

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
NDA 22-090

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Eovist Injection (gadoxetate disodium)

Date: May 19, 2008

To: File for NDA #22-090

From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and pharmacology/toxicology supporting reviews and memoranda provided by Drs. Ouyang and Laniyonu and concur with their conclusions that Eovist may be approved. No additional pharmacology/toxicology studies are necessary.

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/s/

John Leighton
5/19/2008 10:09:33 AM
PHARMACOLOGIST

Supervisory Pharmacologist Memo

NDA: 22-090
Product: Gadoxetate disodium (EOVIST)
Sponsor: Bayer Healthcare Pharmaceutical, Inc.

Gadoxetate disodium (Gd-EOB DTPA; EOVIST) is a disodium salt of a gadolinium (III) complex designated chemically as (4S)-4-(4-Ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9-triazaundecanedioic acid proposed for Magnetic Resonance Imaging (MRI) of the liver to detect and characterize lesions in adults with known or suspected focal liver disease. Because hepatic tumor generally loses the ability to take up gadoxetate disodium, a contrast is achieved between normal and tumor tissues. The proposed dose is 0.025 mmol/kg in adults.

Dr. Yanli Ouyang reviewed the preclinical Pharmacology and Toxicology section of NDA 22-090. This secondary review was based on Dr. Ouyang's review; please see Dr. Ouyang's review for details.

Cardiovascular (including QT_c, hERG potassium channels, and cardiac action potential assessments), and CNS safety evaluations were considered adequate. At clinical intended dose, there was a weak evidence for gadoxetate disodium to prolong ventricular repolarization

Gadoxetate disodium is not metabolized in rats and dogs. Pharmacokinetics studies in these species showed that its elimination from blood was nonlinear, dose dependent and rapid through both renal and hepatic routes with full compensation by remaining route when one route is blocked. Human plasma protein binding was less than 10%. Less than 0.5% of totally administered [¹⁵³Gd] gadoxetate sodium was transferred to the neonates via maternal milk.

Definitive toxicology (single and repeat-dose) studies were conducted in rats and dogs. Dr. Ouyang identified a number of deficiencies in the single dose studies that made study interpretation difficult. However, the 4 week repeat dose toxicity studies were considered adequate, and compensated for the identified deficiencies in the single dose studies. For both rats and dogs studies, the most significant findings was dose-related reversible tubular cell vacuolation of the kidneys (NOAEL; rats, 1.3 MHD; dogs, 2.2 MHD; mmol/m² basis). This is a common finding among Gd-containing contrast agents. In view of recent association of human nephrogenic systemic fibrosis (NSF) with Gd-containing contrast agents, the biological significance of the kidney tubular vacuolation is becoming of increasing importance especially in patients with severe renal failures. Intramuscular injection of gadoxetate disodium in rabbits resulted in interstitial hemorrhage, edema, and focal muscle fiber necrosis. The lesions resolved by day 7.

A full battery of genetic toxicology studies was conducted. Gadoxetate disodium was not mutagenic or clastogenic in these studies.

Reproductive toxicology studies were conducted in rats and rabbits. Gadoxetate disodium was not teratogenic when given intravenously during organogenesis to pregnant rats at doses up to 32 times the human dose (mmol/m² basis), however, an increase in pre implantation loss was noted at doses up to 3.2 times the human dose (mmol/m² basis). Compared to untreated controls, rates of post implantation loss and absorption increased and litter size decreased when pregnant rabbits received Gadoxetate disodium at doses 26 times the recommended human single dose. This occurred without evidence of maternal toxicity.

Dr. Ouyang concluded that the preclinical package of Eovist was complete, and that the studies conducted support the safety and efficacy of Eovist from preclinical pharmacology/toxicology perspectives. She recommends approval of the NDA and suggested changes in the label that would more appropriately reflect findings from preclinical studies.

I concur with Dr. Ouyang's recommendations.

Adebayo Laniyonu, Ph.D.

Supervisory Pharmacologist

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/s/

Adebayo Lanionu
5/17/2008 09:33:03 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: 22-090
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 30 June 2007
PRODUCT: Gd-EOB-DTPA, Gadoxetate disodium
INTENDED CLINICAL POPULATION: Adult patients with known or suspected focal liver disease
SPONSOR: Bayer Healthcare Pharmaceuticals, Inc.
DOCUMENTS REVIEWED: Module 4
REVIEW DIVISION: Division of Medical Imaging and Hematology Products (DMIHP)
PHARM/TOX REVIEWER: Yanli Ouyang, MD, PhD, DABT
PHARM/TOX SUPERVISOR: Adebayo Lanionu, Ph.D.
DIVISION DIRECTOR: Rafel 'Dwayne' Rieves, MD
PROJECT MANAGER: Tiffany Brown, MPH, James Moore, Pharm.
D.

Date of review submission to Division File System (DFS): May 8, 2008

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Approval
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling:

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

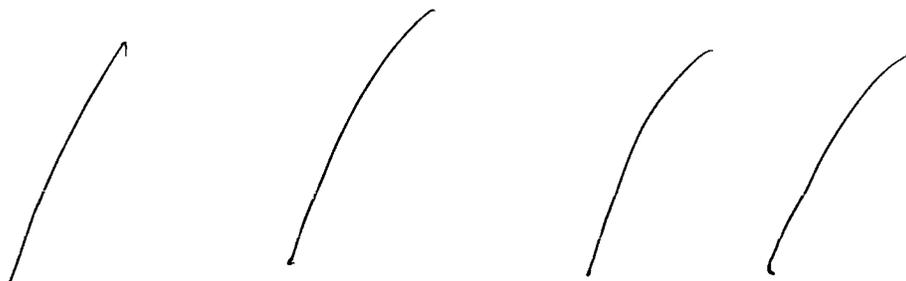


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 Trade Secret / Confidential

 Draft Labeling

 Deliberative Process



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacodynamics

Gd-EOB-DTPA is a paramagnetic compound and its relaxivity in plasma was about 8.7 L/mmol/sec at pH 7, 39°C and 0.47 T. Gd-EOB-DTPA shortened the T1 and T2 relaxation time in target tissues.

Safety Pharmacology

Neurological effects:

- Gait disturbance in 1/3 mice/sex at 800 mg/kg (3.6x based on body surface area), observed at 30 minutes post dose, and resolved by 4 hours
- NOAEL: 400 mg/kg [approximately 0.55 mmol/kg], dose multiple: 1.8x based on body surface area (BSA)

Cardiovascular effects:

In Vitro Electrophysiology Studies

hERG:

- Concentration-related blockage with IC50 31.0±4.9 mM

Action potential duration:

- Concentration-related prolongation
- More pronounced prolongation at 0.3 Hz and 1.0 Hz than at 3.0 Hz

In vivo ECG study:

- Dose-related, transient prolongation in QTc with maximum increase approximately 20 ms
- NOAEL: 0.025 mmol/kg, dose multiple: 0.5 x based on BSA

Pharmacokinetics

- Distribution: the highest levels in liver, kidneys, and GI tract, long duration in kidneys, bone, and testis (detectable radioactivity at 72 hours post single dose and on Day 21 post 5 consecutive doses)
- Limited transfer to the fetus through the placenta (<1% of maximum maternal plasma concentration) and excretion in the milk (<0.5% of injected dose)
- Nonlinear, dose-dependent, and rapid elimination (<0.2% in blood of rats and dogs in 1 and 6 hours, respectively) through both renal and hepatic routes with full compensation by the remaining route when one route was blocked
- Low human plasma protein binding (<10 %)
- Rapid and complete removal from plasma by dialysis in vitro (5.5% remained in plasma after the first cycle and below the detection limit after cycle 5 or 6)

General toxicology

Four-week IV repeat-dose toxicity studies were conducted in both rats (0.2, 0.6, and 2 mmol/kg/day, 12 wk recovery) and dogs (0.1, 0.3, and 1 mmol/kg/day, no recovery phase). Key study findings are summarized below:

Key study findings in rats:

- Dose-related, reversible tubular vacuolation of the kidneys
- Statistically significant increase in kidney weights of rats receiving 2.0 mmol/kg, partially reversible
- Dose-related decrease in testes weight, statistically significant in absolute testis weight in males receiving 2.0 mmol/kg, reversible
- NOAEL: 0.2 mmol/kg/day, dose multiple: 1.3x based on BSA

Key study findings in dogs:

- Drug-related food vomiting, similar incidence in all drug groups
- Dose-related reduction in body weight gain and food consumption at ≥ 0.3 mmol/kg (dose multiples: $\geq 6.5x$ based on BSA)
- Dose-related prolongation in activated partial thromboplastin time at ≥ 0.3 mmol/kg
- Dose-related decrease in alpha-globulins, statistically significant in males receiving 1 mmol/kg only
- Dose-related increase in urinary iron excretion on Day 2
- Reduced absolute liver weight in males receiving 1 mmol/kg (dose multiples: 22x based on BSA), correlating histologically to the reduced glycogen deposition in hepatocytes
- Dose-related, minimal to slight tubular cell vacuolation in the kidneys
- NOAEL: 0.1 mmol/kg/day, dose multiples: 2.2x based on BSA

Genetic toxicology:

Salmonella Typhimurium Reverse Mutation Assay: Negative
Chromosome Aberration Test in Human Lymphocytes in Vitro: Negative
Mouse micronucleus test (using SH L 569 A): Negative

Reproductive and developmental toxicology:

The rat study

- Increase in preimplantation loss in pregnant rats (16.7 %/animal vs. 7.7% for the concurrent controls, up to 15.4 % for the historic controls) given 0.5 mmol/kg/day (dose multiple: 3.2x)
- NOAEL: 0.1 mmol/kg/day, dose multiples: 0.6x based on BSA

The rabbit study

The following embryotoxic effects were noted in rabbits given 2.0 mmol/kg/day (dose multiple: 26 x)

- Increase in implantation resorption (22.6%/group vs. 12.2% for the concurrent controls, 7.2-20.9 % for the historic controls)
- Increase in postimplantation loss (22.6%/group vs. 12.2% for the concurrent controls, 12.0-22.2 % for the historic controls)
- Increase in absorptions (3/group vs. 0 for the concurrent controls, 0-2 for the historic controls)
- Decrease in the number of fetuses/litter (5.1 vs. 7.2 for controls, 6.4-8.6 for historic controls)

NOAEL for the rabbit study: 0.5 mmol/kg/day, dose multiples: 6.5 x based on BSA

Local tolerance:

Local tolerance studies were conducted using single intraarterial, intravenous, paravenous, and intramuscular injections in rats and rabbits. However, an interim histopathological examination was not performed in majority of studies (except for IM study that had a histopathological examination on Day 3). Therefore, acute microscopic lesions may not be revealed appropriately. Key drug related findings are summarized below.

Key findings in intramuscular injection study using SHL 569 B

- Increased incidence (1/4 for the control group vs. 4/4 for the drug group on Day 3, 0/4 vs. 3/4 on Day 7) and severity in the drug group (mild interstitial hemorrhage and slight focal muscle fiber necrosis for the control group vs. slight to moderate interstitial hemorrhage, interstitial edema and focal muscle fiber necrosis for the drug group on Day 3)
- Lesions resolving by Day 7 (4/4, slight to moderate focal muscle fiber necrosis on Day 3 vs. 1/4, slight on Day 7)

Key findings in paravenous injection study using SHL 569 B

- Slight focal proliferation of fibroblasts in the subcutis in ¼ rabbits on Day 7

B. Pharmacologic activity

Gd-EOB-DTPA is a paramagnetic compound that shortened the T1 and T2 relaxation time in target tissues. Gd-EOB-DTPA contains a lipophilic side-chain (ethoxybenzyl, EOB). According to the submission, the EOB group and the anionic characteristic is a prerequisite for Gd-EOB-DTPA uptake into hepatocytes. Because hepatic tumor tissues generally lose the ability to take up Gd-EOB-DTPA, a contrast is achieved between normal and tumor tissues.

C. Nonclinical safety issues relevant to clinical use

CVS safety:

Gd-EOB-DTPA possesses potential risk to prolong ventricular repolarization but the risk is considered low at intended clinical dose.

Kidney lesions and NSF:

Dose-related tubular vacuolation of the kidneys, a common finding among Gd-containing contrast agents, was found in both rats and dogs. It is reversible in nature, generally after a long recovery period. This transient vacuolation is believed to be due to in situ accumulation of Gd-EOB-DTPA. Because the vacuolation was not accompanied by adverse effect on the kidney functions, the Gd-EOB-DTPA-induced tubular vacuolation is not considered to be biological significant by the study report. However, considering the recent association of human nephrogenic systemic fibrosis (NSF) with Gd-containing contrast agents, accumulation of Gd-containing contrast agents in the kidneys especially in the kidneys of patients with severe renal failures might not be such a benign phenomenon. In an exploratory study conducted by the sponsor to evaluate Gd-containing contrast agents' ability to induce NSF-like skin lesions, only minimal or slight calcinosis cutis was identified and the lesion was also noted in animals given Caldiamide (the chelate of a Gd-containing contrast agent). In addition, low Gd concentration was detected in the skin tissue from animals given Gd-EOB-DTPA. No case of NSF is reported during clinical trials and post market surveillances in Europe. Taken together, Gd-EOB-DTPA may be not associated with higher risk of NSF compared to other Gd-containing contrast agents although the risk cannot be excluded. Adequate risk management and post market surveillance should be implemented to minimize the potential risk.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-090

Review number: 001

Sequence number/date/type of submission: 000/June 29, 2007/standard

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Bayer Healthcare Pharmaceuticals, Inc.

Manufacturer for drug substance: Bayer Healthcare Pharmaceuticals, Inc.

Reviewer name: Yanli Ouyang, MD, PhD, DABT

Division name: DMIHP

Review completion date: April 23, 2008

Drug:

Trade name: Gd-EOB-DTPA Injection

Generic name: Gadoteric acid, disodium salt, gadoxetate disodium

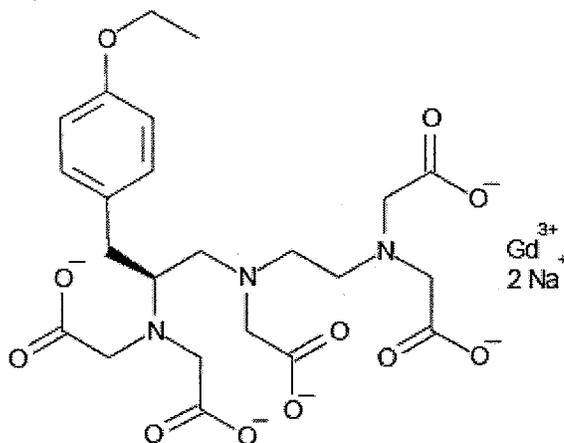
Code name: ZK 139834

Chemical name: (4S)-4-(4-Ethoxybenzyl)-3,6,9-tris(carboxylatomethyl)-3,6,9-triazaundecanedioic acid, gadolinium complex, disodium salt

CAS registry number: N/A

Molecular formula/molecular weight: $\text{GdC}_{23}\text{H}_{28}\text{N}_3\text{O}_{11}\text{Na}_2/725.72$

Structure:



Relevant INDs/NDAs/DMFs: IND 54,875

Drug class: Paramagnetic contrast agent for magnetic resonance imaging (MRI).

Intended clinical population: Gd-EOB-DTPA Injection is indicated for use in T1-weighted magnetic resonance imaging (MRI) of the liver in adults with known or suspected focal liver disease to detect and characterize liver lesions.

Clinical formulation:

Gd-EOB-DTPA (SH L569B) is provided as a sterile clear, colorless to pale yellow aqueous solution in single use vials. Each mL of Gd-EOB-DTPA contains 181.43 mg of Gd-EOB-DTPA (equivalent to 0.25 mol/L Gd-EOB-DTPA) and the excipients caloxetate trisodium, trometamol, hydrochloric acid, and/or sodium hydroxide (for pH adjustment), and water for injection. Gd-EOB-DTPA contains no antimicrobial preservative.

Gd-EOB-DTPA has a pH of 6.8 to 8.0. Pertinent physiochemical data are provided below:

Osmolality at 37°C (Osm/kg H2O)	0.688
Viscosity at 37°C (cP)	1.19
Density at 37°C (g/mL)	1.088

The recommended dose of Gd-EOB-DTPA is 0.1 mL/kg body weight (0.025 mmol/kg body weight).

Gd-EOB-DTPA is to be administered undiluted as an intravenous bolus injection at a flow rate of approximately 2 mL/second. After the injection, the intravenous cannula should be flushed using physiological saline solution.

Gd-EOB-DTPA is intended for single use.

During the development process, the formulation was changed from SH L569A to SH L569B and the compositions of two formulations are described in the table below. In addition to Gd-EOB-DTPA (ZK 139834), both formulations contain

and trometamol

Compositions of SH L569A and SH L569B Drug Products

	SH L569A	Gd-EOB-DTPA Injection (SH L569B)
Gd-EOB-DTPA (ZK 139834)		181.43 mg/mL (0.25 mmol/mL)
Trometamol		
Sodium hydroxide		
HCl		
Water for injection		

Route of administration: intravenous injection

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

Studies reviewed within this submission:

Report #	Study Type	Species	Lot # SHL 569 B	Dose, mmol Gd/kg (Dose Multiple)*
A08413	hERG	CHO cells (in vitro)		1×10^{-3} M, 10×10^{-3} M, 50×10^{-3} M, 100×10^{-3} M
A08301	Action potential duration	Guinea pig papillary muscle (in vitro)	13001 – N2990-1	0.1, 1.0 and 10 mmol/L
A08354	Cardiovascular Effects	Conscious, telemetered Beagle dogs	BB0231	0.025, 0.1, and 0.5 (0.5, 2.2, and 11x)
AZ95	Binding to human plasma	Human plasma (in vitro)	N 51111	0.01, 0.04, 0.10, 0.25, and 1.0 mmol/L
AZ96	Hemodialysis	Human plasma (in vitro)	N 51111	1 mmol/L
A03248	4 wk, IV toxicity	Rat	N7808-1 (BB0231)	0.2, 0.6 and 2.0 (1.3, 3.9, and 13x)
A03186	4 wk, IV toxicity	Dog	N7808-1 (BB0231)	0.1, 0.3, and 1.0 (2.2, 6.5, and 22x)
A18455	Reverse Mutation	Salmonella Typhimurium (in vitro)	N0564B01	Up to 10 mM
A18454	Chromosome Aberration	Human lymphocytes (in vitro)	N0564B01	Up to 10 mM

* The dose multiples to human exposure (0.025 mmol/kg) were calculated based on BSA. The table is constructed by the reviewer.

Studies reviewed by Dr. Susan Wilson for IND 54,875

Report No.	Study Date	Study Title	Species	Test Material Lot
93008	June 2-30 1992	Hemodynamic Evaluation of ZK 139.834 in Normal, Pentobarbital-anesthetized Dogs [Vol. 1.3;pp154-168]	Mongrel dogs	ZK 139 834 – Lot # G/01477
A262	Sept. 14- 17, 1992	Duration of bleeding after single intravenous administration of ZK 135.079 [Gadobutrol] and ZK 139.834 in comparison with Magnevist® [Vol. 1.3; pp.169-180]	Rat – Hans – Schering	SH L 569 A – Batch #G/01479
A263	Sept. 1992	Influence of ZK 139.834 in comparison with Magnevist® on erythrocyte morphology (<i>in vitro</i> investigation with canine blood) [Vol. 1.3; 197-206]	Dog – <i>in vitro</i>	SH L 569A – Batch # G/01479
AAO1	Jan. 20-28, 1994	Acute toxicity (LD ₅₀) after single intravenous injection of ZK 139.834 in female rats [Vol. 1.3; pp. 227-237]	Rat -	SH L 569B – 33021
AB40	July 1993	Neurotropic effects of ZK 139.834 in the Irwin test in mice after single intravenous administration [Vol. 1.3; pp. 238-243]	Micw – NMRI	SH L 569B – Lot # 32011
A238	April-Oct. 1992	Neural tolerance (ED ₅₀) of ZK 139.834 (S-enantiomere) and ZK 155 248 (R-enantiomere) after intracisternal injection in the rat (m + f) administration [Vol. 1.3; pp.244-253]	Rats – HAN-WIST	ZK 139.834 – Batch # WB 4122 Formulation

A878	Jan. 1993	Acute effects of the new magnetic resonance contrast agent [Gd-EOB DTPA] ZK 139.834 on diuresis, saluresis and protein excretion in conscious rats [Vol. 1.3; pp. 254-265]	Rats – Wistar	Batch # 001210 SH L 569 A- Lot #G/01479
A264	July 1991	Renal function and excretion of Gadolinium following a single iv. injection of ZK 139.834 or Magnevist® in rabbits [Vol.1,3; pp.266-281]	Rabbits – New Zealand Whites	ZK 139.834 – Formulatory No. 001117 Batch WB 4095
A433	Sept – Nov. 1992	Acute toxicity of SH L 569A in male mice after a single iv. application [Vol 1.4;pp. 449-460]	Mice/Han-NMRI	SH L 569 A – Batch # GO1480
A382	Sept-Nov. 1992	Acute toxicity of SH L 569A in female mice after a single iv. application [Vol 1.4;pp. 461-470]	Mice/Han-NMRI	SH L 569 A – Batch # GO1480
A389	Sept-Nov. 1992	Acute toxicity of SH L 569A in male rats after a single iv. application [Vol 1.4;pp. 478-489]	Rats/Han-Wistar	SH L 569 A – Batch # GO1480
A346	Sept-Nov. 1992	Acute toxicity of SH L 569A in weaned male rats after a single iv. application [Vol 1.4;pp. 490-500]	Rats/Han-Wistar	SH L 569 A – Batch # GO1480
A428	Oct. 1992	SH L 569 A: Systemic tolerance study in Beagle dogs after a single i.v. administration [Vol 1.4; pp.509-595]	Dogs/ Beagles	SH L 569 A – Batch # GO2084
A038	Dec. 11-24, 1995	Comparative systemic tolerance testing in Beagle dogs after single intravenous application of two SH L 569 B Batches with different manufacturing processes of the complexing agent [Vol 1.4; pp.596-643]	Dogs/ Beagles	SH L 569 B – Batch # 16791/94 Batch # 1681/95
A674	Oct. 12, 1992 – Jan. 7, 1993	Systemic tolerance study in rats after daily intravenous administration over about 4 weeks (16-18 application days, treatment-free weekends) including a reversibility study [Vol 1.4;pp.644-990]	Rats/Han – Wistar	SH L 569 A – Batch # G/00373-1A, G/01480, and G/01479
A485	Nov. 23 - Dec. 17, 1992	Systemic tolerance study in Beagle dogs after daily intravenous administration over about 4 weeks (16-18 application days, treatment-free weekends) including a reversibility study [Vol 1.5;pp.991-1244]	Dogs/ Beagles	SH L 569 A – Batch # G/00373-1A
AL19	Nov. 26, 1993 – Feb. 27, 1994	ZK 139834 – Fertility study in the rat following intravenous administration [Vol. 1.5;pp.1245-1502]	Rats/Sprague Dawley	SH L 569 B – Batch # 33021
AC85	Feb.- April, 1993	SHL 569A: Dose-finding embryotoxicity including teratogenicity study in rats after intravenous administration from day 6 to day 15 of gestation [Vol. 1.5; pp.1503-1585]	Rats/Han: Wist	SH L 569 A – Batch # G02088-1A
AF32	Aug. 1993-Feb.	SHL 569A: Embryotoxicity including teratogenicity study in the rat after intravenous administration from day 6 to day 15 of gestation [Vol. 1.6; pp.1586-2178]	Rats/Han: Wist	SH L 569 A – Batch # G02089-1A
AA67	July – Sept. 1993	SHL 569A: Embryotoxicity including teratogenicity study in the rabbit after intravenous administration from day 6 to day 18 of gestation [Vol. 1.7; pp.2179-2366 and Vol 1.8;]	Rabbits/New Zealand White	SH L 569 A – Batch # G02089-1A
AO19	July 1995-Jan. 1996	SHL 569A: ZK 139834 – Peri- and postnatal study in the rat following intravenous administration (with mating of F1 generation) [Vol. 1.7; pp.2367-2601 and Vol 1.8;pp.2602-2975]	Rats/Sprague Dawley	SH L 569 A – Batch # 51011
A416	Oct. – Nov. 1992	Evaluation of SH L A in the Ames Salmonella/ microsome mutagenicity test [Vol. 1.9;pp.3032-3048]	in vitro	SH L 569 A – Batch # G01480
A473	Dec. 1992 – Feb. 1993	Evaluation of SH L A in the Ames Salmonella/ microsome mutagenicity test [Vol. 1.9;pp.3049-3067]	in vitro	SH L 569 A – Batch # G01480
A474	Feb. 1993	Evaluation of SH L A in a bacterial mutagenicity test with Escherichia coli, strain WP2 uvrA [Vol. 1.9; pp.3068-3080]	in vitro	SH L 569 A – Batch # G01480

