaths in the MD and HD combination groups showed vacuolation in the brain with one of the three also showing neuronal necrosis; degenerative changes in brain are reported in dogs administered metformin
- Exposure to MK-0431 is not altered by co-administration of metformin.
- Exposure to Metformin tends to be 50% higher in females co-administered MK-0431 than metformin alone. However, TK comes from a single female in the MD and HD groups, so only tentative conclusions can be drawn. There is no difference in males.

Reviewer Comments
Merck concludes that all deaths are related to metformin toxicity and not to exacerbated toxicity with the combination. The data in this study do not support that conclusion because earlier deaths with the combination are not adequately explained. To the contrary, the death of one female given metformin alone was associated with a 50% higher metformin AUC than the females given the combination, yet deaths were observed in all groups except control.

Nevertheless, it is feasible that the deaths are related to metformin toxicity for the following reasons:

1. Plasma lactate tended to be higher in surviving dogs and was clearly higher in 2 dogs in the HD combination group, though this may be related to morbidity.

2. The minimally toxic dose of metformin in dogs is 50mg/kg, with death in 50% to 100% of animals at ≥ 100mg/kg [redacted], NDA 20,357), indicating a sharp dose response curve.

3. Metformin exposure in females was higher in this study (≥ 500µg*h/ml) than reported in the metformin NDA (~300 µg*h/ml). Exposure in males was similar to females on day 1 but decreased by week 8.

Exacerbated toxicity of the MK-0431/Metformin combination cannot be excluded based on this data, but neither can it be confirmed.

Study no.: TT 06-6000
Volume #, and page #: eCTD
Conducting laboratory and location: MRL, Chibret, France
GLP compliance: French Ministry of Health GLP standard
QA report: yes (x) no ( )
Drug, lot #, and % purity:
MK-0431, lot L-000224715-010X029
Metformin, lot L-000282095-001L012, from [redacted], purity by HPLC (manufacturer's data)

Methods

Doses:	See table below
Species/source	Beagle dogs from [redacted]
Age:	40-42 weeks
Weight:	6.8-9.6 kg
Toxicokinetic groups	Blood collected after the first dose and in drug week 8
Recovery groups:	No recovery
Route, formulation, dose volume	Oral gavage of both drugs, MK-0431 first, then metformin MK-0431 in acidified water Metformin in 0.5% methylcellulose in water

	Females	Males
Control (vehicles)[a]	3	3
MK-0431 + Metformin		
0 + 50 mg/kg/day[b]	3	3
2 + 50 mg/kg/day[c]	3	3
10 + 50 mg/kg/day[c]	3	3
50 + 50 mg/kg/day[c]	3	3

[a] Control animals received 5 mL/kg of acidified deionized water followed by 5 mL/kg of 0.5% (w/v) methylcellulose in deionized water daily.
[b] Animals received 5 mL/kg of acidified deionized water followed by 5 mL/kg of Metformin dosing formulation daily.
[c] Animals received 5 mL/kg of MK-0431 dosing formulation followed by 5 mL/kg of Metformin dosing formulation daily.

Observation and Times:

Clinical Findings:	Daily observations
Body weights:	Pretest and then weekly
Food consumption:	Four times per week
Ophthalmoscopy	Pretest, drug weeks 6 and 12
EKG:	Pretest, drug weeks 6 and 12
Hematology:	Pretest, drug weeks 4, 9, and 12, fasted state
Clinical chemistry:	Pretest, drug weeks 4, 9, and 12 Lactate and bicarbonate done on week 6, 9, and 12 Some frozen samples from week 4 analyzed in week 6 for bicarbonate and serum electrolytes
Urinalysis:	Overnight urines collected in drug weeks 9 and 12
Gross pathology:	Complete necropsies on all scheduled/unscheduled deaths
Organ weights:	adrenals, brain, heart, ovaries, kidneys, thymus, liver, pituitary, prostate, spleen, testes, thyroid
Histopathology:	From control, metformin alone, and high dose MK-0431/metformin Also from found dead/early sacrifice animals
Adequate Battery:	yes (X), no ( )
Peer review:	yes ( ), no (X)

Results:

Mortality: Deaths occurred in all treated groups including metformin alone (Table 1). There was one death each in the metformin alone group and the low dose MK-0431/metformin group, and two deaths each in the mid- and high-dose MK-0431/metformin groups. Deaths in the combination groups occurred earlier (1-4 weeks) than the metformin alone group (week 6).

Note that only females were effected; mortality did not occur in males.

Females 05-0121 and 05-0143 showed severe physical signs, including lateral recumbency, limb paddling, rigidity, labored breathing and prostration.

Females 05-0133 and 05-0137 showed body weight loss, markedly reduced food intake, and anorexia.