A415	Oct.-Dec. 1992	SH L 569A: Evaluation of gene mutations in mammalian cells in culture: HGPRT-test with V79 cells [Vol. 1.9; pp.3081-3105]	in vitro	SH L 569 A – Batch # G01479
A639	Dec. 1992 – March 1993	Chromosome aberration assay in human lymphocytes in vitro with SH L 569 A (ZK 139.834) [Vol. 1.9; pp.3106-3177]	in vitro	SH L 569 A – Batch # G01480
A555	Nov. 1992	Studies on the mutagenic potential of SH L 569 A in the mouse micronucleus test [Vol. 1.9; pp. 3178-3206]	Mice/NMRI	SHL 569 A – Batch # G/01480
A600	Mar – Apr 1993	Local tolerance test of SH L 569 A in the rat (M + F) after a single intra-arterial injection into the femoral artery [Vol. 1.9; pp.3207-3216]	Rat-Han:WIST (SPF)	SHL 569 A – Batch # G02088-1A
A744	July 1993	Local tolerance test of SH L 569 B in the rat (M + F) after a single intra-arterial injection into the femoral artery [Vol. 1.9; pp.3217-3226]	Rat-Han:WIST (SPF)	SH L 569 B – Batch # 32011
A695	July 1993	Local tolerance test of SH L 569 B following a single i.v. administration into the congested vena marginalis of the ear in rabbits [Vol. 1.9; pp.3253-3260]	Rabbit – New Zealand White	SH L 569 B – Batch # 32011
A393	Nov. 1992	Local tolerance test in the rabbit (M+F) after a single injection into the congested and uncongested marginal vein of the ear [Vol. 1.9; pp.3261-3269]	Rabbit – New Zealand White	SH L 569 A – Batch # G/01480
A394	Nov. 1992	Local tolerance test in the rabbit (M+F) after a single injection into central artery of the ear [Vol. 1.9; pp.3270-3278]	Rabbit – New Zealand White	SH L 569 A – Batch # G/01480
A714	June – July 1993	SHL 569 B: Local irritation test in rabbits after a single intramuscular [Vol. 1.9; pp.3279-3289]	Rabbit – New Zealand White	SH L 569 B – Batch # 32011
A372	Oct. – Nov. 1992	Local irritation test in rabbits after a single intramuscular administration [Vol. 1.9; pp.3290-3300]	Rabbit – New Zealand White	SH L 569 A – Batch # G01480
A406	Nov. 1992	Local tolerance test in rabbit (M+F) after a single paravenous injection [Vol. 1.9; pp.3301-3311]	Rabbit – New Zealand White	SH L 569 A – Batch # G01480
A710	July 1993	Local tolerance test with SH L 569 B after a single paravenous injection [Vol. 1.9; pp.3311-3319]	Rabbit – New Zealand White	SH L 569 B – Batch # 32011
9816	Feb. – Nov. 1991	Pharmacokinetics and biotransformation of ¹⁵³ Gd-labeled ZK 139 834 after single intravenous administration in rats [Vol. 1.3; pp.282-308]	Rats/Wistar HAN	ZK 139 834 – Lot #s WB 4027, WB 4073, and WB 4116
9935	Jan. – May 1992	Pharmacokinetics and biotransformation of ¹⁵³ Gd-labeled ZK 139 834 after single intravenous administration in dogs [Vol. 1.3; pp.309-326]	Dogs/Beagles	ZK 139 834 – Lot #s WB 4027, and WB 4116
A436	Oct. – Dec. 1992	Enterohepatic recirculation of ZK 139 834 in the rat following intravenous application [Vol. 1.3; pp.327-335]	Rats/Wistar HAN	SH L 569 A – Batch # G/01479
A579	Sept. 1993 – July 1994	Biodistribution of ¹⁵³ Gd-labeled ZK 139 834 to tissues and organs following single intravenous administration to rats [Vol. 1.3; pp.336-346]	Rats/Wistar HAN	ZK 139 834 – Batch # WB4068

A580	Sept. 1993 – July 1994	Biodistribution of ¹⁵³ Gd-labeled ZK 139 834 to tissues and organs following repeated intravenous administration to rats [Vol. 1.3; pp.347-359]	Rats - Wistar HAN	ZK 139 834 – Batch # WB4068
A766	Jan.- April 1993	Placental transfer and distribution after intravenous administration of ¹⁵³ Gd-labeled ZK 139 834 to pregnant rats [Vol. 1.3; pp.360-368]	Rats – Sprague Dawley	SH L 569 A – Lot # G/01479
A765	Jan.- April 1993	Transfer to neonates via milk after intravenous administration of ¹⁵³ Gd-labeled ZK 139 834 to lactating rats [Vol. 1.3; pp.369-378]	Rats / Sprague Dawley	SH L 569 A – Lot # G/01479
A435	Oct. 1992 – April 1993	Excretion of ZK 139 834 in the case of impaired liver or kidney function in rats after single intravenous application [Vol. 1.3; pp.404-414]	Rats / Wistar HAN	SH L 569 A – Lot # G/01479

* The table is adapted from the review for IND 54,875.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacodynamics

Gd-EOB-DTPA is a paramagnetic compound and its relaxivity in plasma is about 8.7 L/mmol/sec at pH 7, 39°C and 0.47 T. Gd-EOB-DTPA contains a lipophilic side-chain (ethoxybenzyl, EOB). According to the submission, EOB group in combination with its anionic character is a prerequisite for its uptake into hepatocytes. Because hepatic tumor tissues generally lose the ability to take up Gd-EOB-DTPA, a contrast is achieved between normal and tumor tissues.

Safety Pharmacology

Neurological effects:

- Gait disturbance in 1/3 mice/sex at 800 mg/kg, observed at 30 minutes and resolved at 4 hours
- NOAEL 400 mg/kg [approximately 0.55 mmol/kg], dose multiple 1.8 based on BSA

Cardiovascular effects:

In Vitro Electrophysiology Studies

- hERG:
 - Concentration-related blockage
 - IC50 31.0±4.9 mM
- Action potential duration
 - Concentration-related prolongation
 - More pronounced at 0.3 Hz and 1.0 Hz than at 3.0 Hz

In vivo ECG study

- Dose related, transient increase in QTc with maximum increase approximately 20 ms
- NOAEL: 0.025 mmol/kg, dose multiple: 0.5x based on BSA

2.6.2.2 Primary pharmacodynamics

EOB-DTPA formed a stable complex with the paramagnetic gadolinium ion with high thermodynamic stability ($\log K_{Gd} = -23.46$). The relaxivity in plasma is 8.7 L/mmol/sec at pH 7, 39°C and 0.47 T.

Mechanism of action:

Gd-EOB-DTPA is a paramagnetic compound and develops a magnetic moment when placed in a magnetic field. The magnetic moment produced by Gd-EOB-DTPA results in a local magnetic field, yielding enhanced relaxation rates (shortening of relaxation times) of water protons in the vicinity of the paramagnetic agent, which leads to an increase in signal intensity (brightening) of blood and tissue. In MRI, visualization of normal and pathological tissue depends in part on variations in the radiofrequency signal intensity that occur with 1) differences in proton density; 2) differences of the spin-lattice or longitudinal relaxation times (T1); and 3) differences in the spin-spin or transverse relaxation time (T2). When placed in a magnetic field, Gd-EOB-DTPA shortened the T1 and T2 relaxation time in target tissues.

Gd-EOB-DTPA contains a lipophilic side-chain (ethoxybenzyl, EOB). According to the submission, this group in combination with its anionic character is a prerequisite for its uptake into hepatocytes. Because hepatic tumor tissues generally lose the ability to take up Gd-EOB-DTPA, a contrast is achieved between normal and tumor tissues.

Drug activity related to proposed indication:

Gd-EOB-DTPA is taken up by normal hepatocytes. Because hepatic tumor tissues generally lose the ability to take up Gd-EOB-DTPA, a contrast is achieved between normal and tumor tissues.

2.6.2.3 Secondary pharmacodynamics: N/A

2.6.2.4 Safety pharmacology

Neurological effects: The related studies were reviewed previously by Dr. Wilson and key information based on the previous review is provided below.

Study title: Title: Neurotropic effects of ZK 139834 in the Irwin test in mice after single intravenous administration

Study no.: ZN.93.0641, Report No. AB40

Key study findings:

- Gait disturbance in 1/3 mice/sex at 800 mg/kg, observed at 30 minutes and resolved at 4 hours
- NOAEL: 400 mg/kg [approximately 0.55 mmol/kg], dose multiple: 1.8 based on BSA

Cardiovascular effects:

In Vitro Electrophysiology Studies

Study title: Electrophysiological examination of Eovist on the hERG-mediated potassium current.

Key study findings: Concentration-related blockage with $IC_{50} 31.0 \pm 4.9 \times 10^{-3}$ M

Study no.: SCH_177, report no. A08413

Volume #, and page #: Module 4

Conducting laboratory and location: _____

Date of study initiation: 26 October, 2001

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Eovist (SHL 569B, ZK 139834), Batch #, N/A, purity, N/A

Methods

Patch-clamp experiments were performed using CHO cells stably expressing the hERG in the voltage-clamp mode. Whole-cell currents were recorded. Potassium currents were activated at a frequency of 0.1 Hz by 1-s conditioning pulses to +20 mV followed by a 1-s partial repolarization to -40 mV. Currents were recorded in the absence and presence of test solution. Test solution was applied by bath perfusion after the 14th trace. The 5 min time interval between the 20th and the 50th stimulus was considered for the evaluation of the test solution effect.

Four concentrations of Eovist: 1×10^{-3} M, 10×10^{-3} M, 50×10^{-3} M, 100×10^{-3} M, n=3-4. The half-maximal inhibition concentration (IC_{50}) and corresponding Hill coefficient (nH) were estimated.

Table 1. Eovist solution preparation

Identification Number	Test solution (ml)	vehicle (ml) buffer	Resulting concentration (mmol/L)	Date of preparation
Eovist	0.10	25	1	26/29.10., 05.11.01
	1.00	24	10	26/29.10., 05.11.01
	5.00	20	50	26/29.10., 05.11.01
	10.0	15	100	29.10., 05.11.01

Positive control: Terfenadine

Table 2. Positive control solution preparation

Identification Number	Amount (mg)	vehicle (ml) DMSO	Resulting stock solution (mmol/L)	Further Dilution	Resulting stock solution (mmol/L)	Date of preparation
Terfenadine	10.01	21.22	1	1:10	0.1	22.08.01
			0.1	1:10	0.01	22.08.01

Negative control: DMSO

Results

Positive control:

Terfenadine induced concentration-dependent blockage of the hERG channel with an IC_{50} of 26.51 ± 8.56 nM and an n_H of 0.81 ± 0.20 (Figure 1). According to the study report, this result is in good agreement with findings from the literature (Rampe et. al., 1997).

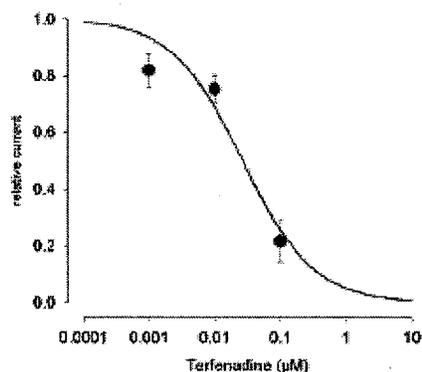


Figure 1. Concentration-dependence of the effect of Terfenadine on the hERG-mediated potassium current. Current amplitudes were measured at the end of the test pulse to -40 mV. Data points are given as mean \pm SEM from 3 experiments.

Negative control:

Control experiments were performed on the same experimental days and under identical conditions as the test solution to verify the stability of the current over time and to evaluate cell condition (Table 3). The results indicated a good cell condition during experimental procedure.

Table 3. hERG-mediated currents under control conditions

Exp.no.	Current amplitude (pA)				Relative current amplitude	
	Curve ₅₀₀ ^a	control ₅₀₀ ^b	Curve ₁₀₀ ^c	Control ₁₀₀ ^d	control ^e	Wash ^f
2ISD11025a1	/	/	/	/	/	/
2ISD11025d1	/	/	/	/	/	/
2ISD11026f1	/	/	/	/	/	/
2ISD11031e2	/	/	/	/	/	/
Mean					0.98	1.06
SD					0.02	0.07

a. Curve value indicating the extrapolated value for the current amplitude measured during the 50th stimulus of experiment. b. Value of the current amplitude in presence of control measured during the 50th stimulus of experiment. c. Curve value indicating the extrapolated value for the current amplitude measured during the 100th stimulus of experiment. d. Value of the current amplitude after wash-out measured during the 100th stimulus of experiment. e. Relative remaining current amplitude calculated according to: $I_{\text{control}50}/I_{\text{curve}50}$. f. Relative remaining current amplitude calculated according to: $I_{\text{control}100}/I_{\text{curve}100}$.

Eovist:

Eovist induced concentration-dependent blockage of the hERG channel with an IC_{50} of $31.0 \pm 4.9 \times 10^{-3}$ M and n_H of 1.3 ± 0.2 (Figure 2).

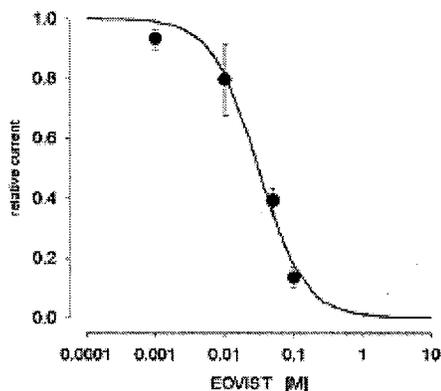


Figure 2. Concentration-dependence of the effect of Eovist on the hERG-mediated potassium current. Current amplitudes were measured at the end of the test pulse to -40 mV. Data points are given as mean \pm SEM from 3-4 experiments.

In most of the experiments, no reversibility of effects could be recorded because patch was disrupted. However, the reversibility was shown in Figure 3 using the time course of current change induced by 50×10^{-3} M Eovist in an undisturbed patch. Figure 3 showed a strong current increase which reached its maximum at stimulus 26 followed by a 57% inhibition. During wash period (the starting point indicated by the dotted line in Fig.), the inhibitory effect recovered to 86% of the control current amplitude.

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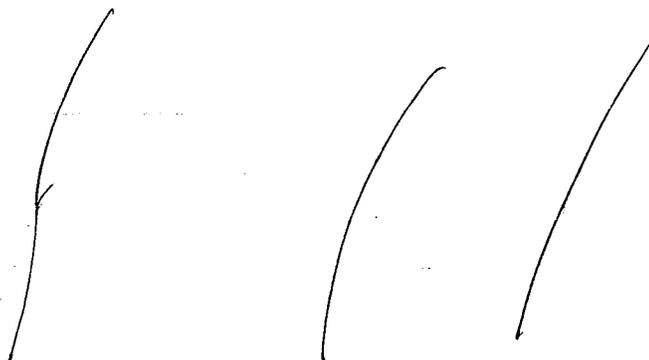


Figure 3. Effect of 50×10^{-3} M Eovist on hERG-mediated current. (A) 50 superimposed original hERG-current traces recorded in presence of Eovist. (B) Onset (indicated by the dashed line) and offset (indicated by the dotted line) of the application of 50×10^{-3} M Eovist. Current amplitude at -40 mV was plotted against time. Time course under control conditions was calculated by a biexponential fit of equation $y = a \cdot \exp(-cx) + b \cdot \exp(-dx)$ (indicated by the solid line).

Reviewer's comments:

Eovist induced concentration-dependent blockage of the hERG channel with an IC_{50} of $31.0 \pm 4.9 \times 10^{-3}$ M. The IC_{50} is greater than 10 μ M, indicating weak evidence of risk.

The study was not conducted in compliance with GLP (no statement).

Study title: Effects of SH L 569 B (Eovist) on cardiac action potential in isolated guinea pig papillary muscle

Key study findings: Concentration-related prolongation in action potential duration, more pronounced at 0.3 Hz and 1.0 Hz than at 3.0 Hz

Study no.: SP20020018, report no. A08301

Volume #, and page #: Module 4

Conducting laboratory and location: Safety Pharmacology, Schering AG, D-13342 Berlin

Date of study initiation: 14 March, 2002

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Eovist (0.25 mol/L, SH L 569 B, ZK 139834), Batch #, 13001 -N2990-1, purity, N/A

Method:

Guinea pig (male, 270 – 410 g) papillary muscles (continuously superfused with Tyrode solution at 35°C) were electrically paced (via silver electrodes at 0.3 Hz, 1 Hz, and 3 Hz) and impaled with — KCl-filled glass micropipettes to monitor membrane potential.

Data were recorded and evaluated using a computerized set up (ISO2 data acquisition system). Six muscles per group were evaluated. The muscles were exposed to Eovist, consecutively at 0.1, 1.0 and 10 mmol/L, for 30-35 min and measurements were then performed.

Positive control: dl-sotalol (100 mcmol/L) was administered to saline-treated muscles (in a fourth test cycle). The muscles were exposed for 15 min.

Test parameters:

Action potential duration at 30%, 60%, and 90% repolarization (APD₃₀, APD₆₀, and APD₉₀), maximum rate of rise of the upstroke (V_{max}), upstroke amplitude (AP-Amp), and diastolic membrane potential (MP). The changes were determined as the mean of 10 measurements per time point and stimulation frequency.

Result:

- Concentration-related prolongation in action potential duration (Table 4 and Figure 4), more pronounced at 0.3 Hz and 1.0 Hz than at 3.0 Hz
- Increase in V_{max} at 10 mmol/L only (Figure 5)
- No consistent effect on membrane potential and action potential amplitude

Table 4. Effect of Eovist on action potential durations in guinea pig papillary muscle in vitro (0.3 Hz, means \pm SD, n=6)

Eovist (0.3 Hz)		APD30 [ms]	
Dose	mean		SD
baseline	102.5		13.7
0.1 mmol/L	124.2		16.2
1.0 mmol/L	133.0		15.0
10 mmol/L	147.5		17.0

Eovist (0.3 Hz)		APD60 [ms]	
Dose	mean		SD
baseline	143.5		12.8
0.1 mmol/L	163.0		22.2
1.0 mmol/L	172.0		21.3
10 mmol/L	189.2		23.3

Eovist (0.3 Hz)		APD90 [ms]	
Dose	mean		SD
baseline	160.0		13.0
0.1 mmol/L	177.7		23.1
1.0 mmol/L	186.3		22.1
10 mmol/L	203.3		23.8

Table 5. Effect of saline or sotalol on action potential durations in guinea pig papillary muscle in vitro (0.3 Hz, means \pm SD, n=6)

saline (0.3 Hz)	APD30 [ms]	
Dose	mean	SD
baseline	104,7	19,0
30 min.	121,2	25,2
60 min.	126,0	24,7
90 min.	127,0	26,3
Sotalol 10^{-4} mol	143,8	27,7

saline (0.3 Hz)	APD60 [ms]	
Dose	mean	SD
baseline	142,8	20,0
30 min.	157,7	26,5
60 min.	162,3	27,4
90 min.	164,5	28,8
Sotalol 10^{-4} mol	196,8	33,5

saline (0.3 Hz)	APD90 [ms]	
Dose	mean	SD
baseline	162,2	14,6
30 min.	175,0	21,9
60 min.	179,3	23,4
90 min.	182,0	24,5
Sotalol 10^{-4} mol	216,3	30,5

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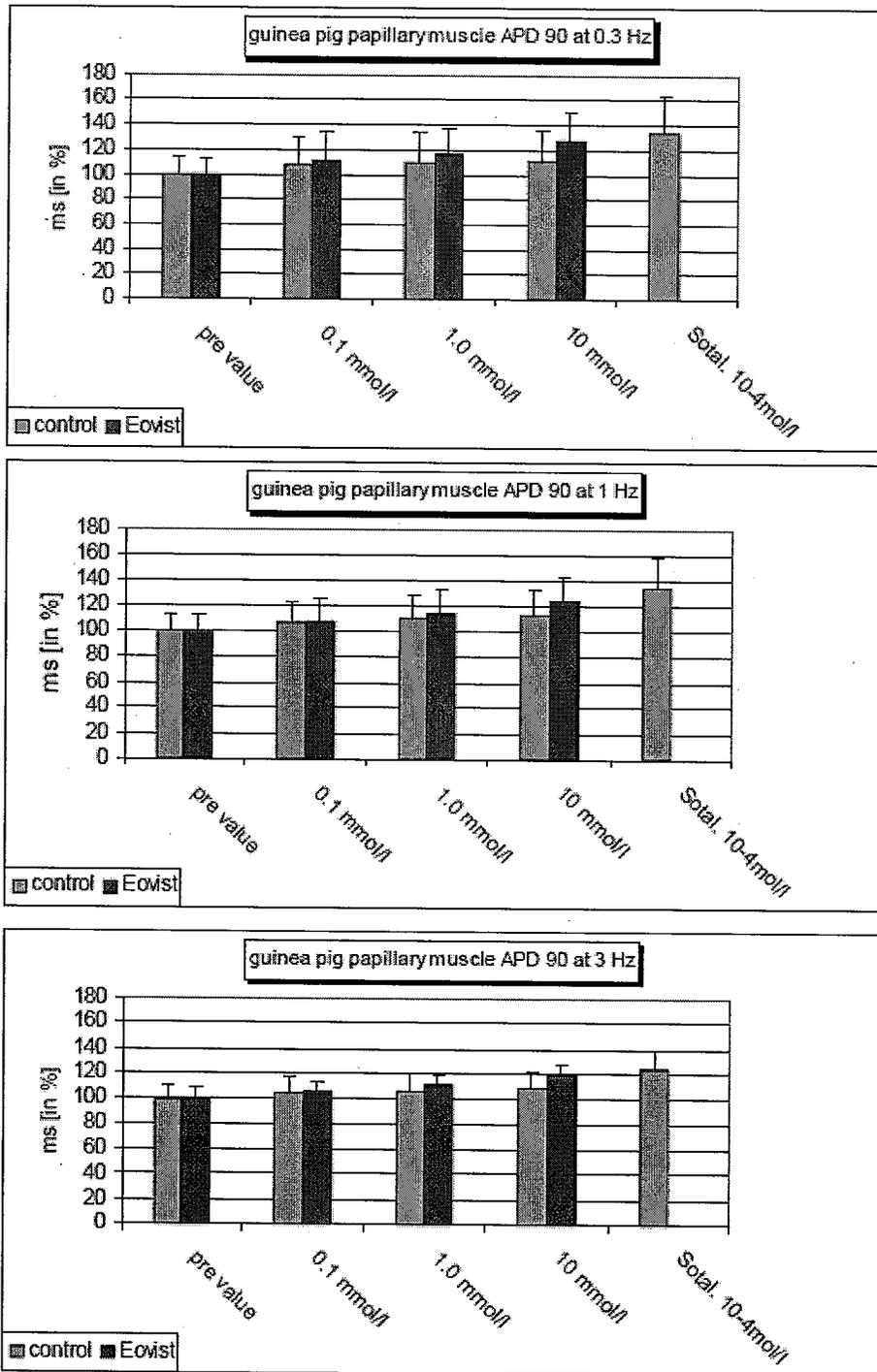


Figure 4. Effect of Eovist on APD90 in guinea pig papillary muscle in vitro (means \pm SD, n=6)

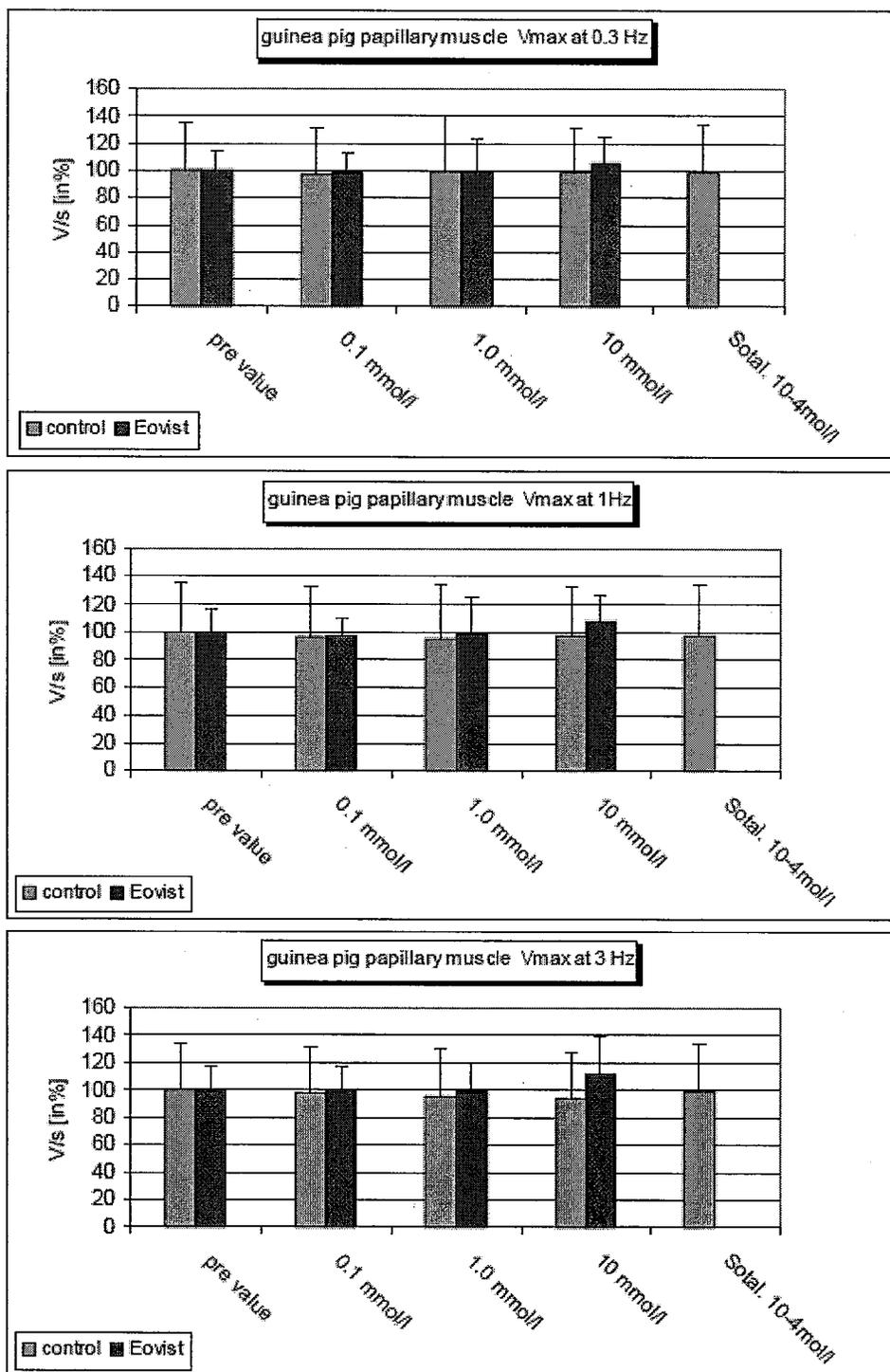


Figure 5. Effect of Eovist on V_{max} in guinea pig papillary muscle in vitro (means ± SD, n=6)

Conclusions:

According to the study report, the results of this study do not provide evidence of a potential of Eovist to prolong repolarization of action potential in cardiac muscle in clinically relevant concentrations.

Reviewer's comments:

Although concentration-related prolongation in action potential duration was noted, notable prolongation was seen at 10 mmol/L only, a concentration exceeding the anticipated maximal therapeutic plasma concentration.

Triangulation analysis was not provided in this study.

The study was not conducted in compliance with GLP (no statement). However, GLP compliance is not required.

In vivo ECG study

Study title: Cardiovascular Effects of Eovist in Conscious, Telemetered Beagle Dogs

Key study findings: Dose related, transient prolongation in QTc. NOAEL was established at 0.025 mmol/kg and the dose multiple to human exposure was 0.5 based on BSA.

Study no.: — Study No: DERA1004, Report #: A08354

Volume #, and page #: Module 4

Conducting laboratory and location: _____

Date of study initiation: 16 July 2001

GLP compliance: Yes, formulation analysis was not performed.

QA report: yes (X) no ()

Drug, lot #, and % purity: Batch # BB0231 (0.25 mmol/mL), purity, 99.9 %

Methods

Doses: One treatment group of 4 dogs (2 male and 2 female) was dosed according to the following ascending regimen:

Day 1 Vehicle (sterile saline, 2 ml/kg)

Day 4 Eovist (0.025 mmol/kg)

Day 8 Eovist (0.1 mmol/kg)

Day 11 Eovist (0.5 mmol/kg)

The dose multiples to human exposure (0.025 mmol/kg) were 0.5, 2, or 11 based on BSA.

Species/strain: Dog/Beagle

Number/sex/group or time point: 2/sex/group

Route, formulation, volume, and infusion rate: intravenous infusion over approximately 10, 10, 50, and 50 s for 0.025, 0.1, and 0.5 mmol/kg and vehicle doses, respectively. The dose volumes were 0.1, 0.4, and 2.0 mL/kg for the 0.025, 0.1, and 0.5 mmol/kg doses, respectively. The vehicle dose volume was 2.0 mL/kg.

Age: 11-13 month old

Weight: 9.49-14.01 kg

For each dose, telemetry data collection commenced at least 30 min before dosing and ended approximately 6 h after dosing. Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and the lead II electrocardiogram variables were measured continuously. Mean arterial blood pressure (MAP) was calculated as $[DBP + \frac{1}{3}(SBP - DBP)]$. The following variables from the lead II electrocardiogram were measured, *calculated and reported: PR interval, RR interval, QRS duration, QT interval QTcF interval, and QTcQ interval (calculated as $QTcQ = QT + \#(1 - RR)$, —)* (# corresponds to a correction factor specific to each individual dog as it represents the slope of the line from a plot of QT against RR interval generated over a range of heart rates on the vehicle dosing day. Although based on the Framingham equation, this factor is specific for each individual dog and does not make the assumptions of uniformity made by the Framingham correction ($QTc = QT + 0.154(1 - RR)$), which has a fixed correction factor). In addition to interval analysis, a visual inspection of all of the ECG waveforms, for disturbances in rhythm and waveform morphology, in all dogs on all of the dosing days was performed.

Results

Arterial Blood Pressure and Heart Rate: No remarkable drug related effect

Lead II ECG: No remarkable drug-related effect on RR and PR intervals, QRS duration, gross morphology, or rhythm.

Dose related, transient (resolved by 30 min post doing) increase in QTc, small magnitude (< 20 ms at doses up to 0.5 mmol/kg) (Table 6).

Table 6. Effects of Vehicle and Eovist on QTcF Interval in Conscious, Telemetered Beagle Dogs

Time (min)	QTcF Interval (ms)			
	Vehicle 2 ml/kg	Eovist 0.025 mmol/kg	Eovist 0.1 mmol/kg	Eovist 0.5 mmol/kg
-15	245.4 ± 3.0	236.9 ± 3.2	247.5 ± 5.1	246.3 ± 5.4
-1	233.7 ± 1.7	239.6 ± 6.0	245.0 ± 4.1	242.6 ± 4.4
0	231.2 ± 2.0	240.4 ± 7.3	241.9 ± 3.2	247.4 ± 8.8
0.5	236.3 ± 2.4	244.2 ± 5.2	250.8 ± 2.9	266.5 ± 5.8**
1	242.9 ± 4.0	244.5 ± 2.5	261.5 ± 5.1*	266.9 ± 6.2**
2.5	250.0 ± 9.1	246.4 ± 3.7	258.2 ± 8.1	262.5 ± 9.5
5	231.5 ± 2.1	247.8 ± 1.0	258.5 ± 13.5	260.3 ± 7.8
10	241.5 ± 3.2	246.0 ± 4.7	243.3 ± 7.7	259.7 ± 8.0
20	231.6 ± 5.7	241.7 ± 3.6	247.0 ± 4.5	256.5 ± 6.7
30	238.7 ± 6.8	241.2 ± 3.6	242.8 ± 5.2	247.3 ± 6.9
60	244.6 ± 3.5	240.5 ± 4.6	255.7 ± 8.5	243.3 ± 8.6
180	248.0 ± 3.3	241.7 ± 5.4	245.0 ± 7.6	254.4 ± 6.0
360	246.3 ± 4.6	251.4 ± 7.1	250.1 ± 4.6	252.0 ± 5.2

Time 0 was the start of dosing. Data are mean ± s.e. mean of results obtained from 4 animals (2 males and 2 females). * and ** indicate $P < 0.05$ and $P < 0.01$, compared to vehicle, respectively.

Table 7. Effects of Vehicle and Eovist on QTcQ Interval in Conscious, Telemetered Beagle Dogs

Time (min)	QTcQ Interval (ms)			
	Vehicle 2 ml/kg	Eovist 0.025 mmol/kg	Eovist 0.1 mmol/kg	Eovist 0.5 mmol/kg
-15	242.3 ± 1.2	233.9 ± 2.6	240.4 ± 4.1	239.3 ± 5.0
-1	227.2 ± 5.8	232.3 ± 1.0	239.6 ± 5.0	237.7 ± 7.0
0	226.3 ± 5.1	233.5 ± 4.8	238.9 ± 5.5	240.0 ± 4.2
0.5	230.0 ± 5.1	237.0 ± 1.2	242.4 ± 5.9*	252.2 ± 3.0**
1	234.8 ± 3.2	235.3 ± 4.3	246.4 ± 6.6	252.1 ± 5.0*
2.5	237.8 ± 5.3	232.6 ± 5.1	243.6 ± 2.8	241.6 ± 1.7
5	228.8 ± 5.6	238.4 ± 3.5	247.9 ± 6.2	248.7 ± 4.3
10	236.9 ± 5.6	237.1 ± 2.7	238.9 ± 6.9	250.6 ± 1.1
20	229.6 ± 5.1	233.6 ± 4.2	239.6 ± 4.3	248.2 ± 2.4*
30	237.5 ± 7.1	235.6 ± 2.4	239.8 ± 4.6	242.1 ± 6.5
60	241.6 ± 4.3	234.4 ± 4.3	250.0 ± 4.0	240.0 ± 6.8
180	234.9 ± 6.0	234.5 ± 5.4	237.5 ± 7.5	246.6 ± 0.8
360	238.8 ± 5.9	244.6 ± 5.7	237.2 ± 3.6	243.0 ± 3.8

Time 0 was the start of dosing. Data are mean ± s.e. mean of results obtained from 4 animals (2 male and 2 female). * and ** indicate $P < 0.05$ and $P < 0.01$, compared to vehicle, respectively.

Conclusion:

According to the study report, intravenous administration of Eovist at 0.025, 0.1, and 0.5 mmol/kg had no marked effect on arterial blood pressure (systolic, diastolic, and mean), heart rate or lead II ECG variables (RR, PR and QT intervals and QRS duration) and waveforms over the duration of the recording period. QTcF and QTcQ interval were both prolonged between 0.5 and 1 min following dosing, with prolongation of QTcQ persisting from 5-20 min after dosing. These changes may be due at least in part to a decrease in QT interval in the vehicle group.

Reviewer's comments:

Dose related, transient increase in QTc were noted. In general, individual correction methodology (QTcQ in this study) is the preferable method. NOAEL was established at 0.025 mmol/kg and the dose multiple to human exposure was 0.5 based on BSA. However, only 4 dogs were used for this study and the power to detect QT prolongation is low (4/sex/group is preferred group size and could, with 80% chance, detect a 5% change (10% if 2/sex/group) in appropriately corrected QT). In addition, Latin square study design with 2/dose/time, 1 week washout time, is the preferable study design.

Summary of cardiovascular effects:

- Concentration-related blockage of the hERG channel with an IC₅₀ of 31.0±4.9 mM.
- Concentration-related increase in action potential duration, notable prolongation at 10 mmol/L only, and more pronounced at 0.3 Hz and 1.0 Hz than at 3.0 Hz
- Dose related, transient increase in QTc in a conscious dog study, NOAEL at 0.025 mmol/kg and the dose multiple to human exposure 0.5 based on BSA.

Taken together, the results showed that Eovist possesses the potential to prolong ventricular repolarization but with weak evidence of risk at intended clinical dose.

Pulmonary effects: N/A

Renal effects: A study was conducted and reviewed previously for the IND. The key findings are summarized below based on the previous review.

Title: Renal function and excretion of Gadolinium following a single i.v. injection of ZK 139, 834 and Magnevist in rabbits.

Study Identification: Report No. A264 [Study No. KM 90227]

Formulation: ZK 139, 834 [Batch No. WB 4095/Formulation No. 001117 – contains both _____]

1 mmol/kg of Gd-EOB-DTPA (dose multiple: 13X based on BSA), single IV, New Zealand White rabbits (2M and 3 F), no concurrent controls.

Key findings:

- Transient increase in creatinine clearance at 2 hr post dosing compared to baseline values (4.92 to 17.14 mL/min, returned to baseline values by 6 hr)
- Transient increase in urine flow (2.70 to 6.90 mL/h, returned to baseline values by 6 hr)
- Increase in the incidence of proteinuria [4/5 (30 mg/dL during 24-48 hr and persisted throughout Day 7) vs. 1/5 (mild) in pretreatment samples]
- Increase in LDH from 2-48 hours (9.46 U/g creatinine at baseline vs. 37.12, 67.27, 12.20, and 36.41 at 2, 6, 24, and 48 hr post dosing, respectively, returned to baseline values during 48-168 hour collection interval)
- Gd detected in the kidneys [0.9 ± 0.47 mcg/g organ (mean \pm SD), $\leq 0.01\%$ of the total dose] and liver [2.29 ± 1.26 mcg/g organ, $\leq 0.09\%$ of the total dose]

Reviewer's comment: This was a single dose study and no concurrent controls were included. These deficiencies made the result interpretation rather difficult. Therefore, the significance of the study findings is unknown.

Gastrointestinal effects: N/A

Abuse liability: N/A

Other: N/A

2.6.2.5 Pharmacodynamic drug interactions: N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The PK studies were mainly conducted using the formulation SHL 569 A and reviewed previously for IND. Two new in vitro studies evaluating protein binding and hemodialysis using the formulation SHL 569 B were reviewed below.

2.6.4.1 Brief summary

The summary is based on the previous IND review and current review.

- In biodistribution studies in rats, the highest levels of radioactivity were detected primarily in the organs of excretion: liver, kidneys, and GI tract. Kidneys, bone and testis had detectable radioactivity at 72 hours post single dose and on Day 21 post 5 consecutive doses.
- Limited Gd-EOB-DTPA was transferred to the fetus through the placenta (<1% of maximum maternal plasma concentration) and excreted in the milk (<0.5% of injected dose).
- Gd-EOB-DTPA did not undergo biotransformation.
- Elimination of Gd-EOB-DTPA were nonlinear, dose-dependent and rapid (<0.2% in blood of rats and dogs in 1 and 6 hours, respectively) through both renal and hepatic routes with full compensation by the remaining route when one route was blocked. There is minimal (<5% of the injected dose) enterohepatic recirculation of the drug in rats.
- Hepatic elimination can be blocked by co-administration of sulfobromophthalein indicating that the organic anion transport mechanism is involved.
- Low human plasma protein binding (<10%), concentration-independent within the investigated range (0.01-1.0 mmol Gd/L)
- Gd-EOB-DTPA was quickly and completely removable from plasma by dialysis in vitro (5.5% remained in plasma after the first cycle and below the detection limit after the cycle 5 or 6).

2.6.4.2 Methods of Analysis N/A

2.6.4.3 Absorption: N/A

2.6.4.4 Distribution

The following summary is based on the previous IND review.

The highest levels of radioactivity were detected primarily in the organs of excretion: liver, kidneys, and GI tract and the longest duration in kidneys, bone, and testis in rats when given a single intravenous dose of radiolabeled Gd-EOB-DTPA [^{153}Gd] at 0.05 mmol Gd/kg. Only kidneys, bone, and testis had detectable radioactivity at 72 hours post single dose or by 21 days following the last dose of 5 consecutive doses (0.05 mmol/kg).

When 0.1 mmol Gd/kg of Gd-EOB-DTPA was administered to dams on Day 15 of pregnancy, <1% of the drug in the maternal plasma was observed in the fetus. Drug levels in the fetus and amniotic fluid for ≤ 3 hours were ≤ 1.0 nmol/g of wet tissue and ≤ 0.6 nmol/mL, respectively, and no longer detectable by 24 hours. Less than 0.5% of the drug was excreted in the milk of lactating rats at a dose of 0.1 mmol Gd/kg. Although radioactivity was detected in the stomach and intestinal contents of the 12-day old nursing pups, radioactivity was not detected in their livers and kidneys.

2.6.4.5 Metabolism

According to the previous IND review, the pharmacokinetics studies indicated that Gd-EOB-DTPA did not undergo biotransformation.

2.6.4.6 Excretion

The following summary is based on the previous IND review.

Based on both rat and dog studies, elimination of Gd-EOB-DTPA were nonlinear, dose-dependent, and rapid (<0.2% in blood of rats and dogs in 1 and 6 hours, respectively) by both renal and hepatic routes with full compensation by the remaining route when one route was blocked.

Based on studies in rats, hepatic elimination could be blocked by co-administration of sulfobromophthalein, indicating that the organic anion transport mechanism is involved.

There was minimal (<5% of the injected dose) enterohepatic recirculation of the drug in rats.

2.6.4.7 Pharmacokinetic drug interactions N/A

2.6.4.8 Other Pharmacokinetic Studies

Study title: Binding of gadoxetate to human plasma at different concentrations

Key study findings:

Low human plasma protein binding (<10 %), concentration-independent within the investigated range (0.01-1.0 mmol Gd/L)

Study no.: KM 98080, Report No. AZ95

Volume #, and page #: Module 4

Conducting laboratory and location: _____

Date of study initiation: May 13, 1998

GLP compliance: No.

QA report: yes () no (X)

Drug, lot #, and % purity: SH L 569 B, Batch number: N 51111, 250 mmol Gd / L, _____
Tromethamol, _____, water

Methods:

The binding of gadoxetate to human plasma proteins was determined by an in vitro ultrafiltration assay. The assay separated the unbound fraction of the drug from plasma proteins by filtering the drug containing plasma through a membrane with a pore size excluding molecules larger than about _____. Five concentrations, 0.01, 0.04, 0.10, 0.25, and 1.0 mmol Gd/L, were used. The concentrations were representative for the range of concentrations occurring in the plasma of a patient after the administration of a typical dose, according to the study report. In addition, an internal reference compound _____ was used to compensate for some parameters like protein volume, osmotic effects, and the Donnan effect which are difficult to control during the determination of the protein binding.

Results: Figure 6.

Low protein binding (7.7 and 9.1 %), concentration-independent within the investigated range (0.01-1.0 mmol Gd/L).

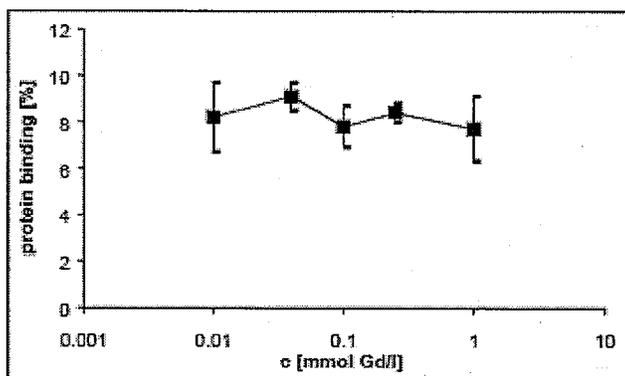


Figure 6. Plasma protein binding of gadoxetate at different concentrations.

Study title: Hemodialysis of gadoxetic acid, disodium (SH L 569 B). In vitro study

Key study findings:

Gadoxetic acid, disodium was quickly and completely removable from plasma by dialysis through a commercial hemodialysis device with 5.5% remaining in plasma after the first

cycle and below the detection limit after the cycle 5 or 6. No significant difference from the freely dialysable (4.0 % remaining in plasma after the first cycle and below the detection limit after the cycle 5) was noted.

Study no.: KM 98080, Report No. AZ96

Volume #, and page #: Module 4

Conducting laboratory and location: _____

Date of study initiation: 15 Oct. 1998

GLP compliance: No.

QA report: yes () no (X)

Drug, lot #, and % purity: gadoxetic acid, disodium (SH L 569 B), Batch number: N 51111, 250 mmol Gd / L, _____ Tromethamol, _____ water

Methods:

An in vitro study

Plasma (300 mL) was cycled stepwise several times through the hemodialysis filter at a flow rate 14 mL/min using a commercial hemodialysis device.

An internal standard _____ was used.

Gadoxetic acid and _____ were dissolved in 300 mL human plasma at a concentration of 1 mmol/L each.

Three independent experiments were performed. New filters were used for each experiment.