Table 1: Mortality in female dogs administered MK-0431 and Metformin

Group (Dose in mg/kg/day)	Animal Number	Drug Week of Death	Found Dead or Early Sacrifice	Cause of Death/Reason Killed
Metformin (50)	05-0133F	6	ES	Body weight loss and persistent anorexia
MK-0431 + Metformin (2 + 50)	05-0137F	3	FD	Undetermined
MK-0431 + Metformin (10 + 50)	05-0121F	1	FD	Undetermined
	05-0111F	4	FD	Undetermined
MK-0431 + Metformin (50 + 50)	05-0125F	4	FD	Undetermined
	05-0143F	4	FD[a]	Undetermined

[a] Animal died prior to euthanasia for early sacrifice.
ES = Early Sacrificed.
F = Female.
FD = Found Dead.

Histological changes in early sacrifice females #05-0121, -0111, and -0143 included vacuolation of the brain in all three and neuronal necrosis in #05-0143. Note that two of these dogs are in the MD combo group and the one with vacuolation and neuronal necrosis is in the HD combo group. Hemorrhage was not reported in these individuals. No other early death or surviving dogs showed histological changes in brain. Metformin administration to dogs has been associated with degenerative changes in the brain, among other organs (ref. NDA 20,357).

Other histological changes found only in early sacrifice females include lymphoid depletion of Peyer's patch, lymph nodes, and thymus, and adrenal vacuolation (1f).

Merck concludes that the deaths are related to metformin alone and not to co-treatment with MK-0431, citing lactic acidosis as a likely mechanism. As evidence, the metformin-

alone death was associated with high plasma lactate and low bicarbonate prior to sacrifice in week 6 compared to the control group. Unfortunately, plasma lactate was not measured in 3 other combination-group deaths where frozen samples were available from week 4. Bicarbonate tended to be lower in those samples, but not substantially. Changes in lactate/bicarbonate in surviving animals is discussed under the clinical chemistry section.

The data indicate lactic acidosis as a possible cause of death in the metformin-alone group, but is not conclusive as to the cause of deaths in the combination groups.

Plasma Lactate and Bicarbonate from Unscheduled Deaths

	Dog number	Plasma Lactate (mg/dL)	Bicarbonate (mM)
CONTROL	*group average*	*9.9*	*23.9*
Metformin	05-0133F	90	12.1
LD MK-0431/metformin	05-0137F	nd	nd
MD MK-0431/metformin	05-0121F	nd	nd
	05-0111F	nd	20.1
HD MK-0431/metformin	05-0125F	nd	17.8
	05-0143F	nd	21.6

nd = sample not taken (05-0137 & -0121) or not analyzed (05-0111, -0125, & -0143)

Clinical signs:

Males and females in all treatment groups (excluding control) showed physical signs starting in drug weeks 1/2. Signs included salivation and occasional emesis shortly after dosing, and an increased incidence of unformed stools. There was no difference noted between the dose groups relative to these clinical signs.

In the high dose MK-0431/metformin group, ataxia and tremors were observed in 2 females and 1 male shortly after dosing but the signs subsided within 4-6 hours. The transient ataxia/tremors associated with dosing were observed from drug weeks 1 to 3. This CNS effect has been identified in several previous toxicity studies in beagle dogs administered 50 mg/kg.

Body weights:

Death of females #05-0133 and 05-0137 from the metformin-alone and LD MK-0431/metformin group was associated with a 1 to 2 kg loss in body weight by drug week 2 and 5, respectively, consistent with reduced food intake and anorexia in these individuals.

Treated females generally gained less weight than the control females except for the individual in the HD MK-0431/metformin group. Males administered the combination tended to gain less weight than the control of metformin-alone males, but the data is variable and not dose-dependent.

The data are not conclusive as to an effect on BW in surviving dogs, though the data in males suggests some effect with combination treatment.

Change in BW in surviving dogs

Study Time	Dose, mg/kg	Females BW gain (kg)	Males BW gain (kg)
13 weeks	Control	+0.6	0.5
	Metformin	+0.1 (n=2)	0.5
	LD MK-0431/metformin	-0.1 (n=2)	-0.3
	MD MK-0431/metformin	-0.3 (n=1)	0 (-0.8 to 0.9)
	HD MK-0431/metformin	+0.6 (n=1)	0.1

Food consumption: Aside from reduced food consumption in the moribund females described above, there was no difference in food intake between the treated and control groups.

Ophthalmoscopy: There were no treatment-related changes reported.

EKG: There were no treatment-related changes reported. Note that primary data was not submitted.

Hematology: There were no treatment-related changes relative to RBC, WBC, and coagulation variables.

Clinical chemistry: There was no substantial change in the standard clinical chemistry panel in the treated groups relative to control or baseline values. Plasma bicarbonate, lactate, and glucose require specific attention, however.

Plasma Bicarbonate: Surviving males and females in all groups had plasma bicarbonate levels between 22-27 mM on drug weeks 4, 6, 9, and 12. Treated and control groups were no different.