The Gd- and _____ concentrations in the dialysate and in the plasma were determined using atomic emission spectrometry at wavelengths of 342.247 nm (Gd) and _____ nm _____

(_____)

Results:

The plasma concentrations of gadoxetic acid, disodium and _____ were reduced to 5.5 ± 3.4 % and 4.0 ± 3.0 % of their initial concentrations after the first cycle (Table 8). The high extraction rate was because during the first passage the dialysate has not yet reached its steady state concentration, according to the study report. The concentration of gadoxetic acid, disodium fell below the detection limit, corresponding to 0.1% of the initial plasma concentration, after cycles 5-6. A total volume of 5.5 - 6 L of dialysate was used during the 9 cycles. It contained 103 ± 1 % and 102 ± 2.1 % of the administered gadoxetic acid, disodium and _____, respectively, demonstrating complete recovery for both compounds.

Table 8. Efficacy of dialysis of gadoxetic acid, disodium and through a hemodialysis filter

dialysis cycle no.	plasma concentration [mmol Gd/L]	plasma concentration [% of initial]	plasma concentration [mmol Yb/L]	plasma concentration [% of initial]
	gadoxetic acid, disodium			
initial	0.98 ± 0.02	100	1.02 ± 0.03	100
1	0.054 ± 0.033	5.5 ± 3.4	0.040 ± 0.030	4.0 ± 3.0
2	0.052 ± 0.009	5.3 ± 1.0	0.037 ± 0.005	3.6 ± 0.6
3	0.0078 ± 0.0027	0.79 ± 0.3	0.0058 ± 0.0010	0.57 ± 0.11
4	0.0033 ± 0.0017	0.34 ± 0.2	0.0023 ± 0.0007	0.22 ± 0.07
5	0.0005 ± 0.0009 *	0.05 ± 0.1	< LOQ	< LOQ
6	< LOQ	< LOQ	< LOQ	< LOQ
7	< LOQ	< LOQ	< LOQ	< LOQ
8	< LOQ	< LOQ	< LOQ	< LOQ
9	< LOQ	< LOQ	< LOQ	< LOQ

LOQ: lower limit of quantification: 0.001 mmol/L Gd or corresponding to 0.1% of the initial value.
 * in two of the three experiments the concentration of gadoxetic acid, disodium was below the LOQ

Reviewer's comments:

This is an in vitro study, which is different from clinical situation in the following aspects:

- 300 mL plasma vs. 5-6 L blood
- Stepwise cycling vs. remixing of the filtered and unfiltered blood
- Flow rates: 14 mL/min plasma, 24 mL/min dialysate vs, 200 and 500 mL/min, respectively

However, according to the study report, these deviations should not significantly affect the dialysability of the compound. In addition, the internal standard (a compound with no protein binding and known to pass hemodialysis filters freely) was used to compensate for the above-mentioned deviations from the clinical situation and comparable results were obtained (Table 8).

The reviewer agreed with the above rationales.

2.6.4.9 Discussion and Conclusions N/A

2.6.4.10 Tables and figures to include comparative TK summary N/A**2.6.5 PHARMACOKINETICS TABULATED SUMMARY N/A****2.6.6 TOXICOLOGY****2.6.6.1 Overall toxicology summary**General toxicology:*Repeat dose toxicity studies:*

Two repeat dose (4 wk, once daily) toxicity studies were conducted in rats and dogs using IV routes. The following key study findings were observed:

Rat (12 wk recovery)

- Dose-related, reversible tubular vacuolation of the kidneys
- Statistically significant increase in kidney weights of rats receiving 2.0 mmol/kg, partially reversible
- Dose-related decrease in teste weight, statistically significant in absolute teste weight in males receiving 2.0 mmol/kg, reversible

NOAEL was established at 0.2 mmol/kg/day and the dose multiple to human exposure (0.025 mmol/kg) was 1.3 based on BSA.

Dog (no recovery group)

- Drug-related food vomiting, similar incidence in all drug groups
- Dose-related reduction in body weight gain and food consumption at ≥ 0.3 mmol/kg
- Dose-related prolongation in activated partial thromboplastin time at ≥ 0.3 mmol/kg
- Dose-related decrease in alpha-globulins, statistically significant in males receiving 1 mmol/kg only
- Dose-related increase in urinary iron excretion on Day 2
- Reduced absolute liver weight in males receiving 1 mmol/kg, correlating histologically to the reduced glycogen deposition in hepatocytes
- Dose-related, minimal to slight tubular cell vacuolation in the kidneys

NOAEL was established at 0.1 mmol/kg/day and the dose multiples to human exposure (0.025 mmol/kg) was 2.2 fold based on BSA.

Genetic toxicology:

- Salmonella Typhimurium Reverse Mutation Assay: Negative
- Chromosome Aberration Test in Human Lymphocytes in Vitro: Negative

- Mouse Micronucleus Test (SH L 569 A): Negative

Carcinogenicity: N/A

Reproductive and developmental toxicology:

The rat study:

- Increase in preimplantation loss in pregnant rats (16.7 %/animal vs. 7.7% for the concurrent controls, up to 15.4 % for the historic controls) given 0.5 mmol/kg/day (dose multiple: 3.2x)
- NOAEL for the rat study: 0.1 mmol/kg/day, dose multiples: 0.6x based on BSA

The rabbit study:

The following embryotoxic effects were noted in rabbits given 2.0 mmol/kg/day (dose multiple: 26 x).

- Increase in implantation resorption (22.6%/group vs. 12.2% for the concurrent controls, 7.2-20.9 % for the historic controls)
- Increase in postimplantation loss (22.6%/group vs. 12.2% for the concurrent controls, 12.0-22.2 % for the historic controls)
- Increase in absorptions (3/group vs. 0 for the concurrent controls, 0-2 for the historic controls)
- Decrease in the number of fetuses/litter (5.1 vs. 7.2 for controls, 6.4-8.6 for historic controls)

NOAEL for the rabbit study: 0.5 mmol/kg/day, dose multiples: 6.5 x based on BSA

Local tolerance:

Local tolerance studies in rats and rabbits were conducted using the formulations SHL 569 A and SHL 569 B using single intraarterial, intravenous, paravenous, and intramuscular injections. . However, an interim histopathological examination was not performed in majority of studies (except for IM study which had a histopathological examination on Day 3), therefore, acute microscopic lesions may not be revealed appropriately. Key drug-related findings are summarized below.

Key findings in intramuscular injection study using SHL 569 B

- Increased incidence (1/4 for the control group vs. 4/4 for the drug group on Day 3, 0/4 vs. 3/4 on Day 7) and severity in the drug group (mild interstitial hemorrhage and slight focal muscle fiber necrosis for the control group vs. slight to moderate interstitial hemorrhage, interstitial edema and focal muscle fiber necrosis for the drug group on Day 3).

- Lesions resolving by Day 7 (4/4, slight to moderate focal muscle fiber necrosis on Day 3 vs. 1/4, slight on Day 7).

Key findings in paravenous injection study using SHL 569 B

- Slight focal proliferation of fibroblasts in the subcutis in ¼ rabbits on Day 7

Key findings in intraarterial injection study using SHL 569 A

- Drug related functional disturbance in the leg injected the drug in ¼ rat, resolved by Day 4
- Dark brownish dots correlating with an “exfoliated parakeratotic scales” with minimal inflammation and hemorrhage in this rat

Key findings in intravenous injection study using SHL 569 A

- Drug related injection site reddening

Key findings in paravenous injection study using SHL 569 A

- Slight to moderate, reversible reddening and swelling in injection sites in drug group only
- White marblization of the subcutis at the injection sites of all four animals in drug group only.
- Moderate to marked focal necrosis accompanied by a moderate to marked cellular proliferating granulation tissue, in part, with giant cells and calcification at the injection sites of all four animals in drug group only.

2.6.6.2 Single-dose toxicity

Seven single IV dose toxicity studies were conducted in mice, rats, and dogs and reviewed previously for IND. All studies used the formulation SHL 569 A except one. All studies were not adequately designed and conducted. For example, all studies used 1-3 animals/sex/group except one (6/sex/group), no sacrifice and histopathological examination were conducted in an early phase and furthermore, histopathological examination in late sacrifices (Day 14 post dosing) were conducted for testes only. Key findings were summarized below based on previous IND review.

- Dose-related mortalities: For example, 0/3, 1/3, and 3/3 mice died at 7.5, 10, and 12.5 mmol Gd/kg, respectively, within 2 days post dosing.
- Dose-related clinical signs such as apathy, changes in respiratory rate, and abnormal positions, reversible in survived animals
- Pulmonary fluid and pale liver and/or kidneys in the prematurely dead animals

2.6.6.3 Repeat-dose toxicity

Two repeat-dose toxicity studies were conducted using SHL569B (ZK 139834) and reviewed separately below.

Study title: SHL569B (ZK 139834) Systemic tolerance study in rats (M+F) after single daily i.v. administration over ca. 4 weeks with a subsequent observation period of ca. 12 weeks

Key study findings:

- Dose-related, reversible tubular vacuolation of the kidneys;
- Statistically significant increase in kidney weights of rats receiving 2.0 mmol/kg, partially reversible;
- Dose-related decrease in teste weight, statistically significant in absolute teste weight in males receiving 2.0 mmol/kg, reversible;
- NOAEL established at 0.2 mmol/kg/day, the dose multiple to human exposure (0.025 mmol/kg) 1.3 based on BSA.

Study no.: TXST20000151, Nonclinical Study Report No. A03248

Volume #, and page #: Module 4 page 1-440

Conducting laboratory and location: Schering AG, Experimental Toxicology, 13342 Berlin, Germany

Date of study initiation: 06 July 2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Batch #: N7808-1 (BB0231), purity, N/A

Methods

Doses: see Table 9.

Table 9. Summary of the Study Design

Group	Number of animals/sex	Compound	Dose per day [mmol/kg ZK 139834]	Concentration [mol/L ZK 139834]	Administration volume [mL/kg]	Day of sacrifice
1 (control)	10M/10F	0.9% (w/v) NaCl-solution	0.0	0.000	8.0	30-32
2	10M/10F	Dilution of SH L569B	0.2	0.166	1.2	30-32
3	10M/10F	SH L569B	0.6	0.250	2.4	30-32
4	10M/10F	SH L569B	2.0	0.250	8.0	30-32
5 (control)	10M/10F	0.9% (w/v) NaCl-solution	0.0	0.000	8.0	113-114
6	10M/10F	SH L569B	2.0	0.250	8.0	113-114

Species/strain: Rat/Wistar (Shoe: WIST)

Number/sex/group or time point (main study): 10/sex/group

Age: N/A

Weight: 246 – 315 g for males and 145 – 187 g for females

Observations and times:

Mortality: Daily.

Clinical signs: Twice daily from Days 1-45, daily thereafter

Body weights: Wk -2, Day 1, then weekly until Wk 4 (group 1-4) or 16 (groups 5 and 6)

Food consumption: Weekly

Ophthalmoscopy: Week 4 for 5 males and 5 females of each group, Week 12 for groups 5 and 6 as well

ECG: Not performed

Hematology: 5 male and 3 to 5 female animals per group in weeks 1, 2, and 4 for groups 1 to 4 and in weeks 2, 3, 5, 9, and 16 for treatment groups 5 and 6

Clinical chemistry: 5 male and 4 to 5 female animals per group, Week 2 and 4 for groups 1 to 4 and Week 3, 5, 9, and 16 for groups 5 and 6. In addition, the serum enzymes AST, ALT and ALP in week 1 (Day 2).

Coagulation: 3 to 5 male and 4 to 5 female animals per group in week 1, 2, and 4 and for groups 5 and 6 additionally in weeks 9 and 16.

Urinalysis: Spontaneous urine samples collected over a period of about 24 hours from 5 male and 4 to 5 female animals per group in week 4 and additionally in week 9 and 16 for groups 5 and 6.

Gross pathology: Terminal and recovery sacrifices.

Organ weights: See histopath table

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Tissues from each rat in the control and 2 mmol/kg/day groups and gross lesions from all rats were examined microscopically.

Results

Mortality: One animal (no. 92F) died due to arterial puncture during blood sampling procedure (on Day 112 of the study), according to the study report.

Clinical signs: No remarkable drug related findings.

Body weights: No remarkable drug related findings.

Food consumption: No remarkable drug related findings.

Ophthalmoscopy: No remarkable drug related findings.

ECG: Not performed.

Hematology: No remarkable drug related findings.

Clinical chemistry: No remarkable drug related findings.

Urinalysis: Dose-related increase in specific gravity, statistically significant at 2.0 mmol/kg ZK 139834 for males at week 4 (groups 4 and 6, $p < 0.05$ to $p < 0.01$) and for females at weeks 4 and 16 (only group 6, $p < 0.05$). Nevertheless, the values remained within the historical ranges and were not considered to be biological significant, according to the study report.

Gross pathology: No remarkable drug related findings.

Organ weights:

Increase in kidney weights in rats receiving 2.0 mmol/kg ZK 139834 at the terminal (absolute weight in females only, $p < 0.05$, and relative weight in both males and females, $p < 0.01$) and recovery sacrifices (absolute and relative weight in males only, $p < 0.05$).

Dose-related decrease in teste weight, statistically significant in absolute teste weight of males receiving 2.0 mmol/kg ZK 139834 at the terminal sacrifice only (3.4±0.4 vs. 3.7±0.3 for the controls, p < 0.05).

Dose-related increase in thyroid gland weights in females, statistically significant in females receiving 2.0 mmol/kg ZK 139834 at the terminal sacrifice only (14±3 vs. 11±3 for the controls, p < 0.05).

Histopathology:

Tubular vacuolation of the kidneys:

- Dose-related increases in both frequency and severity (Table 10)
- Reversible

Table 10. Drug-related histological findings (number of animals affected)

Group	1		2		3		4		5		6		
	Animals sacrificed after the end of treatment										Recovery animals		
	Control		ZK 139834						Control		ZK 139834		
	Dose (mmol/kg ZK 139834)		0		0.2		0.6		2.0		0		2.0
Sex	M	F	M	F	M	F	M	F	M	F	M	F	
	10	10	10	10	10	10	10	10	10	9*	10	10	
Organ/finding	Number of animals examined												
Kidneys - tubular vacuolation	Grade												
	1	-	-	-	-	9	10	-	-	-	-	-	1
	2	-	-	-	-	1	-	-	4	-	-	-	-
	3	-	-	-	-	-	-	6	6	-	-	-	-
	4	-	-	-	-	-	-	4	-	-	-	-	-

* one rat died

- = no findings, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Colloid plug in urinary bladder in 4 (minimal for 2 and slight for 2, n=10) males receiving 2.0 mmol/kg only at terminal sacrifice and in 2 and 4 (minimal, n=10) males receiving 0 and 2.0 mmol/kg, respectively, at recovery sacrifice.

Lung granuloma in 1 (slight), 1 (minimal), and 3 (minimal for 2 and slight for 1) males receiving 0, 0.6, and 2.0 mmol/kg, respectively, at terminal sacrifice.

Minimal liver microgranuloma in 1 and 3 females receiving 0 and 2.0 mmol/kg, respectively, at terminal sacrifice.

Inflammation, thrombophlebitis/thrombarteritis, and hemorrhage in injection sites, comparably present in both control and drug groups, procedure-related.

Toxicokinetics: Not performed.

Other:

Histopathology inventory (optional)

Organ	W	Hist. Ex.						Organ	W	Hist. Ex.					
		1	2	3	4	5	6			1	2	3	4	5	6
Liver	w	x	P	P	x	x	x	Testes	wp	x	P	P	x	x	x
Kidneys	wp	x	x	x	x	x	x	Prostate gland	w	x	P	P	x	x	x
Urinary bladder		x	P	P	x	x	x	Seminal vesicle	wp	x	P	P	x	x	x
Heart	w	x	P	P	x	x	x	Mammary gland		x	P	P	x	x	x
Trachea		x	P	P	x	x	x	Skin (<i>Back - lumbar region</i>)		x	P	P	x	x	x
Lung	w	x	P	P	x	x	x	Thymus	w	x	P	P	x	x	x
Submandibular glands	wp	x	P	P	x	x	x	Spleen	w	x	P	P	x	x	x
Tongue		x	P	P	x	x	x	Mandibular lymph nodes		x	P	P	x	x	x
Esophagus		x	P	P	x	x	x	Sternum (<i>incl. bone marrow</i>)		x	P	P	x	x	x
Stomach		x	P	P	x	x	x	Femur (<i>incl. bone marrow</i>)		x	P	P	x	x	x
Duodenum		x	P	P	x	x	x	Brain	w						
Jejunum		x	P	P	x	x	x	- Cerebrum		x	P	P	x	x	x
Ileum		x	P	P	x	x	x	- Cerebellum		x	P	P	x	x	x
Caecum		x	P	P	x	x	x	- Medulla oblongata		x	P	P	x	x	x
Colon		x	P	P	x	x	x	Spinal cord (<i>cervical</i>)		x	P	P	x	x	x
Rectum		x	P	P	x	x	x	Eyes		x	P	P	x	x	x
Pancreas	w	x	P	P	x	x	x	Harderian glands		x	P	P	x	x	x
Pituitary gland	w	x	P	P	x	x	x	Application site		x	P	P	x	x	x
Thyroid glands with parathyroid glands	wp	x	P	P	x	x	x	Macroscopic findings (<i>if necessary for evaluating a diagnosis</i>)		x	x	x	x	x	x
Adrenal glands	wp	x	P	P	x	x	x	Iliac lymph nodes	wp	P	P	P	P	P	P
Ovaries	wp	x	P	P	x	x	x	Skeletal muscle (<i>M. gastrocnem.</i>)		P	P	P	P	P	P
Uterus	w							Peripheral nerve (<i>N. saphenus</i>)		P	P	P	P	P	P
- Horns		x	P	P	x	x	x	Aorta (<i>thoracic</i>)		P	P	P	P	P	P
- Corpus		x	P	P	x	x	x	Vein (<i>Vena cava caudalis</i>)		P	P	P	P	P	P
- Cervix		x	P	P	x	x	x	Heart - Atria (<i>left and right</i>)		P	P	P	P	P	P
Vagina		x	P	P	x	x	x	Epididymides		P	P	P	P	P	P

Organs/Tissues in bold print = paired examination

W = Weighing, w = organ weight, wp = paired organ weight, Hist. Ex. = Histological examination, 1-6 = treatment group 1-6, x = examined histologically, P = organ/tissue samples preserved

Reviewer’s comments:

Dose-related tubular vacuolation of the kidneys, a common finding among gadolinium contrasts, were observed. It is reversible as evidenced by the fact that only minimal vacuolation was seen in one rat after the recovery period of 12 weeks. According to the study report’s speculation, the transient vacuolation is due to an accumulation of SHL569B after glomerular filtration and subsequent re-transportation (by endocytosis) into the respective cells. Because the vacuolation was not accompanied by any negative influence on the kidney functions, the SH L569B-induced tubular vacuolation is not considered to be biological significant by the study report. However, considering the recent association of human nephrogenic systemic fibrosis (NSF) with gadolinium contrasts, accumulation of gadolinium contrasts in the kidneys especially in the kidneys of patients with severe renal failures might not be such a benign phenomenon. In addition, increased kidney weight was noted at 2.0 mmol/kg group even after 12 week recovery period.

No significant drug related effects were observed at doses 0.2 mmol/kg/day, therefore, NOAEL could be established at 0.2 mmol/kg/day for this study and the dose multiple to human exposure (0.025 mmol/kg) was 1.3 based on BSA.

Study title: SH L569B (ZK 139834) Systemic tolerance study in dogs after daily i.v. administration over ca. 4 weeks.

Key study findings:

- Drug-related food vomiting, similar incidence in all drug groups
- Dose-related reduction in body weight gain and food consumption at ≥ 0.3 mmol/kg
- Dose-related prolongation in activated partial thromboplastin time at ≥ 0.3 mmol/kg
- Dose-related decrease in alpha-globulins, statistically significant in males receiving 1 mmol/kg only
- Dose-related increase in urinary iron excretion on Day 2
- Reduced absolute liver weight in males receiving 1 mmol/kg, correlating histologically to the reduced glycogen deposition in hepatocytes
- Dose-related, minimal to slight tubular cell vacuolation in the kidneys

NOAEL was established at 0.1 mmol/kg/day and the dose multiples to human exposure (0.025 mmol/kg) was 2.2 fold based on BSA.

Study no.: TXST20000152, Report No. A03186

Volume #, and page #: Module 4, page 1-330

Conducting laboratory and location: Schering AG, Experimental Toxicology, 13342 Berlin, Germany

Date of study initiation: 06 July 2000

GLP compliance: Yes.

QA report: yes (X) no ()

Drug, lot #, and % purity: SHL569B (0.25 mol/L), Batch #: N7808-1 (BB0231), purity: N/A

Methods

Doses: 0 [0.9% (w/v) NaCl], 0.1, 0.3, or 1 mmol/kg/day for 28 to 31 days.

Table 11. Summary of the Study Design

Group	Compound	Dose [mmol/kg ZK 139834]	Concentration [mmol/mL ZK 139834]	Application volume [mL/kg SH L569B]	Animal nos. and sex
1	0.9% (w/v) NaCl-solution	0 control	0	4.0	106M, 9462M, 9485M 7146F, 7178F, 7236 F
2	SH L569B	0.1	0.25	0.4	278M, 304M, 9484M 2651F, 7124F, 7210F
3	SH L569B	0.3	0.25	1.2	101M, 9385M, 9416M 1201F, 7094F, 7182F
4	SH L569B	1.0	0.25	4.0	120M, 3121M, 9502M 1195F, 7191F, 7204F

Species/strain: Dog/Beagle
Number/sex/group or time point: 3/sex/group
Route, formulation, volume, and infusion rate: intravenous, dose volume of 4.0, 0.4, 1.2, or 4.0 mL/kg, 9 mL/min.
Satellite groups used for toxicokinetics or recovery: N/A
Age: male: 13 to 26 months, female: 12 to 36 months
Weight: male: 7.6 – 17.2 kg, female: 6.8 – 12.3 kg
Sampling times: N/A
Unique study design or methodology (if any): N/A

Observations and times:

Mortality: Twice Daily

Clinical signs: Twice Daily

Nervous system functions: On Day -11 and on Day 26

a) brain reflexes: (pupillary light reflex, consensual light reflex, palpebral reflex, corneal reflex, gag and cough reflexes), b) spinal nerve reflexes: (flexor reflex, patellar reflex, supratarsal and supracarpal reflex, anal reflex), c) attitudinal reactions: [extensor postural thrust reaction, hopping reaction, placing reaction, Schuster reflex, (tactile reaction), righting reaction, tonic neck reaction], d) sensitivity of the skin of the torso and extremities.

Body weights: On Day -7, Day 1, then once weekly until Day 29

Food consumption: Daily except for Day 1 (technical problem according to the study report)

Ophthalmoscopy: On Day -13 or -12 and on Day 25

Blood pressure: Systolic and diastolic blood pressure using direct measurement in the femoral artery on Days -6 to -5, Day 16 to 18 (week 3, immediately after administration) and on Day 24, 29, 30 or 32 (week 4, about 24 hours after administration).

ECG: on Days -6 to -5, Day 16 to 18 (week 3, immediately after administration) and on Day 24, 29, or 30 (week 4, about 24 hours after administration). The standard limb leads I, II, III, aVR, aVL, and aVF were registered, but only leads I, II, and III were evaluated for statistical calculations. The other leads were checked by visual comparison only.

Heart rate was extrapolated from the number of the R-waves per minute.

Hematology: Blood samples were collected on days -7 (pre-values), 11, and 26.

Clinical chemistry: Blood samples were collected on days -7 (pre-values), 2, 11, 26, and 30 to 32 prior to necropsy. In addition, blood samples were collected at 2, 4, 8, and 24 hours after the first administration from dogs of control and high dose groups and iron level was measured using Electrothermal Atomic Absorption Spectrometry (ETAAS).

Urinalysis: Urine samples were collected over a period of about 24 hours (spontaneous and catheter urine were mixed together) on days -10 (pre-values) and 23. In addition, spontaneous as well as in catheter urine samples were collected over a period of about 24 hours on day 2 for iron measurement.

Gross pathology: Days 30-32.

Organ weights: See histopath table

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Tissues from dogs of the control and high dose groups, and other dogs with drug-related necropsy findings and/or relevant alterations in organ weights (absolute and relative) or “target organs” were examined microscopically.

Results

Mortality: None.

Clinical signs: Food vomiting was noted in the animals receiving the drug only (0/6, 2/6 (2F), 4/6 (2M&2F), and 3/6 (1M&2F) at 0, 0.1, 0.3, and 1 mmol/kg, respectively). Only single/sporadic vomiting was noted at 0.1 mmol/kg group while multiple/frequent at higher dose groups.

Body weights: Dose-related reduction at ≥ 0.3 mmol/kg (Table 12)

Table 12. Summary of Body Weight Gain (kg, mean \pm SD, n=3, Days 1-29)

	0 mmol/kg	0.1 mmol/kg	0.3 mmol/kg	1 mmol/kg
Males	0.3 \pm 0.1	0.4 \pm 0.1	-0.2 \pm 0.4	-0.8 \pm 0.5**
Females	0.0 \pm 0.2	0.5 \pm 0.3	-0.3 \pm 0.7	-0.6 \pm 0.3

**p<0.01, the table was prepared by the reviewer based on the data in the submission.

Food consumption: Dose-related reduction at ≥ 0.3 mmol/kg (Table 13)

Table 13. Summary of Food Consumption (g/d, mean \pm SD, n=3)

	0 mmol/kg	0.1 mmol/kg	0.3 mmol/kg	1 mmol/kg
Males	438 \pm 125	404 \pm 63	296 \pm 89	229 \pm 53*
Females	274 \pm 45	277 \pm 27	237 \pm 37	231 \pm 54

*p<0.05, the table was prepared by the reviewer based on the data in the submission.

Ophthalmoscopy: No remarkable drug-related findings.

Blood pressure: No remarkable drug-related changes.

ECG: Dose related decrease in heart rate was noted in males during Days 24-30 (Table 14).

Table 14. Summary of Heart Rate (beats/min, mean \pm SD, Days 24-30, n=3)

	0 mmol/kg	0.1 mmol/kg	0.3 mmol/kg	1 mmol/kg
Males	135 \pm 12	111 \pm 8	96 \pm 1**	87 \pm 14**
Females	137 \pm 17	108 \pm 21	143 \pm 10	98 \pm 21

**p<0.01, the table was prepared by the reviewer based on the data in the submission.

No other remarkable drug-related changes.

Hematology:

Dose-related prolongation in activated partial thromboplastin time (APTT) was noted on Days 11 and 26 with similar magnitudes and trends. The data derived from Day 26 are presented in Table 15. Slight decrease in fibrinogen in male ($p < 0.05$, 203 ± 22 for controls vs. 130 ± 8 mg/100 mL for animals receiving 1.0 mmol/kg on Day 26) and female (n.s.) animals was also observed at the 1.0 mmol/kg group on Days 11 and 26. There is no clear dose response in decrease in fibrinogen.

Table 15. Summary of APTT (Sec, Mean \pm SD, Day 26, n=3)

	0 mmol/kg	0.1 mmol/kg	0.3 mmol/kg	1 mmol/kg
Males	15.0 \pm 1.4	15.6 \pm 0.6	16.8 \pm 0.3	19.6 \pm 0.7**
Females	15.5 \pm 0.5	15.6 \pm 0.5	16.6 \pm 0.9	18.4 \pm 0.8**

** $p < 0.01$, the table was prepared by the reviewer based on the data in the submission.

No other remarkable drug-related findings.

Clinical chemistry:

Dose-related decrease in relative alpha-globulins (Table 16) was observed on Days 11 and 26.

Table 16. Summary of Relative alpha-Globulins (% , Mean \pm SD, Day 26, n=3)

	0 mmol/kg	0.1 mmol/kg	0.3 mmol/kg	1 mmol/kg
Males	17.2 \pm 0.4	17.1 \pm 0.2	16.5 \pm 0.6	13.5 \pm 0.9**
Females	18.0 \pm 1.1	17.9 \pm 1.2	18.3 \pm 4.2	14.2 \pm 0.4

** $p < 0.01$, the table was prepared by the reviewer based on the data in the submission.

No other remarkable drug-related findings.

Urinalysis: Dose-dependent increase in urinary iron excretion on Day 2 (Table 17).

Table 17. Summary of Urinary Iron Excretion (Mean/SD, n=3)

Group	$\mu\text{g Fe/mL}$		$\mu\text{g Fe/24 h}$	
	males	females	males	females
1 (Control)	0.21/0.14	0.23/0.16	34.55/23.40	11.59/2.42
2 (0.1 mmol/kg ZK 139834)	0.58/0.20	0.55/0.22	43.13/19.29	37.85/12.86
3 (0.3 mmol/kg ZK 139834)	0.79/0.20 *	1.12/0.12 *	76.17/16.13	77.72/30.42
4 (1.0 mmol/kg ZK 139834)	1.33/0.16 **	1.33/0.55 **	131.85/28.49 *	99.36/61.05 *

* $p < 0.05$, ** $p < 0.01$, measured in total urine samples collected over a period of about 24 hours on Day 2.

According to the study report, the qualitative analysis revealed that iron in urine was excreted most likely as Fe-EOB-DTPA, which was formed in the body as a result from recomplexation. Due to analytical difficulties, the amount of iron excreted as Fe-EOB-DTPA could not be determined exactly, but it could be calculated as being in the range of 50 – 300 mcg iron in 24 hours.

Gross pathology: No remarkable drug related findings.

Organ weights: The absolute liver weight was significantly reduced in male animals at 1 mmol/kg ZK 139834. This correlates histologically to the reduced glycogen deposition in hepatocytes and is considered a consequence of the reduced food intake especially expressed in male animals.

Histopathology:

A minimal to slight vacuolation of tubular cells in the kidneys was noted in 1/3 males (minimal) in the 0.1 mmol/kg group and 2/3 male (minimal or slight) and 1/3 female (minimal) dogs treated with the 1.0 mmol/kg ZK 139834. In addition, minimal tubular basophilia was noted in 1/3 females in 0.1 mmol/kg and 1/3 males in 1.0 mmol/kg group, minimal interstitial nephritis in 1/3 males in 0.3 mmol/kg group, minimal pyelitis in 1/3 males in 0.1 and 1 mmol/kg groups, minimal hyaline casts in 1/3 females in 1.0 mmol/kg group and moderate focal necrosis in 1 female each (1/3) in 0.1 and 0.3 mmol/kg groups.

A complete depletion of glycogen in hepatocytes was noted in male animals receiving 1.0 mmol/kg ZK 139834, which was considered to be a consequence of the reduced food intake accompanied by body weight loss by the study report. Minimal (1/3) or moderate (2/3) pigmentation of Kupffer cells was also noted in males receiving 1.0 mmol/kg ZK 139834, which was not observed in male dogs in control and lower dose groups. However, the pigmentation of Kupffer cells was noted in females in both control (2/3, minimal) and drug groups (0.3 mmol/kg: 1/3, slight and 1.0 mmol/kg: 1/3, slight). In addition, moderate local liver necrosis was noted in 1/3 female in both 0.1 and 0.3 mmol/kg groups.

Lung and pancreas granuloma was noted in one female in 0.1 mmol/kg (1/3, moderate), and lung granuloma in one male in 1.0 mmol/kg (1/3, minimal) groups.

Moderate inflammation and marked tubular atrophy in testes was noted in one male in 0.3 mmol/kg group. Minimal luminal dilation in testes was noted in 2/3 males in 1.0 mmol/kg group. Periductular edema (minimal or slight) and hemorrhage (minimal or moderate) in mammary gland were noted in 1/3 female in 0.1 mmol/kg group and 2/3 females in 1.0 mmol/kg group.

Dermatitis, lymphohistiocytic infiltration, phlebitis/periphlebitis, and hemorrhage were observed at the injection sites and the incidence and severity were comparable between the control and high dose groups, which were considered to be related to the procedure.

Toxicokinetics: Not performed.

Other:

Histopathology inventory (optional)

- liver (W) *	- adrenal glands (W)
- gall bladder	- uterus (W) [horns, corpus, cervix]
- kidneys (W) *	- ovaries (W)
- urinary bladder	- vagina
- heart (W) *	- testes (W) [B] *
- heart - atria	- epididymides
- lungs (W)	- prostate gland (W)
- aorta (thoracic)	- mammary gland
- vein (V. cava caudalis)	- skin (back - lumbar region)
- trachea	- thymus (W)
- esophagus	- spleen (W)
- tongue	- iliac lymph nodes (W)
- submandibular glands (W)	- mandibular lymph nodes
- stomach	- bone [femur with joint, sternum] (both with bone marrow)
- duodenum	- brain (w) [cerebrum, cerebellum, medulla oblongata]
- jejunum	- spinal cord (cervical)
- ileum	- intraorbital lacrimal glands
- caecum	- peripheral nerve (N. Saphenus)
- colon	- eyes
- rectum	- skeletal muscle (M. gastrocnem.)
- pancreas (W)	- application site
- pituitary gland (W) [B]	- and any other organ/tissue with macroscopic alterations, if necessary for evaluating a diagnosis
- thyroid glands with parathyroid glands (W)	

(W) = Organ weight was recorded, [B] = Bouin's fixative, bold print = examined histologically in controls and top dose group, * = additional histological examination in low- and intermediate-dose group

Conclusions:

According to the study report, no toxicological relevant adverse effects were observed after daily intravenous administration of SH L569B to dogs at the low dose level of 0.1 mmol/kg ZK 139834 over a period of 28 to 31 days. Minor clinical signs after this dose are interpreted as signs of malaise being of minor biological significance.

From the mid dose of 0.3 mmol/kg ZK 139834 upwards beginning impaired general condition in the form of decreased food consumption and body weight gain were regarded as signs of beginning general toxicity. After the high dose of 1.0 mmol/kg ZK 139834 additionally slight effects on blood coagulation were observed.

The high dose of 1.0 mmol/kg ZK 139834 is regarded to represent the maximum tolerated dose in the dog because of the prominent reduction in food consumption as well as body weight gain associated with effects on alpha-globulins, activated partial thromboplastin time and fibrinogen. The effects on the coagulation parameters are considered to be possibly due to the _____ which is used _____ ingredient of the formulation.

The vacuolization of tubular cells in the kidneys noted in the present study represents a known finding in rats treated with various contrast media, which is regarded to represent a storage phenomenon of the test compound in the respective cells without having any impact on kidney functions. Because this finding was not observed in a previous dog study when dogs were given 1.0 mmol/kg ZK 139834 5 days per week, the observed vacuolation in the present study (7 days per week) can be regarded as biologically non-relevant in respect of the intended single use of SH L569B in humans, according to the study report.

Reviewer's comments:

_____ is used as _____ ingredient in the formulation. According to the study report, dose-related reduction in APTT and fibrinogen, and increase in urinary iron excretion are possibly due to the _____. In addition, the excreted iron in urine samples was identified as complex-bound Fe-EOB-DTPA. Therefore, the _____ may be associated with some SH L569B-related adverse effects.

NOAEL was established at 0.1 mmol/kg/day and the dose multiples to human exposure (0.025 mmol/kg) was 2.2 fold based on BSA.

2.6.6.4 Genetic toxicology

Two in vitro genetic toxicology studies were conducted using SHL 569 B and reviewed below. In addition, the genetic toxicology studies were also conducted using the formulation SHL 569 A and is enclosed in the appendix.

Study title: Salmonella Typhimurium Reverse Mutation Assay with SHL 569 B Including the Impurity _____

Key findings: SHL 569 B did not induce significant increase in revertant colony numbers in all test strains either in the presence of S-9 or in the absence of S-9.

Study no.: _____ study number 803105, Schering Study Number: TXEX20030029, Report #: A18455

Volume #, and page #: Module 4, page 1-32

Conducting laboratory and location: _____

Date of study initiation: 16 October 2003

GLP compliance: Yes. No stability report was provided.

QA report: yes (X) no ()

Drug, lot #, and % purity: SHL 569 B, containing the active ingredient ZK 139834 and the impurity _____ at a level of _____ (w/v), Batch #, N0564B01, purity, N/A

Methods

Plate incorporation test (experiment I) and the pre-incubation test (experiment II)

Strains/species/cell line: *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537).

Doses used in definitive study:

Experiment I: 0.08, 0.16, 0.312, 0.625, 1.25, 2.5, 5, or 10 mM /plate (=58.05 – 7257.1 mcg/plate of the active ingredient ZK 139834 and 1.36 – 170 mcg/plate of the impurity _____)

Experiment II: 0.16, 0.312, 0.625, 1.25, 2.5, 5, or 10 mM /plate (=116.1 – 7257.1 mcg/plate of the active ingredient ZK 139834 and 2.72 – 170 mcg/plate of the impurity _____)

Basis of dose selection:

A pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction.

Cytotoxic effects, evident as a reduction in the number of revertants, were observed and summarized in Table 18.

Table 18. Summary of Cytotoxicity

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	2.5 - 10	5 - 10	/	5
TA 1537	5 - 10	5 - 10	5 - 10	5 - 10
TA 98	/	/	/	/
TA 100	10	10	/	10
TA 102	1.25 - 5	2.5 - 5	1.25 - 10	2.5 - 10

/= no cytotoxicity observed, concentrations as mM/plate.

Negative controls: None.

Solvent controls: Phosphate buffer.

Positive controls: See Table 19.

Table 19. Summary of Positive Control Agents*

Assay	Chemicals	Conc. (mcg/plate)	Responding strains
Nonactivation	4-nitro-o-phenylene-diamine (4-NOPD)	10.0	TA 98
	Sodium azide (NaN ₃)	10.0	TA 100, 1535
	4-nitro-o-phenylene-diamine (4-NOPD)	50.0	TA 1537
	Methyl methane sulfonate (MMS)	4.0 (mcL/plate)	TA 102
Activation	2-aminoanthracene (2-AA)	2.5	TA 98, 100, 1535, 1537
	2-aminoanthracene (2-AA)	10.0	TA 102

*Prepared by the reviewer based on the submission.

Incubation and sampling times: at least 48 hours at 37° C in the dark.
 Pre-incubated for 1h at 37°C for the pre-incubation test.

Results

Study validity: The study was valid for the following reasons:

1. Concentration selection was acceptable because the highest concentration used was > 5 mg/plate and cytotoxicity as evidenced by reduction in the number of revertant colonies was observed;
2. The negative control counts fell within the historical ranges except that in experiment I, the counts for the negative control of strain TA 100 were slightly above the historical control range (198 vs. 77-189). This deviation was rather small and not considered to have significant impact on the study result interpretation.
3. The positive controls induced significant increases in revertant colony numbers and fell within the historical ranges except for TA 1535 in the absence of S-9 in the Exp. I (931 vs. 68-814 for historical range), and 1537 (448 for Exp. I and 232 for Exp. II vs. 47-175,) in the presence of S-9. This higher counts were considered not compromising to the interpretation of the data with negative results.
4. Triplicate cultures;
5. The colonies were counted using the _____

Study outcome:

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with SH L 569 B including the impurity _____ at any dose level in the presence or absence of S9 mix (Tables 20-21).

Table 20. Summary of Revertant Colony Numbers in the Absence of S9

Concentration mM/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative Control	23	22	9	8	25	36	198	172	232	229
Solvent Control	24	18	8	13	21	29	208	153	234	214
Positive Control#	931	807	59	73	147	210	570	700	1319	1134
0.08	/	/	/	/	/	/	/	/	226	/
0.16	28	21	9	11	23	31	210	181	243	251
0.312	27	22	10	11	23	27	206	179	207	230
0.625	29	22	11	11	22	29	218	183	200	204
1.25	20	22	7	6	24	26	175	178	75	113
2.5	10	14	5	6	23	27	183	176	34	41
5	4	12	2	5	14	23	141	150	0	11
10	7	10	2	0	14	18	74	120	/	0

see Table 19.

Table 21. Summary of Revertant Colony Numbers in the Presence of S9 Mix

Concentration mM/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative Control	16	15	7	9	31	37	184	182	237	266
Solvent Control	14	18	10	13	19	28	208	204	227	226
Positive Control**	342	314	448	232	628	467	1201	591	1320	1094
0.08	/	/	/	/	/	/	/	/	253	/
0.16	13	23	8	9	26	29	220	222	272	244
0.312	13	17	8	10	24	32	246	227	250	284
0.625	14	13	8	10	19	23	269	188	248	243
1.25	13	15	9	8	26	24	184	219	144	206
2.5	10	13	7	6	24	20	214	160	48	103
5	4	8	2	4	14	17	152	146	11	18
10	5	9	3	0	11	17	45	62	/	0

** see Table 19.

Conclusion:

During the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, SH L 569 B including the impurity _____ at a level of _____ (w/v) is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

Reviewer's comments:

The reviewer concurred with the conclusion.

Study title: Chromosome Aberration Test in Human Lymphocytes in Vitro with SHL 569 B including the impurity _____

Key findings: SHL 569 B was negative in the chromosome aberration assay in human peripheral blood lymphocytes with or without S9 under the experiment condition.

Study no.: _____ **Study #:** 803103, Schering **Study #:** TXEX20030027, Report #: A18454

Volume #, and page #: Module 4, page 1-36

Conducting laboratory and location: _____

Date of study initiation: N/A. **Date of study completion:** January 13, 2004

GLP compliance: Yes.

QA report: yes (X) no ()

Drug, lot #, and % purity: SHL 569 B, containing the active ingredient ZK 139834 and the impurity _____, at a level of _____ (w/v), Batch #, N0564B01, purity, N/A

Method

Strains/species/cell line: Human peripheral blood lymphocytes from blood samples obtained from healthy donors receiving no medication. The blood was collected from a female donor (43 years old) and a male donor (40 years old) for experiment I and II, respectively.

Doses used in definitive study: Table 22

Table 22: SHL 569 B concentrations in the chromosome aberration assay*

Exp.	Prep. interval	Exposure period	Concentrations in % (v/v)					
			without S9 mix					
I	22 hrs	4 hrs	0.5	1.0	1.5	2.0	3.0	4.0
I	22 hrs	22 hrs	0.5	1.0	1.5	2.0	3.0	4.0
II	46 hrs	46 hrs			1.0	2.0	3.0	4.0
with S9 mix								
I	22 hrs	4 hrs	0.5	1.0	1.5	2.0	3.0	4.0
II	46 hrs	4 hrs			1.0	2.0	3.0	4.0

* 4 % (v/v) of SHL 569 B corresponds to 10 mM of the active ingredient.

Basis of dose selection: Range-finding study. The cytogenetic evaluation of concentrations higher than 1 % (v/v) in the 46 hrs preparation interval (without S9 mix) was impossible due to strong cytotoxicity (low metaphase numbers, partially paralleled by poor metaphase quality).

Negative controls: culture medium (DMEM:F12).

Positive controls:

Positive control in the absence of S9 was EMS [ethylmethane sulfonate, 550 mcg/mL (4.43 mM, 22 hrs preparation interval) and 440 mcg/mL (3.54 mM, 46 hrs preparation interval)].

The positive control in the presence of S9 was cyclophosphamide (CPA, 15.0 mcg/mL (0.053 mM, 46 hrs preparation interval) and 22.5 mcg/mL (0.079 mM, 22 hrs preparation interval).

Incubation and sampling times: Table 23.

Table 23. Summary of Exposure Time

	without S9 mix			with S9 mix	
	Exp. I		Exp. II	Exp. I	Exp. II
Exposure period	4 hrs	22 hrs	46 hrs	4 hrs	4 hrs
Recovery	18 hrs	—	—	18 hrs	42 hrs
Preparation interval	22 hrs	22 hrs	46 hrs	22 hrs	46 hrs

Result

Study validity: The study was valid for the following reasons:

- 1). Concentration selection, cell treatments, and culture conditions were acceptable (Tables 22 and 23); the highest concentration used was 10 mmol.
- 2). Negative control cultures gave a negative result and values were within historic range (0-4%).
- 3). Positive controls induced statistically significant increase in the frequency of structure aberrations ($p < 0.001$ compared to the negative control).
- 4). Cytotoxicity is characterized by the percentages of mitotic suppression in comparison to the controls by counting 1000 cells per culture in duplicate (Tables 24 and 25).