Plasma Glucose: Treated and control groups were no different at drug weeks 4, 9, and 12. This appears to rule out hypoglycemia as a contributing factor.

Plasma Lactate: There is a tendency toward higher plasma lactate in all female groups administered metformin and in males in the MD and HD MK-0431/metformin groups. The data in males suggests higher plasma lactate in response to co-administration of MK-0431 and metformin, but is not conclusive.

The historical range for plasma lactate is 28.8 to 33.4 mg/dL. One male and one female, both in the HD MK-0431/metformin group slightly exceeded the high range on at least one occasion.

Table: Plasma Lactate in Surviving Females

TREATMENT GROUP & ANIMAL NUMBER	DRUG WEEK 6	9	12
Control			
05-0127F	6.5	9.7	7.5
05-0141F	6.5	6.2	6.5
05-0147F	7.7	8.5	15.6
MEAN	6.9	8.1	9.9
STD DEV	0.7	1.8	5.0
Metformin 50 mg/kg/day			
05-0113F	17.3	16.0	14.5
05-0145F	18.8	16.2	11.0
MK-0431 2mg/kg/day +Metformin			
05-0131F	16.9	24.1	19.1
05-0151F	13.6	21.2	18.0
MK-0431 10mg/kg/day +Metformin			
05-0123F	9.8	9.4	11.0
MK-0431 50mg/kg/day +Metformin			
05-0135F	20.8	34.9	23.9

Plasma Lactate in Male Dogs (all survived, n=3/group)			
	Week 6	Week 9	Week 12
CONTROL	8.1	*10.2*	*8.3*
Metformin	7.9	11.6	10.1
LD MK-0431/metformin	7.9	11.2	9.7
MD MK-0431/metformin	9.1	16.8	13.2
HD MK-0431/metformin*	9.1	24.1*	20.2*

*Plasma lactate in dog #05-0138 was 37 and 31 mg/dL at weeks 9, 12 respectively, skewing the mean upward.

Historical Control Values for Dogs, Plasma Lactate and Bicarbonate

Historical Control Values for Dog (06-Feb-2006 to 20-Feb-2006)

Age	No. of Tests	No. of Animals	Median Value	Range of Actual Values		95% Spread 2.5%	97.5%
Bicarbonate mmol/L							
Female							
26 to 39 Weeks	73	73	23.4	18.7	26.6	19.5	26.4
Combined Ages	73	73	23.4	18.7	26.6	19.5	26.4
Male							
26 to 39 Weeks	74	74	24.0	20.7	27.7	20.8	27.2
Combined Ages	74	74	24.0	20.7	27.7	20.8	27.2
Both Sexes							
26 to 39 Weeks	147	147	23.8	18.7	27.7	20.0	26.9
Combined Ages	147	147	23.8	18.7	27.7	20.0	26.9
Lactate mg/dL							
Female							
26 to 39 Weeks	53	53	12.1	5.4	28.8	5.5	28.7
Combined Ages	53	53	12.1	5.4	28.8	5.5	28.7
Male							
26 to 39 Weeks	54	54	13.0	4.5	33.4	4.8	33.2
Combined Ages	54	54	13.0	4.5	33.4	4.8	33.2
Both Sexes							
26 to 39 Weeks	107	107	12.7	4.5	33.4	5.4	32.1
Combined Ages	107	107	12.7	4.5	33.4	5.4	33.1

Urinalysis: Treated and control groups showed no difference in urinary volume, specific gravity, urobilinogen, glucose, protein, blood, ketones, or sediments.

Gross pathology: Treated and control groups showed no difference. (scheduled sacrifices)

Organ weights: Treated and control groups showed no difference. (scheduled sacrifices)

Histopathology:

Histological changes were present only in early sacrifice females, and are described under the 'Mortality' section above.

To summarize, females #05-0121 and -0111 had brain vacuolation and female 05-0143 showed brain vacuolation and neuronal necrosis. Although these dogs are in the MD and HD combo groups, such changes in brain have been previously described in dogs following administration of metformin (ref. NDA 20,357).

Other histological changes found only in early sacrifice females include lymphoid depletion of Peyer's patch, lymph nodes, and thymus, and adrenal vacuolation (1f).

Toxicokinetics:

MK-0431: Exposure as AUC at all dose groups is consistent with TK from prior studies. There is no sex difference, and exposure slightly decreases by drug week 8. Co-administration of metformin did not alter TK of MK-0431.

Metformin: Exposure as AUC was ~50% higher in females given metformin alone compared to females given the combination, which may suggest some PK interaction in females. This was not seen in males. In addition, exposure in females tends to be higher than in males by drug week 8, though not substantially. Note that TK comes from a single female in the MD and HD groups, so only tentative conclusions can be drawn.

Exposure to MK-0431

Mean Plasma MK-0431 Toxicokinetic Parameters – Drug Day 1

	MK-0431 + Metformin (mg/kg/day)		
	Females		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	11.0 ± 1.61	59.8 ± 9.65	315 ± 37.4
C_{max} (µM)	2.24 ± 0.391	11.3 ± 1.02	66.6 ± 9.56
T_{max} (hr)	1.0 ± 0.0	0.8 ± 0.2	0.5 ± 0.0
	Males		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	12.2 ± 0.593	71.0 ± 4.35	368 ± 30.9
C_{max} (µM)	2.36 ± 0.130	11.6 ± 0.578	58.7 ± 0.623
T_{max} (hr)	1.0 ± 0.0	0.8 ± 0.2	0.7 ± 0.2
	Sexes Combined		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	11.6 ± 0.815	65.4 ± 5.35	342 ± 24.8
C_{max} (µM)	2.30 ± 0.186	11.4 ± 0.529	62.7 ± 4.63
T_{max} (hr)	1.0 ± 0.0	0.8 ± 0.1	0.6 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Mean Plasma MK-0431 Toxicokinetic Parameters – Drug Week 8

	MK-0431 + Metformin (mg/kg/day)		
	Females		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	9.12 ± ID	56.3* ± ID	252* ± ID
C_{max} (µM)	1.47 ± ID	7.08* ± ID	35.8* ± ID
T_{max} (hr)	0.8 ± ID	2.0* ± ID	1.0* ± ID
	Males		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	7.84 ± 2.20	42.9 ± 16.5	313 ± 29.1
C_{max} (µM)	1.24 ± 0.358	7.54 ± 3.30	46.5 ± 4.33
T_{max} (hr)	1.0 ± 0.0	0.8 ± 0.2	0.8 ± 0.2
	Sexes Combined		
	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	8.35 ± 1.40	46.2 ± 12.2	298 ± 25.6
C_{max} (µM)	1.33 ± 0.254	7.43 ± 2.34	43.9 ± 4.07
T_{max} (hr)	0.9 ± 0.1	1.1 ± 0.3	0.9 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
* The data reported is from 1 animal (n=1).
ID = Insufficient data available for calculation.
Values are the mean ± SEM.

Exposure to Metformin

Mean Plasma L-000282095 (Metformin) Toxicokinetic Parameters – Drug Day 1

	MK-0431 + Metformin (mg/kg/day)			
	Females			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	755 ± 72.6	519 ± 57.1	592 ± 59.3	528 ± 63.6
C_{max} (µM)	205 ± 13.0	159 ± 23.3	154 ± 13.2	134 ± 10.5
T_{max} (hr)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.2
	Males			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	445 ± 75.8	558 ± 39.4	585 ± 34.4	541 ± 86.7
C_{max} (µM)	112 ± 33.6	182 ± 17.6	190 ± 15.5	164 ± 18.8
T_{max} (hr)	1.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.2
	Sexes Combined			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	600 ± 83.7	539 ± 32.2	588 ± 30.7	534 ± 48.2
C_{max} (µM)	158 ± 26.4	171 ± 14.8	172 ± 12.1	149 ± 11.8
T_{max} (hr)	1.2 ± 0.2	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Mean Plasma L-000282095 (Metformin) Toxicokinetic Parameters – Drug Week 8

	MK-0431 + Metformin (mg/kg/day)			
	Females			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	807 ± ID	517 ± ID	503* ± ID	330* ± ID
C_{max} (µM)	131 ± ID	136 ± ID	65.7* ± ID	49.3* ± ID
T_{max} (hr)	1.5 ± ID	1.0 ± ID	3.0* ± ID	2.0* ± ID
	Males			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	432 ± 96.3	363 ± 121	358 ± 132	469 ± 65.7
C_{max} (µM)	124 ± 45.2	91.9 ± 38.9	80.7 ± 28.9	99.9 ± 18.2
T_{max} (hr)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.0 ± 0.0
	Sexes Combined			
	0[a] + 50[b]	2[a] + 50[b]	10[a] + 50[b]	50[a] + 50[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	582 ± 165	424 ± 81.0	394 ± 100	435 ± 58.0
C_{max} (µM)	131 ± 37.0	109 ± 27.7	76.9 ± 20.8	87.3 ± 18.1
T_{max} (hr)	1.4 ± 0.2	1.2 ± 0.2	1.8 ± 0.5	1.3 ± 0.3

[a] Dose of MK-0431.
[b] Dose of Metformin.
* The data reported is from 1 animal (n=1).
ID = Insufficient data available for calculation.
Values are the mean ± SEM.

Exploratory 5-week oral tolerability study with Metformin in female dogs
Control and 50mg/kg

Key study findings:

- One female was found dead on day 16 following body weight loss and reduced food intake.
- One female was sacrificed on day 23 for physical signs; plasma lactate was markedly elevated and bicarbonate reduced just prior to sacrifice, likely an effect of morbidity than true drug-related lactic acidosis.
- The timing of deaths is similar to deaths in response to the combination with MK-0431 in study #TT 06-6000.
- Mean plasma lactate increased ≤ 2 fold in metformin-treated females (high end of historical range), but bicarbonate was unchanged.