Table 24. Cytotoxicity of SHL 569 B (Experiment I)

Concentration in % (v/v)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of negative control
without S9 mix				
Negative control	4 hrs	22 hrs	18.3	100
0.5	4 hrs	22 hrs	15.0	82
1.0	4 hrs	22 hrs	13.3	72
1.5	4 hrs	22 hrs	12.8	70
2.0	4 hrs	22 hrs	16.3	89
3.0	4 hrs	22 hrs	13.4	73
4.0	4 hrs	22 hrs	17.2	94
Negative control	22 hrs	22 hrs	15.9	100
0.5	22 hrs	22 hrs	19.7	124
1.0	22 hrs	22 hrs	19.6	123
1.5	22 hrs	22 hrs	13.7	86
2.0	22 hrs	22 hrs	10.9	68
3.0	22 hrs	22 hrs	9.3	58
4.0	22 hrs	22 hrs	8.2	52
with S9 mix				
Negative control	4 hrs	22 hrs	13.2	100
0.5	4 hrs	22 hrs	13.0	98
1.0	4 hrs	22 hrs	14.4	109
1.5	4 hrs	22 hrs	12.5	94
2.0	4 hrs	22 hrs	13.6	103
3.0	4 hrs	22 hrs	13.4	102
4.0	4 hrs	22 hrs	13.0	98

* mean value of two cultures in %

Table 25. Cytotoxicity of SHL 569 B (Experiment II)

Concentration in % (v/v)	Exposure time	Preparation interval	Mitotic cells per 1000 cells*	% of negative control
without S9 mix				
Negative control	46 hrs	46 hrs	9.9	100
1.0	46 hrs	46 hrs	7.9	80
2.0	46 hrs	46 hrs	2.9	29
3.0	46 hrs	46 hrs	0.0	0
4.0	46 hrs	46 hrs	0.0	0
with S9 mix				
Negative control	4 hrs	46 hrs	12.3	100
1.0	4 hrs	46 hrs	9.6	78
2.0	4 hrs	46 hrs	12.3	100
3.0	4 hrs	46 hrs	14.5	118
4.0	4 hrs	46 hrs	11.8	96

* mean value of two cultures in %

At least 100 metaphase per culture (duplicates) were scored for structural chromosomal aberrations, except in experiment II with and without S9 mix after treatment with the

**Table 26. Structural chromosome aberrations
(Experiment I, preparation interval 22 hrs, +S9, exposure period 4 hrs)**

Slide no.	Cells scored	% Aberrant cells incl. gaps excl. gaps* with ex- changes			Aberrations											
					gaps		chromatid type				chromosome type				other	
					g	lg	b	f	d	ex	lb	lf	ld	cx	ma	cd
					with S9 mix											
Negative control: DMEM:F12																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	1	0
1+2	200	1.5	0.5	0.0	2	0	0	0	0	0	0	0	0	0	1	0
Positive control: CPA 22.5 µg / mL																
1	100				11	0	9	1	0	5	0	1	0	0	2	0
2	100				2	1	10	2	0	0	3	3	0	0	0	0
1+2	200	20.5	15.5	2.5	13	1	19	3	0	5	3	4	0	0	2	0
Test item: 2.0 % (v/v)																
1	100				0	0	3	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.5	1.5	0.0	0	0	3	0	0	0	0	0	0	0	0	0
Test item: 3.0 % (v/v)																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1+2	200	1.0	0.5	0.0	1	0	0	1	0	0	0	0	0	0	0	0
Test item: 4.0 % (v/v)																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.0	0.5	0.0	1	0	1	0	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations: g = gap, lg = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, lb = iso-break, f = fragment, lf = iso-fragment, d = deletion, ld = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

**Table 27. Structural chromosome aberrations
(Experiment I, preparation interval 22 hrs, -S9, exposure period 22 hrs)**

Slide no.	Cells scored	% Aberrant cells incl. gaps excl. gaps* with ex- changes			Aberrations												
					gaps		chromatid type				chromosome type				other		
					g	lg	b	f	d	ex	lb	lf	ld	cx	ma	cd	
					without S9 mix												
Negative control: DMEM:F12																	
1	100				0	0	0	0	0	0	0	0	0	0	0	0	
2	100				0	0	0	0	0	0	0	0	0	0	0	0	
1+2	200	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0	
Positive control: EMS 550 µg / mL																	
1	100				7	0	11	2	0	4	1	1	0	0	0	0	
2	100				1	0	10	1	0	5	7	1	0	0	0	0	
1+2	200	19.0	16.5	4.0	8	0	21	3	0	9	8	2	0	0	0	0	
Test item: 1.0 % (v/v)																	
1	100				0	0	1	0	0	0	0	0	0	0	0	0	
2	100				2	0	2	0	0	0	0	0	0	0	0	0	
1+2	200	2.5	1.5	0.0	2	0	3	0	0	0	0	0	0	0	0	0	
Test item: 2.0 % (v/v)																	
1	100				2	0	3	0	0	0	1	0	0	0	0	0	
2	100				0	0	2	0	0	0	0	0	0	0	0	0	
1+2	200	3.0	2.5	0.0	2	0	5	0	0	0	1	0	0	0	0	0	
Test item: 3.0 % (v/v)*																	
1	200				3	0	2	0	0	0	0	1	0	0	3	0	
2	200				3	1	6	0	0	0	0	2	0	0	0	0	
1+2	400	4.75	3.5	0.0	6	1	8	0	0	0	0	3	0	0	3	0	
Test item: 4.0 % (v/v)*																	
1	200				1	0	1	0	0	0	0	0	0	0	4	0	
2	200				2	0	7	2	0	0	0	1	0	0	1	1	
1+2	400	4.75	4.0	0.0	3	0	8	2	0	0	0	1	0	0	5	1	

* inclusive cells carrying exchanges ;

200 metaphase per slide were scored due to inhomogeneous results

Abbreviations: g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 28. Structural chromosome aberrations
 (Experiment II, preparation interval 46 hrs, -S9, exposure period 46 hrs;
 preparation interval 46 hrs, +S9, exposure period 4 hrs)

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps	excl. gaps*	with ex-changes	gaps		chromatid type				chromosome type				other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
without S9 mix																
Negative control: DMEM/F12																
1	100				0	0	3	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	0	0	0	0	0	0	0
1+2	200	2.5	2.0	0.0	1	0	4	0	0	0	0	0	0	0	0	0
Positive control: EMS 440 µg / mL^a																
1	50				2	0	10	1	0	6	0	0	0	0	2	0
2	50				3	0	13	0	0	3	0	1	0	0	1	0
1+2	100	29.0	27.0	8.0	5	0	23	1	0	9	0	1	0	0	3	0
Test item: 1 % (v/v)																
1	100				2	0	2	0	0	0	0	0	0	0	0	0
2	100				2	0	0	1	0	0	0	0	0	0	0	0
1+2	200	3.0	1.5	0.0	4	0	2	1	0	0	0	0	0	0	0	0
with S9 mix																
Negative control: DMEM/F12																
1	100				1	0	2	0	0	0	0	1	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1+2	200	1.5	1.5	0.0	1	0	2	0	0	0	0	1	0	0	0	0
Positive control: CPA 15 µg / mL^{##}																
1	50				2	0	5	1	0	1	0	1	0	0	3	0
2	100				0	0	13	1	0	8	4	7	0	1	2	0
1+2	150	22.67	22.0	5.33	2	0	18	2	0	9	4	8	0	1	5	0
Test item: 2 % (v/v)																
1	100				0	0	1	0	0	0	0	1	0	0	0	0
2	100				1	0	0	2	0	0	0	0	0	0	0	0
1+2	200	2.5	2.0	0.0	1	0	1	2	0	0	0	1	0	0	0	0
Test item: 3 % (v/v)																
1	100				1	0	2	1	0	0	0	0	0	0	0	0
2	100				0	0	2	0	0	0	0	0	0	0	0	0
1+2	200	2.5	2.0	0.0	1	0	4	1	0	0	0	0	0	0	0	0
Test item: 4 % (v/v)																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	1	0	0	0	0	0	0	0	0
1+2	200	1.0	0.5	0.0	1	0	0	1	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

50 metaphase plates per culture were scored due to strong clastogenic effects

in one culture 50 metaphase plates were scored due to strong clastogenic effects

Abbreviations: g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 29. Biometry of Experiment I

	Test group versus negative control	preparation interval	exposure period	S9 mix	p-value
Test group	2.0 % (v/v)	22 hrs	4 hrs	-	n.c.
"	3.0 % (v/v)	22 hrs	4 hrs	-	n.c.
"	4.0 % (v/v)	22 hrs	4 hrs	-	n.c.
"	1.0 % (v/v)	22 hrs	22 hrs	-	0.062
"	2.0 % (v/v)	22 hrs	22 hrs	-	0.015 ^s
"	3.0 % (v/v)	22 hrs	22 hrs	-	0.002 ^s
"	4.0 % (v/v)	22 hrs	22 hrs	-	0.001 ^s
"	2.0 % (v/v)	22 hrs	4 hrs	+	0.187
"	3.0 % (v/v)	22 hrs	4 hrs	+	n.c.
"	4.0 % (v/v)	22 hrs	4 hrs	+	n.c.
Positive control versus negative control					
EMS	550.0 µg/mL	22 hrs	4 hrs	-	< 0.001 ^s
EMS	550.0 µg/mL	22 hrs	22 hrs	-	< 0.001 ^s
CPA	22.5 µg/mL	22 hrs	4 hrs	+	< 0.001 ^s

n.c. not calculated as the aberration rate is equal or lower than the control rate

^saberration rate is statistically significant higher than the control rate

Table 30. Biometry of Experiment II

	Test group versus negative control	preparation interval	exposure period	S9 mix	p-value
Test group	1.0 % (v/v)	46 hrs	46 hrs	-	n.c.
"	2.0 % (v/v)	46 hrs	4 hrs	+	0.362
"	3.0 % (v/v)	46 hrs	4 hrs	+	0.362
"	4.0 % (v/v)	46 hrs	4 hrs	+	n.c.
Positive control versus negative control					
EMS	440.0 µg/mL	46 hrs	46 hrs	-	< 0.001 ^s
CPA	15.0 µg/mL	46 hrs	4 hrs	+	< 0.001 ^s

n.c. not calculated as the aberration rate is equal or lower than the control rate

^saberration rate is statistically significant higher than the control rate

Numerical aberrations

No significant increase in the frequency of polyploid cells was noted.

Conclusion:

According to the study report, in the study described and under the experimental conditions reported, SHL 569 B did not induce structural chromosomal aberrations in human lymphocytes in vitro.

Therefore, SHL 569 B including the impurity — at a level of — (w/v) is considered to be non-clastogenic in this chromosome aberration test.

Reviewer's comments:

The reviewer concurred with the conclusion described above.

Human peripheral blood lymphocytes from blood samples obtained from one donor only, which is considered acceptable although it is a concern considering human genetic diversity.

Inhomogeneous results were noted at 3% and 4% groups in Experiment I with exposure period 22 hrs, preparation interval 22 hrs and without S9 mix. Two hundred metaphase plates per slide were scored. Considering the fact the CA frequencies were within the historic ranges no further regulatory action is recommended.

In addition, the mouse micronucleus test (Title: Studies on the mutagenic potential of SH L 569 A in the mouse micronucleus test; Study Identification: Report No. A555, Study No. TX 92.321) was conducted using SH L 569 A and the review is enclosed in the appendix. The results were negative.

2.6.6.5 Carcinogenicity N/A

In general, the drug will be used as a single dose, therefore, no carcinogenicity study was conducted or requested.

2.6.6.6 Reproductive and developmental toxicology

The following reproductive and developmental toxicology studies were conducted using the formulation SHL 569 A or SHL 569 B and the previous review enclosed in the appendix.

The related key information is provided below mainly based on the previous review enclosed in the appendix and some additional information based on current review of the submissions.

Title: ZK 139.834 – Fertility study in the rat following intravenous administration

Study Identification: Report No. AL19 [Study No. TX 93266]

Formulation: SH L 569 B

Key study findings:

No adverse effects were observed on fertility, general reproductive performance, early embryonic development, fetal weights, sex distribution, or external fetal anatomy at doses up to 1 mmol/kg (dose multiple: 6.5X based on BSA).

Title: SH L 569A: Embryotoxicity including teratogenicity study in rats after intravenous administration from day 6 day to day 15 of gestation

Study Identification: Report No. AF32 [Study No. TX 93183]

Formulation: SH L 569 A

Dose: 0, 0.1, 0.5, and 5.0 mmol/kg/day, dose multiple: 0.6, 3.2, and 32x based on BSA

Key study findings:

Drug-related maternal toxicities were noted in rats of 5 mmol/kg/day group (36 rats) as evidenced by death (4 rats), unscheduled sacrifice (1 due to moribund condition), reduction in body weight gain. Clinical signs such as abdominal position, apathy, gait disturbances, decreased motor activity and piloerection were noted in 1 to 8 rats depending on the time points observed or signs. In addition, 4 dams were found to be non-pregnant. The NOAEL for maternal toxicity was 0.5 mmol/kg/day and dose multiple was 3.2X time based on a BSA.

There was an increase in preimplantation loss in dams at 0.5 mmol/kg group as %/animal. The loss in the controls was 7.7% while 16.7 %/animal at 0.5 mmol/kg/day group. The historic control for preimplantation loss was up to 15.4 %/ animal.

There was an increase in skeletal variations in all dose groups (65-72%) compared to the control group (44%). The difference reached statistical significance on a "fetus-based analysis" but not on a litter –based analysis. The variations was predominantly an increase in incomplete ossification most notably of the frontal (fetal incidence: 3.1, 19.3*, 14.2*, and 20.7*%, *: p<0.05) and parietal (fetal incidence: 33.3, 62.3*, 53.5*, and 59.8*%, *: p<0.05) skull bones.

Reviewer's comments:

Increased incidence of incomplete ossification was noted in drug groups but without clear dose response relationship. In addition, the incidences were within historical controls (up to 25% for frontal and up to 68% for parietal). Therefore, the effect was not considered adverse.

Title: SH L 569A: Embryotoxicity including teratogenicity study in the rabbit after intravenous administration from day 6 day to day 18 of gestation

Study Identification: Report No. AA67 [Study No. TX 93.176]

Formulation: SH L 569 A

Dose: 0, 0.1, 0.5, and 2.0 mmol/kg/day, dose multiple: 1.3, 6.5, and 26 x based on BSA

Key study findings:

No maternal toxicity observed.

Statistically significant increase in embryotoxicity was noted at the 2.0 mmol/kg/day group as evidenced by an increase in postimplantation loss and absorptions. There were 1 and 3 abortions in 0.1 and 2.0 mmol/kg/day groups, respectively (concurrent control: 0, historic control: 0-2 abortion, 0-9.5%). The mean incidences of the percent of implantations that were resorbed were 22.6% and 12.2% for the group receiving 2.0

mmol/kg/day and saline respectively (historic control: 7.2-20.9 %/group). The mean incidences of postimplantational loss were 22.6% and 12.2% for the group receiving 2.0 mmol/kg/day and saline, respectively (historic control: 12.0-22.2 %/group). Consequently, the number of fetuses per litter at 2.0 mmol/kg/day was decreased from a control mean of 7.2 to 5.1 (historic control: 6.4-8.6).

Increased incidences of skeletal variations such as unossified middle phalanges of the digits and the incompletely ossified 5th sternbrae were noted in all treatment groups without clear dose response relationship. In addition, the incidence was within the historic range although large variability among historic controls was noted. For example, fetal incidences of unossified right middle phalanges of the digits were 13.9, 42.5*, 31.1*, and 55.6* % (* p<0.05) for animals in 0, 0.1, 0.5, and 2.0 mmol/kg/day groups, respectively. The fetal incidences from historic controls ranged from 13.9 to 61.7%.

Title: SH L 569A: ZK 139.834- Peri- and postnatal study in the rat following intravenous administration (with mating F1 generation)

Study Identification: Report No. A019 [Study No. TX 94133]

Formulation: SH L 569 A

Dose: 0, 0.4, 1.2, and 3.6 mmol/kg/day, dose multiples: 0.65, 1.9, and 5.8x

Key study findings:

Drug-related clinical signs and reduced weight gain were observed in dams of 3.6 mmol/kg/day group. The signs included heavy bleeding, ataxia, blue coloration, hypoactivity, and rapid respiration.

No significant, direct drug-related adverse effects in F1 and F2 generations

2.6.6.7 Local tolerance

Fourteen local tolerance studies were conducted using the formulations SHL 569 A and SHL 569 B (4) and the studies were previously reviewed and the review is enclosed in the appendix and briefly summarized below. Unfortunately, an interim histopathological examination was not performed in majority of studies, therefore, acute microscopic lesions may not be revealed appropriately. In addition, only summary reports were provided. However, considering the totality of findings including clinical findings and previous nonclinical and clinical findings for the products in this class, no additional studies or reports are required.

A.

Title: Local tolerance test of SH L 569 B in the rat (M + F) after a single intra-arterial injection into the femoral artery

Study Identification: Report No. A744 [Study No. TX 93.144]

Formulation: SH L 569 B

Dose: 0.1 mL

Key study findings: No drug-related findings. However, the histopathological examination was performed on Day 15 only.

B.

Title: Local tolerance test of SH L 569 B following a single i.v. administration into the congested vena marginalis of the ear in rabbits

Study Identification: Report No. A695 [Study No. TX 93.141]

Formulation: SH L 569 B

Dose: 0.5 mL

Key study findings: No drug-related findings but histopathologic examination was performed on Day 8.

C.

Title: SH L 569 B: Local irritation test in rabbits after a single intramuscular

Study Identification: Report No. A714 [Study No. TX 93.142]

Formulation: SH L 569 B

Dose: 1.0 mL

Key study findings: Table 31.

Increased incidence (1/4 for the control group vs. 4/4 for the drug group on Day 3, 0/4 vs. 3/4 on Day 7) and severity in the drug group (slight to moderate for the drug group vs. slight to mild for the control group on Day 3), resolving by Day7 (4/4, slight to moderate focal muscle fiber necrosis on Day 3 vs. 1/4, slight on Day 7).

Table 31. Summary of the results on Day 3*

Findings/observations at injection sites	Substance	A 0.9% (w/v) NaCl-solution (control)				B ZK 139.834 (0.25 Mol/l) (SH L 569 B)			
		Animal number	960 M	959 M	35 F	11 F	960 M	959 M	35 F
Day 3									
Macroscopic findings		0		0	0				
- reddening			1-2			1-2	2-3	2	2-3
- hemorrhage							3	2-3	3
Histologic findings		-		-	-				
- interstitial hemorrhage			2			2	3	3	2
- interstitial edema						2	2	1	2
- focal muscle fibre necrosis			1			3	1	2	1

Day 7								
Macroscopic findings	0	0	0	0				0
- reddening					1	1	1	
- hemorrhage					1	1	1	
Histologic findings	--	--	--	--				--
- interstitial hemorrhage					3	2	1	
- interstitial edema					2	2	3	
- focal muscle fibre necrosis							1	
- focal fibrosis with histiocytic infiltration and phagocytosis of degenerated/necrotic muscle fibres and hemoglobin					2	2	3	
- focal fat replacement of muscle fibres							2	

*0: no finding/observation, 1: slight, 2: mild/small, 3: moderate

D.

Title: Local tolerance test with SH L 569 B after a single paravenous administration in the rabbit

Study Identification: Report No. A710 [Study No. TX 93.143]

Formulation: SH L 569 B

Dose: 1.0 mL

Key study findings: Slight focal proliferation of fibroblasts in the subcutis in ¼ rabbits, but histopathological examination performed on Day 7 only.

Only summary report provided and no individual data presented.

E.

Title: Local tolerance test of SH L 569 A in the rat (M + F) after a single intra-arterial injection into the femoral artery

Study Identification: Report No. A600 [Study No. TX 93.023]

Formulation: SH L 569 A

Dose: 0.1 mL

Key study findings:

One of 4 animals receiving the drug exhibited functional disturbance in the injected leg. The functional disturbance was characterized by a loss of the holding and grasping reflexes on Day 2 and not using the leg when walking on Days 2-3. The functional disturbance resolved by Day 4. Dark brownish dots on the plantar surface were noted in this animal from Days 13-15, which correlated with an “exfoliated parakeratotic scales” with minimal inflammation and hemorrhage noted on histopathology. No arterial lesions were observed.

The histopathological examination was performed on Day 15 only.

F.

Title: Local tolerance test in the rabbit (M+F) after a single injection into the congested and uncongested marginal vein of the ear

Study Identification: Report No. A393 [Study No. TX 92.310]

Formulation: SH L 569 A

Dose: 0.5 mL

Key study findings:

No drug-related local irritation was observed following injection into the uncongested ear vein.

Moderate reddenings was observed following injection into the congested ear vein on Days 1-4 and were not associated with histopathological changes. However, the histopathologic examination was performed on Day 8 only.

Only summary report was provided.

G.

Title: Local tolerance test in the rabbit (M+F) after a single injection into central artery of the ear

Study Identification: Report No. A394 [Study No. TX 92.311]

Formulation: SH L 569 A

Dose: 0.5 mL

Key study findings: Drug related reddenings and increased vessel injection were observed on Days 1-3 and no histopathological changes were noted. However, the histopathologic examination was performed on Day 8 only.

H.

Title: Local tolerance test in rabbit (M + F) after a single paravenous injection

Study Identification: Report No. A406 [Study No. TX 92.313]

Formulation: SH L 569 A

Dose: 1.0 mL

Key study findings:

Slight to moderate reddening and swelling in injection sites in drug group only. Reversible.

White marblization of the subcutis at the injection sites of all four animals in drug group only.

Moderate to marked focal necrosis accompanied by a moderate to marked cellular proliferating granulation tissue, in part, with giant cells and calcification at the injection sites of all four animals in drug group only.

Histopathological examination performed on Day 7 only.

2.6.6.8 Special toxicology studies: N/A**2.6.6.9 Discussion and Conclusions***CVS safety-potential to prolong ventricular repolarization*

Both in vitro electrophysiology and in vivo ECG studies suggest that Gd-EOB-DTPA possesses potential to prolong ventricular repolarization as evidenced by concentration-related blockage of hERG and prolongation of action potential duration, and dose-related prolongation of QTc. However, Gd-EOB-DTPA induced blockage of hERG and prolongation of action potential duration occurred at high concentrations only and the magnitudes of Gd-EOB-DTPA induced prolongation of QTc are small (max ~ 20 msec). Correlatively, prolongation of QTc was not noted at intended clinical doses but noted in patients given higher doses during the clinical trials. Therefore, Gd-EOB-DTPA possesses potential to prolong ventricular repolarization but the evidence of risk at intended clinical dose was weak.

Tubular vacuolation of the kidneys and potential NSF risk

Dose-related tubular vacuolation of the kidneys is a common finding among Gd-containing contrast agents. It is reversible in nature but in general after a long recovery period only. For example, vacuolation was still noted after the recovery period of 12 weeks in the 4 week rat study in the current submission. According to the study report, the transient vacuolation was due to an accumulation of Gd-EOB-DTPA after glomerular filtration and subsequent re-transportation (by endocytosis) into the respective cells. Because the vacuolation was not accompanied by adverse effect on the kidney functions, the Gd-EOB-DTPA-induced tubular vacuolation is not considered to be biological significant by the study report. However, considering the recent association of human nephrogenic systemic fibrosis (NSF) with Gd-containing contrast agents, accumulation of Gd-containing contrast agents in the kidneys especially in the kidneys of patients with severe renal failures might not be such a benign phenomenon. In addition, kidneys had detectable radioactivity at 72 hours post single dose of radiolabelled Gd-EOB-DTPA and on Day 21 post 5 consecutive doses of Gd-EOB-DTPA.

NSF is a new disease entity identified in patients with advanced renal failure. Although the etiology of NSF is still under investigation, Gd-containing contrast agents were noted to be associated with NSF. In an exploratory study conducted by the sponsor to evaluate Gd-containing contrast agents on NSF-like skin lesions (as a Safety Update submitted to IND 54,875), only minimal or slight calcinosis cutis was identified in 50% animals given Gd-EOB-DTPA. Although the lesion was not noted in animals given saline and a few other Gd-containing contrast agents, the lesion was also noted in animals given Caldiamide (the chelate of a Gd-containing contrast agent). In addition, low Gd concentration was detected in the skin tissue from animals given Gd-EOB-DTPA. No case of NSF is reported during clinical trials and post market surveillances from European countries. Taken together, Gd-EOB-DTPA may be not associated with higher

risk of NSF than other Gd-containing contrast agents although the risk can not be excluded. Adequate risk management and post market surveillance should be implemented to minimize the potential risk.

related issues

[redacted] in the current formulation] is used as a [redacted] ingredient in the formulation and [redacted]

According to the study report, dose-related reduction in APTT and fibrinogen, and increase in urinary iron excretion are possibly due to the [redacted]

In addition, the excreted iron in urine samples was identified as complex-bound Fe-EOB-DTPA. Therefore, the [redacted] may be associated with some Gd-EOB-DTPA -related adverse effects.

Unresolved toxicology issues (if any): N/A

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY N/A

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Taken together, Gd-EOB-DTPA possesses potential risk to prolong ventricular repolarization but the risk is considered low at intended clinical dose.

Although Gd-EOB-DTPA, similar to other Gd-containing contrast agents, causes reversible tubular vacuolation of the kidneys, no adverse effect on the kidney functions was identified.

Embryotoxic effects as evidenced by increase in pre- and post-implantation loss were noted.

Taken together, from pharmacology and toxicology perspective, an approval decision for this NDA is recommended.

Recommendations: Approval

2 Page(s) Withheld

 Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

APPENDIX/ATTACHMENTS

The attached studies were reviewed by Dr. Susan D. Wilson for IND 54,875. The pertinent summaries and additional information were included in the NDA review. The full IND reviews for reproductive and developmental toxicity studies, in vivo genotoxicity study, and local tolerance studies that full written reviews were not provided in the NDA review are attached unedited.