Reviewer Comments:
This study shows that 50mg/kg metformin is not well-tolerated in dogs, with 2 of 5 females dying by drug day 23. Merck suggests that metformin-induced lactic acidosis underlies morbidity/mortality in the dogs, but the reviewer concludes that morbidity in the dogs underlies elevations in plasma lactate. The NDA —— (Glucophage) review states that animals (rodent) studies have provided conflicting results relative to lactate production, and that death of dogs administered metformin are associated with symptoms of GI distress, vascular lesions, and degenerative changes in the brain, heart, kidney, and skeletal muscle (NDA ——, which are reasonable alternative explanations for the deaths.

The mechanism of metformin-related death is less important than the occurrence of death; a 50mg/kg dose with an exposure of ≥ 400 μM*h appears too high for combination toxicity studies in dogs.

The reviewer concludes that the deaths of female dogs given the combination of MK-0431 and metformin may be due to metformin toxicity rather than exacerbated toxicity of the combination.

Study no.: TT-06-6018
Volume #, and page #:
Conducting laboratory and location: MRL, Chibret, France
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity:
Metformin, lot L-000282095-001L012, from [redacted]

Methods

Doses:	Control and 50 mg/kg groups
Species/source	Beagle Dogs from [redacted]
Age:	34-38 weeks
Weight:	5.5-9.6 kg
Number/sex/group: (main study)	5 females
Toxicokinetic groups	After first dose and drug week 3
Route, formulation, dose volume	Oral gavage of drug in 0.5% methylcellulose/water

Results:

Mortality: Two females died during the study. One female (#05-0277) was found dead before dosing on drug day 16, and the other (#06-0047) was sacrificed after dosing on drug day 23 due to marked physical signs.

Female 05-0277: This female lost 1.2 kg body weight and food intake decreased markedly before death. Plasma lactate was elevated, but not substantially.

Female 06-0047: This female had labored breathing and lateral recumbency prior to sacrifice. Plasma lactate was markedly increased (177 mg/dL), bicarbonate decreased (13mM), and glucose, potassium, and chloride decreased prior to its sacrifice on day 23. These findings may be secondary to morbidity rather than directly drug-related.

Clinical signs: Surviving females showed emesis and peri-dose salivation. The incidence of emesis was greatest during week 1.

Body weights: Female 05-0277 lost 1.2 kg BW (above). No other female showed a change in BW relative to pre-test values.

Food consumption: Female 06-0047 showed reduced food intake in drug weeks 1 and 2 prior to death. No other female showed a change in food intake.

Clinical chemistry:

Blood samples were collected 24 hours post-dose (trough drug levels) on all days except day 17, where samples were taken at 1 hour post-dose (near-peak drug levels).

Blood was also drawn immediately before sacrifice of female #06-0047 of the metformin group prior to its sacrifice on day 23.

There were no changes in the standard clinical chemistry panel. Plasma lactate and bicarbonate require detailed evaluation:

Plasma Bicarbonate: No change is seen in control or metformin groups up to drug day 29.

Plasma Lactate: Plasma lactate in metformin-treated females tended to be higher relative to pre-test values and to the control group, particularly during the first 2-3 weeks of dosing. The magnitude of the increase is ~2-fold compared to pre-test values and is in the high end of the historical range. Plasma lactate did not exceed ~3.5mM; clinical lactic acidosis is defined by a plasma lactate concentration of 5mM.

Plasma lactate reportedly increased to 177 mg/dL (20mM) in female #06-0047 (metformin group) and bicarbonate reportedly decreased (13mM) prior to sacrifice on day 23. These values, which are not obvious in the Table below, came from a blood sample taken just prior to sacrifice of the dog. This suggests that large acute changes in plasma lactate are associated with the moribund condition rather than directly related to drug treatment.

Plasma Bicarbonate

TREATMENT GROUP & ANIMAL NUMBER	PRETEST PERIOD	DRUG DAY 8	15	17	22	29
Control						
05-0229F	22.2	24.9	24.3	21.8	22.1	23.3
06-0013F	23.2	24.0	25.3	26.1	25.6	24.0
06-0043F	22.3	23.2	23.8	22.7	23.2	22.5
06-0045F	25.1	23.7	25.9	28.0	24.1	24.4
06-0049F	22.2	23.5	23.8	22.9	21.8	23.6
MEAN	23.0	23.9	24.6	23.3	23.0	23.6
STD DEV	1.2	0.7	0.9	2.6	0.9	0.7
50 mg/kg/day						
05-0241F	21.3	19.4	21.1	20.1	20.7	22.3
05-0265F	22.8	24.3	24.0	24.2	25.2	24.6
05-0277F		23.0	27.6			
06-0011F	22.5	25.0	27.5	27.8	26.4	24.9
06-0047F	21.1	24.0	21.1	25.4	22.6	
MEAN	21.9	23.1	24.7	24.4	23.7	23.9
STD DEV	0.9	2.2	2.8	3.2	2.6	1.4