REPRODUCTIVE TOXICOLOGY

Title: ZK 139.834 – Fertility study in the rat following intravenous administration [Vol. 1.5; pp 1245-1502]

Study Identification: Report No. AL19 [Study No. TX 93266]

Site:

In-life Phase Study Dates: Nov. 26, 1993 – Feb. 15, 1994

Formulation: SH L 569 B [Batch No. 33021; Expiry Date – Aug. 1994]

Certificate Analysis: Yes (X) Certificate #15581/93 dated Oct. 18, 1993 not included in submission [in protocol amendment p. 1482 says date Jul. 27, 1993] No ()

Final Report (X) Oct. 27, 1995 **Draft Report ()**

GLP: Yes (X) In compliance with Japanese MHW Good Laboratory Practice Standards for Safety Studies on Drugs, No. 313 and Good Laboratory Practice Regulations: German Chemical Law No ()

Objective: To determine potential impairment of fertility in rats following exposure to Gd-EOB-DTPA.

Test Material / Group Designation	Dose and Regimen (2 ml/min)		Sex	Species/ Strain	N	Study Duration**
	mmol Gd/kg	ml/kg				
Group 1 – Control (NaCl)	-	4	M/F	rats/Sprague Dawley - CD (SD)	25/25	Males were treated 60 days prior to and during the mating period
Gd-EOB-DTPA Group 2*	0.1	1.0				Females were treated 14 days prior to, during and 7 days post mating
Group 3	0.3	1.2				
Group 4	1.0	4.0				

*SH L 569 B at this dose was diluted 1:2.5 NaCl solution to a concentration of 0.1 mmol/ml vs. 0.25 mmol/mol for Groups 3 and 4

**Males were sacrificed shortly after mating and females were sacrificed on Day 20 post coitus.

Parameter Evaluated	Time Point(s)
Clinical observations	daily
Morbidity/Mortality	2X/day
Body weight Males Females	-weekly -weekly prior to coitus, Days 0, 7, 14, and 20 post coitus

Uterine/implantation data – pregnancy status, no. of corpora lutea, gravid uterine weight, no. and position of implantations, early and later resorptions, dead fetuses, live fetuses	Day 20 post coitus
Fetal data – external fetal abnormalities, fetal weight, fetal sex	Day 20 post coitus
Organ Weights – testes, epididymides	Day 20 post coitus

Results – Parameters were not discussed if there were no compound related effects. Changes in several parameters were statistically significant compared to control values but either the changes did not exhibit a dose dependent relationship or it was felt by the Reviewer that the magnitude of the change was not physiologically relevant.

Necropsy – Dilated renal pelvis was observed in all groups of male rats including control but not in females. The incidence was 1, 2, 5, and 5 per 25 rats at 0, 0.1, 0.3, and 0.5 mmol/kg/day, respectively. No correlation with histopathological changes or changes in serum chemistry could be determined since these parameters were not assessed. [Reviewer's Comment – The Sponsor indicates that although the incidence is increased, the frequency is within historical controls. The historical control values for this lab were not provided. The sponsor will be asked to provide this information.]

Reproductive Parameters – The fecundity, fertility, and insemination index [percent] tended to be decreased in the treated rats compared to the controls but this decrease was generally <10%. There was a 1-2% increase in percent preimplantation loss. There were 2 dams which lost their litters following administration of 0.3 mmol/kg/day. The accounted for the increase in early and late resorptions and dead fetuses observed at this dose. However, a similar finding was not seen at 1.0 mmol/kg/day.

Sponsor's Conclusion (numbered) and Reviewer's Comments

(1) At doses up to 1 mmol/kg, no adverse effects were observed on fertility, general reproductive performance, early embryonic development, fetal weights, sex distribution, or external fetal anatomy. **Reviewer's Comment** – In general, the Reviewer concurs. The changes observed in fecundity, fertility, and insemination index were considered to be attributable to biological variability. The increase in the percent preimplantation loss at 0.3 mmol/kg/day was considered to be minimal and not a treatment related effect since it was not observed at 1.0 mmol/kg/day. The increase in the incidence of renal pelvis dilation is not an uncommon finding with a reported background incidence. Of note, is the fact that there were no changes in testicular weight.

Title: SH L 569A: Dose-finding embryotoxicity including teratogenicity study in rats after intravenous administration from day 6 day to day 15 of gestation [Vol. 1.5; pp 1503-1585]

Study Identification: Report No. AC85 [Study No. TX 93.061]

Site: Experimentelle Toxikologie, Schering AG; Berlin, Germany

Study Dates: Feb. – April, 1993

Formulation: SH L 569 A[Batch No. G02088-1A; Expiry Date – Feb. 1994]

Certificate Analysis: Yes (X) Certificate #21965/92 dated Feb. 19, 1993 not included
in submission No ()

Final Report (X) March 3, 1995 **Draft Report** ()

GLP: Yes () No (X) [Exempt since a dose finding study]

Objective: (1) To determine potential embryotoxicity and fetal toxicity in rats following exposure to Gd-EOB-DTPA. (2) The Sponsor also states that the design was chosen to determine if the "extremely high postnatal mortality observed....[in study TX 92.256] represented a reproducible phenomena."

**APPEARS THIS WAY
ON ORIGINAL**

Test Material / Group Designation	Dose and Regimen		Sex	Species/ Strain	N	Study Duration**
	mmol Gd/kg	ml/kg				
Group 1 – Control (NaCl)	-	10	F	rats/Han: WIST (SPF)	8	females were treated Days 6- 15 of gestation
Gd-EOB-DTPA						
Group 2	0.5	1.0				
Group 3	1.0	2.0*				
Group 4	5.0	10.0				

*p.1504 – Table 1: Treatment scheme indicates that the dose volume for Group 3 was 0.3 ml/100 g body weight/day. The Reviewer assumes that this is a typographical error and should read 0.2 since there is no indication that the drug was diluted.

**The in life study phase was from Day 0 post coitus [p.c.] to Day 22 post partum [p.p.].

Parameter Evaluated	Time Point(s)
Clinical observations	daily
Body weight	Days 0, 6, 15, 21 p.c. and Days 1, 8, 15, and 22 p.p.
Necropsy – pups and dams	Day 33 or 34 p.p.
Uterine/implantation data – no. and position of implantations, prenatal loss, number of still and live born pups	Day 33 or 34. p.p.
Fetal Examination – weight	Days 1, 8, 15, and 22 post partum

Results

Clinical Observations – Moderate to marked tail necrosis at the site of injection was observed in 1/8 and 2/8 dams at 1.0 and 5.0 mmol/kg/day, respectively. There was a nonsignificant decrease in body weight gain for Days 6-15 in dams receiving 5.0 mmol/kg/day.

Reproductive Parameters – Though not statistically significant, the pup weights during lactation tended to be less in the treated groups than in control groups. The magnitude of decreases did not exhibit a dose dependent relationship. In the group which were administered 1.0 mmol/kg/day of Gd-EOB-DTPA, there was an increase in pup loss during Days 1-22 of lactation from a control value of 15.9% γ 22.5 to 27.5% γ 23.1. In the control group only 2 litters had >25% loss, whereas in the 1.0 mmol/kg/day treatment group there 5 litters with >25% loss. The pup loss at 5.0 mmol/kg/day was comparable to the control group.

Sponsor's Conclusion (numbered) and Reviewer's Comments

1. No effects on outcome of pregnancy nor postnatal development was observed at doses up to 5.0 mmol/kg.

- There was an increase in pup loss during lactation at 1.0 mmol/kg which was considered not to be treatment related because of a lack of a dose dependent response.

Reviewer’s Comment – In general, the Reviewer concurs. It should be noted that the NOAEL for maternal toxicity, based on histopathology [injection site necrosis], was 0.5 mmol/kg which translates into approximately 20X/3.6X MHD (maximum human dose) based on a body mass/surface area dose. This finding is consistent with the local tolerance studies which showed that perivenous administration resulted in local irritation. As noted above, there was an increase in pup loss during lactation at 1.0 mmol/kg/day. However, the loss at 5.0 mmol/kg/day was comparable to control. This would indicate that due to a lack of a dose response, this finding is not related to treatment.

Title: SH L 569A: Embryotoxicity including teratogenicity study in rats after intravenous administration from day 6 day to day 15 of gestation [Vol. 1.6; pp 1586-2178]

Study Identification: Report No. AF32 [Study No. TX 93183]

Site: Schering AG; Berlin, Germany

In-life Phase Study Dates: Aug. 1993 - Feb. 1994

Formulation: SH L 569 A[Batch No. G02089-1A: Stability to Feb. 1994]

Certificate Analysis: Yes () Certificate #03558/93 dated Mar. 19, 1993
No ()

Final Report (X) July 24, 1996

Draft Report ()

GLP: Yes (X) – OECD Principles of GLP and Germany Chem G Guidance for GLP standards

No ()

Objective: (1) To determine potential embryofetotoxicity in rats following exposure to Gd-EOB-DTPA.

Test Material / Group Designation	Dose and Regimen		Sex	Species/ Strain	N	Study Duration
	mmol Gd/kg	ml/kg				
Group 1 – Control (NaCl)	-	10	F	rats/Han: WIST (SPF)	24 – section subgroup	Dams treated Days 6-15: Sacrificed Day 21 of gestation:
Gd-EOB-DTPA Group 2**	0.1	2.0			12 – lactation subgroup	Dams treated Days 6-15: dams allowed to deliver, F1 raised to sexual maturity, bred, and males and females* sacrificed on Day 21 of gestation
Group 3	0.5	1.0				
Group 4	5.0	10.0				

*N = 25-26

**diluted with saline

Parameter Evaluated	Time Point(s)
Clinical observations	Daily Days 1-21 gestation Days 0-22 of lactation
Body weight	Days 0, 6, 15, 21 of gestation

	Days 1, 8, 15, 22 of lactation
Uterine/implantation data* – corpora lutea, no. and position of implantations, early and late resorptions, viable and dead fetuses	Day 21 of gestation
Fetal Examination (section subgroup) – fetal weight, sex distribution, gross external abnormalities, visceral abnormalities, and skeletal abnormalities	Day 21 of gestation Days 1, 8, 15, 22 of lactation
Necropsy	Day 21 of gestation Approximately Day 22 of lactation
Uterine/implantation data* - corpora lutea, no. and position of implantations, early and late resorptions, viable and dead fetuses	Day 21
Fetal Examination (section subgroup) – fetal weight, sex distribution, gross external abnormalities, visceral abnormalities, and skeletal abnormalities	Day 21 of gestation
Pup Examination – birth, weight, sex, day of pinna detachment, beginning hair growth, teeth eruption, complete fur, separation of eyelids, descent of testes or patency of vagina, post weaning, daily observations	
Behavioral studies (N=2/sex/litter) Surface righting test Inclined plane test Auditory function, bar holding test Visual function, rotating rod test Open field test Water T-maze	Day 8 [post natal] Day 13 Day 22 Day 35 Day 48 Days 60-65
F1 Generation Mating	Approximately Day 90 post partum
F1 Necropsy	Day 21 of gestation (males and females)

*determined on 24, 23, 24, and 18 dams at 0, 0.1, 0.5, and 5.0 mmol/kg/day, respectively

Results - Parameters were not discussed if there were no compound related effects. Changes in several parameters not discussed were either not exhibit dose dependent or it was felt by the Reviewer that the magnitude of the change was not physiologically relevant.

Clinical Observations – Of the 36 dams administered 5.0 mmol/kg/da 4 dams were found dead and 1 was sacrificed in moribund condition between Days 6-12 p.c.. Additional signs at this dose in 1-8 dams included abdominal position, apathy, gait disturbances, decreased motor activity, decreased body temperature, decreased body weight gain without concurrent significant change in gravid uterine weights, and piloerection.

Body Weight – At 5.0 mmol/kg/day there was a significant reduction in body weight gain. The decrease was greatest during the period of dosing on Days 6-15. No clear treatment related trend was observed during the lactation period.

Necropsy – The lungs in the dams which died had reddish discoloration and tended not to collapse and be watery on the cut surface. It is not known whether there was a casual relationship to drug administration.

Fetal Visceral Examination – At 5.0 mmol there was an increase in severely dilated renal pelves [anomaly] with 4/94 fetuses (4.3% and 16.7% fetal and litter incidence) affected compared to 0 in the control group. The difference reached statistical significance on a “fetus-based analysis” but not on a litter-based analysis. The fetal incidence of dilated renal pelves [variation] varied from approximately 25 – 50% for both control and treated groups. It should be noted that the incidence of severely dilated renal pelves in the F2 fetuses was 4.6% and 27.3% in the control groups.

Fetal Skeletal Examination – There was an increase in skeletal anomalies in all dose groups (65-72%) compared to the control group (44%). The difference reached statistical significance on a “fetus-based analysis” but not on a litter-based analysis. The anomaly was predominantly an increase in incomplete ossification most notably of the frontal and parietal skull bones. According to the Sponsor, this indicates retarded ossification and has been associated with retarded development. Since there were no other indications of developmental retardation and since the incidence was within historical controls, the Sponsor maintains that these findings were not considered a test article related effect. [Reviewer’s Comment – The Sponsor will be requested to submit the historical control data to support this contention]. There was also an increase in the following variations: (1) metatarsals and phalanges not ossified at 0.5 mmol/kg/day, and (2) sternebra variations in all dose groups compared to controls. [Evaluation of F2 would suggest that this incidence was also within historical controls.]

Reproduction and Delivery Data

P Generation – Cesarean Section Group – There was a minimal increase in preimplantation loss in dams at 0.5 and 5.0 mmol/kg. The loss in the control was 7.7% vs. 16.7 and 12.9% / animal at 0.5 mmol/kg/day respectively. The difference was not apparent expressed at % per group. There was an increase in postimplantation loss/total resorptions in the dams administered 5.0 mmol/kg/day of Gd-EOB-DTPA. The % of postimplantation loss per group [mean] and per animal [median] were 4.4 and 0% for control dams and 8.4 and 7.1% for dams receiving test article.

Lactation Group – At 5.0 mmol/kg/day there was an increase in prenatal loss from a control value of 2.9% /group to 10.1%. There was an increase in % loss/litter [median] during lactation in all dose groups when expressed as median value for % per litter from 4.2% in control litters to 8.3 – 18.4% for the group receiving drug. The increase was not dose dependent with the greatest loss observed in the litters from dams administered 0.5 mmol/kg/day. When expressed as percent per group, total losses from Days 1-22 p.p. were increased in the groups receiving either 0.1 mmol/kg/day [21.1%] or 0.5 mmol/kg/day [20.4%] but not at 5.0 mmol/kg/day [7.6%] when compared to the control group [12.1%].

Sponsor’s Conclusion (numbered) and Reviewer’s Comments

- (1) Maternal toxicity was observed at 5.0 mmol/kg/day including apathy, gait disturbances, decreased motor activity, abdominal position, piloerection, decreased body weight gain, and death. **Reviewer’s Comments** – The NOAEL for maternal

toxicity was 0.5 mmol/kg/day which is 20X/3.3X MHD based on a body mass/surface area dose equivalency. The clinical signs observed in this study are consistent with those observed in the acute and repeat dose toxicity studies.

- (2) The Sponsor suggests that since the overall incidence of visceral variation was lower at 5.0 mmol/kg/day than in the saline control group, the finding of severely dilated renal pelves as considered to be a chance finding. **Reviewer's Comment**– As noted above, the incidence of renal pelvis dilation in the F2 generation was greater than that observed at in the F1 dams administered 5.0 mmol/kg/day. This weakens the argument that this increase is a treatment related effect.
- (3) There were no toxicity observed in reproductive outcome, F1 embryofetal or pup development, and F1 reproductive capabilities. **Reviewer's Comment** – There were mild increases in prenatal loss in both the Cesarean Section and Lactation groups predominantly at 0.5 mmol/kg/day. Preimplantation losses were also mildly increased at 0.5 mmol/kg/day. It should be noted that manner of data expression [e.g, % / group or % / animal] impacted whether changes were observed and/or the magnitude of the changes. The significance of the non dose dependent increase in pup loss during lactation is unclear. A similar finding was observed in the Study No. TX 93.061: SH L 569A: Dose-finding embryotoxicity including teratogenicity study in rats after intravenous administration from day 6 to day 15 of gestation.

Additional Comments – There was also an increase in the frequency of skeletal anomalies [primarily unossified frontal and parietal skull bones] in all treatment groups compared to the controls. As noted by the Sponsor, this finding has been associated with retarded development. The Reviewer agrees with the Sponsor that other indications of developmental retardation [e.g. behavioral studies] were not clearly indicated. Therefore, the significance of this finding is unclear. Of concern is that a NOAEL for this finding was not identified in this study.

d. Title: SH L 569A: Embryotoxicity including teratogenicity study in the rabbit after intravenous administration from day 6 day to day 18 of gestation [Vol. 1.7; pp 2179-2366 and Vol 1.8;]

Study Identification: Report No. AA67 [Study No. TX 93.176]

Site: Experimentelle Toxicologie, Schering AG; Berlin, Germany

Study Dates: July – Sept. 1993

Formulation: SH L 569 A [Batch No. G02089-1A: Stability to Feb. 1994]

Certificate Analysis: Yes (X) Certificate #03558/93 dated Mar. 19, 1993 [not included in submission No ()

Final Report (X) June 8, 1994 **Draft Report ()**

GLP: Yes (X) – OECD Principles of GLP and Germany Chem G Guidance for GLP standards No ()

Objective: (1) To determine potential embryofetotoxicity in rabbits following exposure to Gd-EOB-DTPA.

Test Material / Group Designation	Dose and Regimen		Sex	Species/ Strain	N	Study Duration*
	mmol ml/kg Gd/kg					
Group 1 – Control (NaCl)	-	4	F	rabbits/ New Zealand White	20	Dams treated Days 6-18
Gd-EOB-DTPA						
Group 2	0.1	0.2				
Group 3	0.5	1.0				
Group 4	2.0	4.0				

*Sacrificed Day 28 of gestation

Parameter Evaluated	Time Point(s)
Clinical observations	Daily Days 1-28 of gestation
Body weight	Days 0, 6, 15, 21 of gestation
Necropsy	Day 28 of gestation
Uterine/implantation data* – corpora lutea, no. and position of implantations, early and late resorptions, viable and dead fetuses	Day 28 of gestation
Fetal Examination (section subgroup) – fetal weight, sex distribution, gross external abnormalities, visceral abnormalities (50%), and skeletal abnormalities (50%)	Day 28 of gestation

*determined on 17, 19, 2, 14 dams at 0, 0.1, 0.5, and 2.0 mmol/kg/day

Results

Clinical Observations – Reduced or no defecation occurred in all dose groups at a slightly greater frequency than in the control group. Nasal catarrh was observed in all groups.

Reproduction Data – There was a statistically significant increase in embryoletality at 2.0 mmol/kg/day as evidenced by an increase in postimplantation loss and absorptions. There was 1 abortion and 1 doe with total fetal loss in the control does and 3 abortion and 2 does with total fetal loss at 2.0 mmol/kg/day. The mean incidence of the percent of implantations which were resorbed was 22.6% and 12.2% for the group receiving 2.0 mmol/kg/day and saline respectively. Consequently the number of fetuses per litter at 2.0 mmol/kg/day was decreased from a control mean of 7.2 to 5.1.

Fetal Body Weight – There was a trend towards a decrease in fetal body weight in all dose groups when compared to the saline control groups. The difference did not reach statistical significance.

Skeletal Examination – There was an increase in total skeletal anomalies (bodies and heads) at both 0.1 and 0.5 mmol/kg/day when compared to the control group, reaching statistical significance at the higher dose. The fetal incidence for total skeletal anomalies of the body was 13.1, 16.3, and 23.4% and the litter incidence was 7, 11 and 14% at 0,

Test Material / Group Designation	Dose and Regimen		Sex	Species/ Strain	N	Study Duration*
	mmol Gd/kg	ml/kg				
Group 1 – Control (NaCl)	-	7.2	F	Rats/Sprague Dawley	25	Dams treated Days 15 p.c. – Day 21 p.p.
Gd-EOB-DTPA						
Group 2**	0.4	7.2				
Group 3	1.2	2.4				
Group 4	3.6	7.2				

*one sample of test article was found not to be within the $\pm 10\%$ acceptable range for Gd concentration

**diluted 1:8 with saline daily to a concentration of 0.056 mmol/ml

***dams delivered and raised their offspring until weaning, at least 1 male and 1 female pup per litter designated as F1's, were mated at approximately 10 wks., and the F1 females were allowed to litter and raise offspring, the F1 males were sacrificed shortly after mating results were determined

Parameter Evaluated	Time Point(s)
Clinical observations	Daily
Body weight – P F1	Days 0, 6, 15, 20 of p.c.; Days 1, 4, 7, 14 and 21 p.p. Weekly during pre-mating, the Days 0, 6, 10, 15, 20 p.c.; and Days 1, 4, 7, 14 and 21 p.p.
Food Consumption	Days 0, 3, 6, 8, 10, 13, 18, and 20 p.c., Days 1, 4, 7, 9, 11, and 13 p.p.
Necropsy – dams F1 pups F1 females, F2 pups	After weaning of F1 pups After completion or physical development or last functional test except those retained for mating After weaning of F2 pups
Organ weights – prostate, testes, epididymides of F1 males	
Pregnancy and parturition data – gestation duration, abnormalities of nesting or nursing behavior, status of pups at birth, implanatation site numbers for both P and F1	term
Pup Examination – weight (both F1 and F2) Survival indices, external abnormalities (both F1 and F2), physical development, mortality, sex, pinna unfolding, incisor eruption, eye opening, descending of testes or opening of vagina, preputial separation (F1 only)	Days 1, 4, 7, 14, and 21 p.p. Daily
Behavioral studies (N=all available/litter) – F1 Surface righting reflex Gripping reflex Auditory function, pupillary reflex Open field test (N=2) Water maze test (N=2)	Day 8 p.p. Day 17 p.p. Day 21 p.p. Day 35 Day 35-42
F1 Generation Mating	Approximately 10 weeks p.p.

Results

Clinical Observations – Heavy bleeding at the injection stie in 17/25 dams [Days 1-3 of treatment] and ataxia in 24/25 dams [generally for up to 8 days] was observed at 3.6

mmo/kg/day. Hypoactivity and rapid respiration were also associated with treatment at this dose in 1 dam.

Body Weight Gain and Food Consumption – There was a mild, statistically significant decrease in food consumption 3.6 mmol/kg/day from Day 15-20 p.c. Body weight gain was also significantly decreased in this dose group at the same time point from a control value of 61.16 ± 15.77 [s.d.] to 49.90 ± 10.31 g.

Reproductive Function/Fetal Development – For the P dams, there was a mild nonstatistically significant decrease in both the number of pups delivered and implantation sites at 3.6 mmol/kg/day. The Sponsor indicates that these values were still within reference ranges. The total and mean numbers of implantation sites were 343 and 16.33 ± 3.84 for the control dams and 299 and 14.24 ± 3.02 in the dams receiving 3.6 mmol/kg/day. No adverse effects were observed in F1 fetal development, growth, and reproductive performance except for hydrocephaly observed in only 1 of the pups and a mild increase in the incidence of pup loss during lactation in the high dose group dams. No adverse effects were observed in F2 embryofetal development.

Sponsor's Conclusion (numbered) and Reviewer's Comments

- (1) No treatment related effects were observed in the F-1 and F-2 generations at doses of ≤ 3.6 mmol/kg/day of Gd-EOB-DTPA.
- (2) Maternotoxicity in the P generation was observed at 3.6 mmol/kg/day and included heavy bleeding at the injection site, ataxia, and a decrease in food consumption and body weight gain.
- (3) Maternotoxicity was observed primarily at beginning of the drug administration period. This may reflect habituation to exposure or an increased sensitivity during pregnancy.