Plasma Lactate

TREATMENT GROUP & ANIMAL NUMBER	PRETEST PERIOD	DRUG DAY 0	15	17	23	29
Control						
05-0229F	12.9	9.2	8.7	11.7	17.4	28.2
06-0013F	12.0	6.4	9.6	4.6	10.0	8.8
06-0043F	12.0	8.7	7.5	6.0	13.2	9.3
06-0045F	11.8	12.4	13.1	6.4	12.6	18.4
06-0049F	8.4	12.5	11.6	6.3	23.4	9.0
MEAN	11.4	9.8	10.1	7.0	15.4	14.7
STD DEV	1.7	2.6	2.2	2.7	5.2	8.5
50 mg/kg/day						
05-0241F	7.4	25.8	17.1	10.7	30.9	12.2
05-0263F	6.6	11.7	15.5	22.6	14.9	17.2
05-0277F		28.0	12.0			
06-0011F	17.9	14.2	14.6	7.8	18.8	19.6
06-0047F	10.9	15.2	13.0	10.2	15.8	
MEAN	10.7	19.0	15.2	14.8	20.1	16.3
STD DEV	5.2	7.4	1.5	6.8	7.4	3.8

Historical Control Data for Female Dogs: Lactate and Bicarbonate

Historical Control Values for Dog (06-Feb-2006 to 20-Feb-2006)

Age	No. of Tests	No. of Animals	Median Value	Range of Actual Values	95% Spread 2.5%	97.5%
Bicarbonate mmol/L						
Female						
26 to 39 Weeks	74	74	23.4	18.7 - 26.6	19.5	26.4
Lactate mg/dL						
Female						
26 to 39 Weeks	54	54	12.2	5.4 - 28.8	5.5	20.7

Gross pathology: There were no gross changes in the early sacrifice/found dead animals.

Histopathology: No histopathology was performed.

Toxicokinetics: Exposure was somewhat lower than in the combination toxicity study (402 vs. ≥ 500 µM*h), but still somewhat higher than reported in NDA 20,357 (300 µM*h).

Mean Plasma Metformin Toxicokinetic Parameters - Drug Week 3

	Metformin (mg/kg/day)
	Females
	50
$AUC_{0\text{-}24\ hr}$ (µM•hr)	402 ± 77.2
C_{max} (µM)	119 ± 36.2
T_{max} (hr)	0.88 ± 0.13
Values are the mean ± SEM.	

MK-0431 + Metformin: 16 week oral toxicity in female dogs
Control, 20 mg/kg metformin alone or with MK-0431 at 2, 10, 50 mg/kg

Key study findings:

- The dose of metformin was lowered to 20 mg/kg based on lethality observed in prior studies at 50 mg/kg.
- There were no findings in dogs administered MK-0431 + metformin that substantially differed from dogs administered metformin alone or the control vehicles.

Reviewer Comments:
Dogs tolerated the 20mg/kg dose of metformin, evidenced by no reduction in body weight or food intake, and no resultant deaths. Exposure at 20mg/kg metformin in dogs is similar to exposure at 2000 mg metformin in humans, which is a more appropriate design for combination toxicity studies with an unapproved entity (MK-0431).

The lack of any difference between the control, metformin-alone, and combination treatment groups suggests that the combination of MK-0431 and metformin does not exacerbate existing toxicities (e.g., tremors/ataxia at 50mg/kg MK-0431) or produce new toxicities not seen with either drug alone. This study provides further evidence that deaths observed in study #TT 06-6000 were due to metformin toxicity at 50 mg/kg, and not to co-administration with MK-0431. There is a slight possibility that the earlier deaths with MK-0431 + metformin in study #TT 06-6000 express exacerbated toxicity.

Study no.: TT 06-6017
Volume #, and page #: eCTD
Conducting laboratory and location: MRL, Chibret, France
Date of study initiation:
GLP compliance: Compliant with French Ministry of Health GLP regulations
QA report: yes (X) no ( )
Drug, lot #, and % purity:
MK-0431: lot L-000224715-010X029
Metformin: lot L-000282095-001L012, [redacted]

Methods

Doses:	<table><tr><th></th><th>Females</th></tr><tr><td>Control (vehicles)[a]</td><td>5</td></tr><tr><td>MK-0431 + Metformin</td><td></td></tr><tr><td>0 + 20 mg/kg/day[b]</td><td>5</td></tr><tr><td>2 + 20 mg/kg/day[c]</td><td>5</td></tr><tr><td>10 + 20 mg/kg/day[c]</td><td>5</td></tr><tr><td>50 + 20 mg/kg/day[c]</td><td>5</td></tr></table> NOTE: Dogs were administered metformin alone for the first 2 weeks to assess tolerability. Combination treatment commenced on week 3
Species/source	Beagle Dogs, female, from [redacted]
Age:	32-35 weeks
Weight:	5.9-8.8kg
Number/sex/group: (main study)	5 females per group
Toxicokinetic groups	Drug day 1 (metformin alone) and drug weeks 3 and 15 (combination)
Recovery groups:	None
Route, formulation, dose volume	Oral gavage of MK-0431, then metformin MK-0431 in 0.1mM HCl in water Metformin in 0.5% methylcellulose in water

Observation and Times:

Clinical Findings:	Daily
Body weights:	Pretest and then weekly
Food consumption:	2-4 times weekly
Ophthalmoscopy	Pretest, drug weeks 8, 14
EKG:	Pretest, drug weeks 8, 14
Hematology:	Blood collected from fasted dogs: Pretest, drug weeks 6, 10, 14 Standard panel
Clinical chemistry:	Blood collected from fasted dogs: Pretest, drug weeks 2, 6, 10, 14 Week 2 analysis limited to serum bicarbonate, sodium, potassium, chloride, and lactate
Urinalysis:	Overnight urines collected in drug weeks 10, 14
Gross pathology:	Necropsies on all animals
Organ weights:	adrenals, brain, heart, liver, pituitary, spleen, ovaries, kidneys, thymus, thyroid
Histopathology:	Control, metformin alone, and high dose combination evaluated
Adequate Battery:	yes (X), no ( )

Results:

Mortality: None

Clinical signs:

Ataxia, tremors, or both occurred in all dogs in the HD combination group starting in drug week 3 (first week of combination treatment). The signs occurred within 30 minutes of dosing and lasted about 3 hours post-dose. Transient, dose-related ataxia/tremor has been documented in dogs administered 50 mg/kg MK-0431 in standard toxicology studies. The Sponsor states that such signs were not exacerbated by metformin compared to historical experience with MK-0431 alone, but note that a concurrent 50mg/kg MK-0431 group was not employed.

Body weights: No change in BW was observed.

Food consumption: No change in food intake was observed.

Ophthalmoscopy: No findings observed. (primary data not shown)

EKG: No changes observed. (primary data not shown)

Hematology: No changes observed. (Red cell mass, coagulation, white cell differential)

Clinical chemistry: No changes observed. (Liver and kidney markers; serum proteins)

Serum bicarbonate: No changes; values ranged from 23-25 mg/dL for all groups.

Serum lactate: No changes; values ranged from 5-21 mg/dL for all groups without a dose- or time-dependence.

Urinalysis: No changes observed. (volume, pH, specific gravity, protein, bilirubin, glucose, blood, sediments)

Gross pathology: No changes observed.

Organ weights: No changes observed. (see *Methods* for organ list)

Histopathology:
Control, metformin-alone, and the HD combination groups were evaluated. No treatment-related change was observed.

Findings considered not treatment-related:

- Minimal focal adhesion in the heart of 1 HD combination female.

Toxicokinetics:

Metformin exposure: Exposure to metformin was similar in all groups on day 1 and week 5, but tended to increase in the combination groups by week 15 (~200 µM*h in control vs. 287 µM*h in HD combo group). Metformin PK does not appear to change substantially with co-administration of MK-0431, despite the small increase in AUC.

Note that metformin exposure in dogs administered 20 mg/kg (~200µM*h) is similar to humans administered 2000 mg (160 µM*h).

Mean Plasma L-000282095 (Metformin) Toxicokinetic Parameters – Drug Day 1

	MK-0431 + Metformin (mg/kg/day)			
	Females			
	0[a] + 20[b]	2[a] + 20[b]	10[a] + 20[b]	50[a] + 20[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	182 ± 16.4	196 ± 7.48	192 ± 11.0	212 ± 18.4
C_{max} (µM)	43.9 ± 8.47	53.3 ± 3.10	45.1 ± 2.78	58.1 ± 5.53
T_{max} (hr)	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	0.9 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Mean Plasma L-000282095 (Metformin) Toxicokinetic Parameters – Drug Week 3

	MK-0431 + Metformin (mg/kg/day)			
	Females			
	0[a] + 20[b]	2[a] + 20[b]	10[a] + 20[b]	50[a] + 20[b]
$AUC_{0\text{-}24\ hr}$ (µM•hr)	187 ± 24.8	201 ± 10.2	191 ± 11.3	220 ± 10.9
C_{max} (µM)	39.6 ± 7.57	45.7 ± 6.03	44.5 ± 4.64	51.9 ± 6.30
T_{max} (hr)	1.1 ± 0.2	1.2 ± 0.5	1.1 ± 0.2	1.3 ± 0.4

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Mean Plasma L-000282095 (Metformin) Toxicokinetic Parameters – Drug Week 15

	MK-0431 + Metformin (mg/kg/day)			
	Females			
	0[a] + 20[b]	2[a] + 20[b]	10[a] + 20[b]	50[a] + 20[b]
$AUC_{0\text{-}24\ hr}$ (μM•hr)	191 ± 23.8	224 ± 19.3	239 ± 13.1	287 ± 7.77
C_{max} (μM)	44.9 ± 9.05	50.7 ± 6.21	67.7 ± 9.00	73.8 ± 3.58
T_{max} (hr)	1.3 ± 0.3	1.3 ± 0.3	1.1 ± 0.2	1.1 ± 0.2

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

MK-0431 Exposure: Exposure to MK-0431 increased with dose in a near-linear manner. AUC did not change substantially from the first to last week of administration, and did not change with co-administration of metformin.

Mean Plasma MK-0431 Toxicokinetic Parameters – Drug Week 3

	MK-0431 + Metformin (mg/kg/day)		
	Females		
	2[a] + 20[b]	10[a] + 20[b]	50[a] + 20[b]
$AUC_{0\text{-}24\ hr}$ (μM•hr)	10.2 ± 0.368	50.7 ± 1.78	307 ± 18.9
C_{max} (μM)	1.64 ± 0.159	9.62 ± 0.729	56.6 ± 3.86
T_{max} (hr)	1.2 ± 0.5	0.6 ± 0.1	0.7 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Mean Plasma MK-0431 Toxicokinetic Parameters – Drug Week 15

	MK-0431 + Metformin (mg/kg/day)		
	Females		
	2[a] + 20[b]	10[a] + 20[b]	50[a] + 20[b]
$AUC_{0\text{-}24\ hr}$ (μM•hr)	10.4 ± 0.404	52.9 ± 1.56	310 ± 12.1
C_{max} (μM)	1.80 ± 0.155	11.1 ± 0.609	58.1 ± 2.65
T_{max} (hr)	0.9 ± 0.3	0.7 ± 0.1	0.6 ± 0.1

[a] Dose of MK-0431.
[b] Dose of Metformin.
Values are the mean ± SEM.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

None.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Todd Bourcier
8/31/2006 11:02:47 AM
PHARMACOLOGIST

Karen Davis-Bruno
8/31/2006 11:26:40 AM
PHARMACOLOGIST

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA #21-995
Original Submission
Januvia (sitagliptin phosphate)
Merck Research Laboratories
Date Received: 16 Dec 2005

NDA #:	21-995
Submission date:	16 December 2005
Drug:	Januvia (sitagliptin phosphate; MK-0431)
Dosage form:	Tablets 25, 50, 100mg
Indication:	Type 2 Diabetes
IND#:	65,495

A complete Pharm/Tox development program, including pharmacology and pharmacokinetic studies, single and repeat dose toxicity, carcinogenicity, reprotoxicity and genotoxicity studies, has been carried out with sitagliptin. Study species included mice, rats, rabbits, and dogs. Phase 3 clinical trials evaluated doses of 100 and 200mg QD as monotherapy (18 and 24 months) and in combination with metformin or pioglitazone. The recommended human dose is 100 mg/day which produces a Cmax of 950nM (trough, 100nM) and $AUC_{0\text{-}24h}$ of 8.5 μg*h/mL. The 50mg and 25mg doses are recommended for patients with moderate and severe renal insufficiency, respectively. The NDA was submitted in electronic format and is well organized.

The 3 month monkey study required for all DPP4 inhibitors is ongoing.

It is suggested that the draft label conform to the Final Rule formatting for new drug labels.

Pharmacology/Toxicology supports the filing of NDA 21-995.

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		eCTD format Summary report Tabulated Summaries Study reports
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		eCTD format
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		eCTD
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission, communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA (genotoxicity, reprotoxicity, chronic toxicity of adequate duration, carcinogenicity)	X		All necessary studies have been submitted. • In vivo metabolites, humans/tox species • 6 month rat, 12 month dog toxicology • Genotoxicity • 2 yr Carcinogenicity • Reprotoxicity: Segments I, II, III 3 month monkey study is ongoing.
Have electronic files of the carcinogenicity studies been submitted for statistical review?	X		

5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		

6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		Yes, but conforms to the old template. It would be in Merck's interest to submit labeling in the new template now rather than later. Dose multiples provided for carci/mutagenesis and reprotox sections. Other Animal Tox not reported. Monkey Tox results not ready yet; no class effect statement provided.
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file: N/A			

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/s/

Todd Bourcier
2/8/2006 05:35:10 PM
PHARMACOLOGIST

Jeri El Hage
2/10/2006 10:43:16 AM
PHARMACOLOGIST