Reviewer's Comment– In general, the Reviewer concurs with the following exceptions. There was a mild statistically significant decrease in implantations and number of viable pups delivered in the dams administered 3.6 mmol/kg/day. The NOAEL based on this finding would be 1.2 mmol/kg/day which is 48X/6X MHD on a body mass/surface area dose equivalency.

Summary of Reproduction Toxicology – The NOAEL for maternal toxicity in the rat was 0.1 mmol/kg/day based on moderate to marked tail necrosis [dose-finding study] and 1.2 mmol/kg/day based on other clinical signs including death, apathy, hypoactivity, ataxia, and decreased body weight gain. These findings would be consistent with the acute and repeat dose toxicity, and local tolerance studies. The currently intended formulation for clinical use [SH L 569 B] is less concentrated than the formulation used in this study [Sh L 569 A] which should reduce the irritating properties of the drug. Using the conservative NOAEL of 0.1 mmol/kg/day, the MHD will be 20X/3X based on body mass/surface area dose.

Study Protocol No. 93410: Placental transfer and distribution after intravenous administration of ^{153}Gd -labeled ZK 139.834 to pregnant rats, indicated that at 10 minutes post drug administration [peak maternal plasma values of 109.4 ± 18.1 nmol eq/ml], approximately 1% of maternal plasma values of the radiolabel were detected in the fetus. It would appear that Gd-EOB-DTPA does not readily cross the placental barrier, and, therefore, minimal fetal effects would be anticipated. There were, however, several concerns raised by these studies.

One of the primary concerns was the increased incidence of delayed ossification primarily of the frontal and parietal bones in both rats and rabbits. There was at least 50% and 100% increase in the fetal incidence in the rat and rabbit, respectively. A NOAEL was not determined for either species but would be <0.1 mmol/kg/day. This lesion is of concern because, as indicated by the Sponsor, it has been associated with developmental retardation. However, behavioral tests in the rat pups did not clearly indicate any changes. It should also be noted that the changes generally (1) did not exhibit a dose dependent relationship and (2) were not statistically significant. However, since this finding was observed in both species it does warrant concern. The relationship to treatment is unclear.

A second finding observed in both species was a mild increase in prenatal loss, e.g. pre and/or post-implantation loss. In general, this finding was observed at the high dose of 5.0 and 2.0 mmol/kg/day in the rat and rabbit, respectively. The NOAEL would, therefore, be 0.5 mmol/kg/day for both species which represents 20X /3X and 20X/7X MHD for the rat and rabbit, respectively, on a body mass/surface dose. In the rat, pre-implantation loss was also increased based on % litter at 0.5 mmol/kg/day. The incidence of pre-implantation loss was not increased for either dose when expressed as % per group.

There was also data which suggested that there was an increase in postnatal mortality in 2 rat studies. In addition, the Sponsor refers to an "extremely high postnatal mortality" in Study No. TX 92.256 [**Reviewer's Comment** – This study was not submitted. The Sponsor will be requested to provide a final report for this study.] The increase in lactational mortality in both studies was not dose dependent. Therefore, the significance of this finding is unclear.

In conclusion, the primary concern arising from the data presented in the reproductive toxicology studies is the occurrence for an increase in the incidence in incomplete ossification of primarily the frontal and parietal bones. As with the increase in lactational mortality, a NOAEL was not identified. With respect to reproductive performance, although changes were noted, they occurred at doses which were 3X and 7X MHD in the rat and rabbit, respectively, based on a surface area dose. These changes were generally mild and since the drug will be administered primarily as a single dose and these studies incorporated a repeat dose regimen, these findings would represent a worst case scenario.

GENOTOXICITY

Title: Studies on the mutagenic potential of SH L 569 A in the mouse micronucleus test [Vol. 1.9; pp. 3178-3193]

Study Identification: Report No. A555 [Study No. TX 92.321]

Site: Schering AG Experimentelle Toxikologie, Berlin, Germany

Study Dates: Nov. 1992

Formulation: SH L 569 A [Batch # G/01480]; Expiry date, Feb. 17, 1993

Certificate Analysis: Yes (X) Certificate of analysis # dated Oct 1, 1992 [Not submitted] No ()

Final Report (X) Sept. 17, 1993 **Draft Report ()**

GLP: Yes (X) – OECD Principles of GLP, Germany - Chem G; Chem VwV-GLP No ()

Objective: To evaluate the clastogenic potential of SH L 569 A *in vivo* in the mouse micronucleus assay

Test Material / Group Designation	Dose and Regimen (0.2 ml/min)		Sex	Species/ Strain	N
	mg or mmol/kg	ml/kg			
Group 1 – Negative Control (NaCl)	-	8	M/F	Mice- NMRI	10/10
Gd-EOB-DTPA	mmol Gd/kg				
Group 2	1	2			
Group 3	2	4			
Group 4	4	8			
Group 5 – Positive Control – Cyclophosphamide	mg/kg (i.g.) 30				5/5

Parameter Evaluated	Timing
Bone marrow smears [May-Gruenwald and Giemsa solutions] – micronuclei per 2000 polychromatic and 1000 normochromatic erythrocytes, polychromatic:nomochromatic erythrocyte ratio	24 and 48 hours for the negative control and test article groups 24 hours for the positive control group

Results – There were no signs of toxicity nor statistically or biologically significant alterations in the parameters indicated in the table above following test article administration.

Sponsor's Conclusion (numbered) and Reviewer's Comments

- Gd-EOB-DTPA was considered to be neither mutagenic nor myelosuppressive up to a dose of 4 mmol Gd/kg in this test system. **Reviewer's Comment** - The Reviewer concurs. This dose is 160X/13X MHD on a body mass/surface area dose equivalency.

SPECIAL TOXICOLOGY:

- Title:** Local tolerance test of SH L 569 A in the rat (M + F) after a single intra-arterial injection into the femoral artery [Vol. 1.9; pp. 3207-3216]
Study Identification: Report No. A600 [Study No. TX 93.023]

Site: AG Experimentelle Toxikologie, Berlin, Germany

Study Dates: Mar – Apr 1993

Formulation: SH L 569 A [Batch # G02088-1A]; Expiry date, May 3, 1993

Certificate Analysis: Yes (X) Certificate of analysis # 19101-92-dated Nov. 20, 1992 [Not submitted] No ()

Final Report (X) July 26, 1993 **Draft Report ()**

GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()

Objective: To evaluate the potential for both local intolerance and functional disturbance in the rat following intra-arterial administration of test article

Test Material/ Group Designation	Dose* (bolus)	Sex	Species/Strain	N	Study Duration
Group 1 – Negative Control (NaCl)	0.1 ml	M/F	Rats – HAN:WIST (SPF)	2/2	15 days post injection
Group 2 - Gd-EOB-DTPA	0.1 ml				

*Blood flow was impeded during injection by ligature placement proximal to the puncture site ligature was maintained for 5 minutes post injection

Parameter Evaluated	Timing
Clinical observations – hind legs	Daily from Days 2-15 post injection
Functional evaluation of hind legs – holding and grasping reflex, interdigital pain sensitivity, coordination test	Daily from Days 2-15 post injection
Histopathology – femoral artery at the injection site and arteries in the metatarsal region	Day 15

Results – One of 4 animals receiving test article exhibited functional disturbance in the left hind leg characterized by holding and grasping reflex loss on Day 2 and not using the limb when walking on Days 2-3. Functional disturbances resolved by Day 4. Observation revealed focal areas of hyperpigmentation on the plantar surface from Days 13-15 which correlated with an “exfoliated parakeratotic scales” with minimal inflammation and hemorrhage noted on histopathology. No arterial lesions were observed.

Reviewer’s Comments – Study Design and Data Presentation – The study design was adequate. Data was presented in a textual format which was adequate for this study. The Sponsor will be requested to provide data in tabular presentation for the NDA submission.

Sponsor’s Conclusion (numbered) and Reviewer’s Comments –

1. Intra-arterial administration of SH L 569 A resulted in “clear intolerance reactions in the form of transient functional disturbances and local changes”.
2. The etiology of these changes remain hypothetical.

Reviewer’s Comment – The Reviewer concurs.

1. The application volume used is approximately 8X the MHD and therefore, this study was “very rigorous”.

Reviewer’s Comments – Although this extrapolation may not be appropriate, the Reviewer does agree that the test method [e.g. transient occlusion of blood flow] results in prolonged contact with the artery and can, therefore, be considered rigorous. This study suggests that under the right conditions, SH L 569 A can result in an inflammatory response and exfoliation with a potential for reversible functional disturbance.

- b. **Title:** Local tolerance test of SH L 569 B in the rat (M + F) after a single intra-arterial injection into the femoral artery [Vol. 1.9; pp. 3217-3226]
Study Identification: Report No. A744 [Study No. TX 93.144]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: July 1993
Formulation: SH L 569 B[Batch # 32011]; Expiry date, Nov. 24, 1993
Certificate Analysis: Yes (X) Certificate of analysis # 09151/93 dated June 17, 1993 [Not submitted] No ()
Final Report (X) Dec. 15, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
Objective: To evaluate the potential for both local intolerance and functional disturbance in the rat following intra-arterial administration of test article.

Test Material/ Group Designation	Dose* (bolus)	Sex	Species/Strain	N	Study Duration
Group 1 – Negative Control (NaCl)	0.1 ml	M/F	Rats – HAN:WIST (SPF)	2/2	15 days
Group 2 - Gd-EOB-DTPA	0.1 ml				

*Blood flow was impeded during injection by ligature placement proximal to the puncture site ligature was maintained for 5 minutes post injection

Parameter Evaluated	Timing
Clinical observations – hind legs	Daily from Days 2-15
Functional evaluation of hind legs – holding and grasping reflex, interdigital pain sensitivity, coordination test	Daily from Days 2-15
Histopathology – femoral artery at the injection site and arteries in the metatarsal region	Day 15

Results – Plantar surface focal hyperpigmentation [histologically parakeratosis] was observed in ¼ control rats on Days 8-15. Neither lesions nor functional disturbances were observed in any test article rats.

Reviewer’s Comments – Study Design and Data Presentation – The study design was adequate. Data was presented in a textual format which was adequate for this study. The Sponsor will be requested to provide data in tabular presentation for the NDA submission.

Sponsor’s Conclusion (numbered) and Reviewer’s Comments –

1. Intra-arterial administration of SH L 569 B did not result in local toxicity employing this methodology.
2. The occurrence of focal parakeratosis in a control animal in this study suggests that the parakeratosis described in Study Report A744 may not be related to the test article.

Reviewer’s Comment – The Reviewer concurs.

- c. **Title:** Local tolerance test of SH L 569 B following a single i.v. administration into the congested vena marginalis of the ear in rabbits [Vol. 1.9; pp. 3253-3260]
Study Identification: Report No. A695 [Study No. TX 93.141]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: July 1993
Formulation: SH L 569 B [Batch # 32011]; Expiry date, Nov. 24, 1993
Certificate Analysis: Yes (X) Certificate of analysis # 09151/93 dated June 17, 1993 [Not submitted] No ()
Final Report (X) Sept. 29, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
Objective: To evaluate the potential for local intolerance in the ear of a rabbit following intravenous administration of SH L 569 B into the congested vena marginalis.

Test Material/ Group Designation	Dose* (bolus)	Sex	Species/Strain	N	Study Duration
Left ear – Negative Control (NaCl)	0.5 ml	M/F	Rabbits – New Zealand Whites	2/2	8 days
Right ear - Gd-EOB-DTPA	0.5 ml				

* Congestion was achieved by clamping the proximal 1/3 of the ear with clamp removal immediately following injection

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 8
Histopathology	Day 8

Results – No treatment related local irritation was observed. Lesions which were observed [Focal perifollicular inflammatory inflammation, slight focal degeneration in the ear cartilage] occurred, according to the Sponsor at the same frequency and severity for both control and test article ears.

Reviewer’s Comments – Study Design and Data Presentation – The study design was adequate. Data was presented in a textual format which was adequate for this study. The Sponsor will be requested to provide data in tabular presentation for the NDA submission.

Sponsor’s Conclusion (numbered) and Reviewer’s Comments –

1. Intravenous administration of SH L 569 B into the congested ear vein did not result in local intolerance.

Reviewer’s Comment – The Reviewer concurs.

- d. **Title:** Local tolerance test in the rabbit (M+F) after a single injection into the congested and uncongested marginal vein of the ear [Vol. 1.9; pp. 3261-3269]
Study Identification: Report No. A393 [Study No. TX 92.310]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: Nov. 1992
Formulation: SH L 569 A [Batch # G01480]; Expiry date, Feb. 17, 1993
Certificate Analysis: Yes (X) Certificate of analysis # 15378/92 dated Oct. 1, 1992 [Not submitted] No ()
Final Report (X) April 20, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
Objective: To evaluate the potential for local intolerance in the ear of a rabbit following intravenous administration of SHL 569 A into the congested and congested vena marginalis.

Test Material/ Group Designation	Dose* (30 sec.)	Sex	Species/Strain	N	Study Duration
Left ear – Negative Control (NaCl)	0.5 ml	M/F	Rabbits – New Zealand Whites	2/2	8 days
Gd-EOB-DTPA – right ear					
Group 1 – congested ear vein	0.5 ml				
Group 2 – uncongested ear vein	0.5 ml				

* Congestion was achieved by clamping the proximal 1/3 of the ear with clamp removal immediately following injection

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 8
Histopathology	Day 8

Results – No treatment related local irritation was observed following injection into the uncongested ear vein. Lesions observed following injection into the congested ear vein included slight to moderate hyperemia and vessel injection on Days 1-4 and were not associated with histopathological changes

Reviewer's Comments – Study Design and Data Presentation – The study design and data presentation was adequate.

Sponsor's Conclusion (numbered) and Reviewer's Comments –

- Intravenous administration of SH L 569 A into the congested, but not the uncongested, ear vein induced a mild and transient local intolerance.

Reviewer's Comment – The Reviewer concurs.

- Title:** Local tolerance test in the rabbit (M+F) after a single injection into central artery of the ear [Vol. 1.9; pp. 3270-3278]
Study Identification: Report No. A394 [Study No. TX 92.311]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: Nov. 1992
Formulation: SH L 569 A [Batch # G/01480]; Expiry date, Feb. 17, 199[3]
Certificate Analysis: Yes (X) Certificate of analysis # 15378/92 dated Oct. 1, 1992 [Not submitted] **No ()**
Final Report (X) June 21, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP **No ()**
Objective: To evaluate the potential for local intolerance in the ear of a rabbit following intravenous administration of SH L 569 A into the central artery.

Test Material/ Group Designation	Dose* (30 sec.)	Sex	Species/Strain	N	Study Duration
Left ear – Negative Control (NaCl)	0.5 ml	M/F	Rabbits – New Zealand Whites	2/2	8 days
Right ear - Gd-EOB-DTPA	0.5 ml				

* Congestion was achieved by clamping the proximal 1/3 of the ear with clamp removal immediately following injection

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 8
Histopathology	Day 8

Results – Test article related lesions included slight hyperemia and vessel injection on Days 1-3 and were not associated with histopathological changes.

Reviewer's Comments – Study Design and Data Presentation – The study design and data presentation was adequate.

Sponsor's Conclusion (numbered) and Reviewer's Comments –

- Intra-arterial administration of SH L 569 A into the central artery of the ear induced a mild and transient local intolerance.

Reviewer’s Comment – The Reviewer concurs.

- f. **Title:** SH L 569 B: Local irritation test in rabbits after a single intramuscular [Vol. 1.9; pp. 3279-3289]
- Study Identification:** Report No. A714 [Study No. TX 93.142]
- Site:** Schering AG Experimentelle Toxikologie, Berlin, Germany
- Study Dates:** June – July 1993
- Formulation:** SH L 569 B [Batch # 32011]; Expiry date, Nov. 24, 1993]
- Certificate Analysis:** Yes (X) Certificate of analysis # 09151/93 dated June 17, 1993 [Not submitted] No ()
- Final Report (X)** Oct. 15, 1993 **Draft Report ()**
- GLP: Yes (X)** – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
- Objective:** To evaluate the potential for local intolerance in the rabbit following intramuscular administration of SH L 569 B.

Test Material/ Group Designation	Dose (Sacrosapinal Muscle)	Sex	Species/Strain	N	Study Duration
Negative Control (NaCl)	1.0 ml	M/F	Rabbits – New Zealand Whites	4/4	7 days
Gd-EOB-DTPA	1.0 ml				

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 7
Histopathology - conducted only tissues with macroscopic changes	Day 3 and 7

Results

Macroscopic Lesions - Mild reddening and/or moderate interstitial hemorrhage were observed on Days 3 and 7 in 4/4 and 3/4 article sites, respectively, the severity of the lesions had decreased by Day 7.

Histopathological Lesions – Mild to moderate interstitial hemorrhage, slight to moderate edema, slight focal muscle fiber necrosis and/or degeneration was observed on Days 3 and 7 in 4/4 and 3/4 test article sites, respectively. Additional lesions on Day 7 included local fibrosis, histiocytic infiltration, necrotic fiber necrosis phagocytosis, and hemoglobin. The area of necrosis was decreasing on Day 7.

Macroscopic and histological lesions were noted in only 1 control site on Day 3.

Reviewer’s Comments – Study Design and Data Presentation – The study design and data presentation was adequate.

Sponsor’s Conclusion (numbered) and Reviewer’s Comments –

1. Inadvertent intramuscular administration of SH L 569 B has the potential to induce slight to moderate focal necrosis.

Reviewer’s Comment – The Reviewer concurs. Lesions were resolving by Day 7.

- g. Title:** SH L 569 B: Local irritation test in rabbits after a single intramuscular [Vol. 1.9; pp. 3290-3300]
- Study Identification:** Report No. A372 [Study No. Tx 92.312]
- Site:** Schering AG Experimentelle Toxikologie, Berlin, Germany
- Study Dates:** Oct. – Nov. 1992
- Formulation:** SH L 569 A [Batch # G01480]; Expiry date, Feb. 17, 1993]
- Certificate Analysis:** Yes (X) Certificate of analysis # 15378/92 dated Oct. 1, 1992 [Not submitted] No ()
- Final Report (X)** Oct. 15, 1993 **Draft Report ()**
- GLP: Yes (X)** – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
- Objective:** To evaluate the potential for local intolerance in the rabbit following intramuscular administration of SH L 569 A.

Test Material/ Group Designation	Dose (Sacrosplinal Muscle)	Sex	Species/Strain	N	Study Duration
Negative Control (NaCl)	1.0 ml	M/F	Rabbits – New Zealand Whites	4/4	7 days
Gd-EOB-DTPA	1.0 ml				

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 7
Histopathology - conducted only tissues with macroscopic changes	Day 3 and 7

Results

Macroscopic Lesions - Slight to mild reddening and slight to moderate marblization were observed on Days 3 and 7 in the test article sites.

Histopathological Lesions – Mild to moderate interstitial hemorrhage and edema, and moderate to marked focal muscle fiber necrosis with “formation of a demarcation zone” was noted on Days 3 and 7. The demarcation zone was an area of an inflammatory reaction characterized by WBC and histiocyte infiltration, fibroblast proliferation and necrotic tissue phagocytosis. The degree of necrosis was decreased and the demarcation zone was more prominent on Day 7.

Reviewer’s Comments (Study Design and Data Presentation) – The study design and data presentation was adequate.

Sponsor’s Conclusion (numbered) and Reviewer’s Comments –

1. Inadvertent intramuscular administration of SH L 569 A has the potential to induce moderate focal necrosis.

Reviewer’s Comment – The Reviewer concurs. Lesions were resolving by Day 7.

- h. Title:** Local tolerance test in rabbit (M + F) after a single paravenous injection [Vol. 1.9; pp. 3301-3311]
Study Identification: Report No. A406 [Study No. TX 92.313]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: Nov. 1992
Formulation: SH L 569 A [Batch # G01480]; Expiry date, Feb. 17, 1993
Certificate Analysis: Yes (X) Certificate of analysis # 15378/92-dated Oct. 1, 1992 [Not submitted] No ()
Final Report (X) Oct. 15, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP
No ()
Objective: To evaluate the potential for local intolerance in the rabbit following paravenous administration of SH L 569 A.

Test Material/ Group Designation	Dose* (bolus)	Sex	Species/Strain	N	Study Duration
Left - Negative Control (NaCl)	1.0 ml	M/F	Rabbits – New Zealand Whites	2/2	7 days
Right - Gd-EOB-DTPA	1.0 ml				

*Paravenous to the vena saphena lateralis

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 7
Histopathology - injection sites	Day 3 and 7

Results

Clinical Observations - Slight to moderate reddening and swelling were observed in the test article sites. Reddening and swelling of the leg distal to the injection site were noted in 2 rabbits and bluish-red skin discoloration at the injection site was observed in 1 rabbit. By Day 5, all local changes had resolved in 3 of 4 animals.

Macroscopic Lesions - White marblization of the subcutaneous tissue in all animals at the injection site of the test article.

Histopathological Lesions – The lesions seen were moderate to marked focal necrosis accompanied by a moderate to marked inflammatory reaction.

Reviewer's Comments (Study Design and Data Presentation) – An interim sacrifice would have allowed to determine whether the lesion was resolving or ongoing at Day 7.

Sponsor's Conclusion (numbered) and Reviewer's Comments –

1. Inadvertent paravenous administration of SH L 569 A has the potential to induce moderate to marked focal necrosis.

Reviewer's Comment – The Reviewer concurs.

- i. **Title:** Local tolerance test with SH L 569 B after a single paravenous administration in the rabbit [Vol. 1.9; pp. 3311-3319]
Study Identification: Report No. A710 [Study No. TX 93.143]
Site: Schering AG Experimentelle Toxikologie, Berlin, Germany
Study Dates: July 1993
Formulation: SH L 569 B [Batch # 32011]; Expiry date, Nov. 24, 1993
Certificate Analysis: Yes (X) Certificate of analysis # 09151/93 dated June 17, 1993 [Not submitted] No ()
Final Report (X) Oct. 12, 1993 **Draft Report ()**
GLP: Yes (X) – OECD Principles of GLP, Germany - ChemG; Chem VwV-GLP No ()
Objective: To evaluate the potential for local intolerance in the rabbit following paravenous administration of SH L 569 B.

Test Material/ Group Designation	Dose* (bolus)	Sex	Species/Strain	N	Study Duration
Left - Negative Control (NaCl)	1.0 ml	M/F	Rabbits – New Zealand Whites	2/2	7 days
Right - Gd-EOB-DTPA	1.0 ml				

*Paravenous to the vena saphena lateralis

Parameter Evaluated	Timing
Clinical observations	Immediately, 2 and 4 hours post injection, then daily until Day 7
Histopathology - injection sites	Day 3 and 7

Results

Histopathological Lesions – Lesions were observed in 1 of 3 rabbits and included a slight focal proliferation of fibroblasts in the subcutis.

Reviewer's Comments (Study Design and Data Presentation) – An interim sacrifice would have allowed determination of whether the lesions were apparent at early time points but had resolved by Day 7.

Sponsor's Conclusion (numbered) and Reviewer's Comments –

1. Inadvertent paravenous administration of SH L 569 B may have the potential to induce minimal local intolerance.

Reviewer's Comment – The Reviewer concurs.

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this page is the manifestation of the electronic signature.**

/s/

Yanli Ouyang
5/19/2008 06:43:58 AM
PHARMACOLOGIST

Adebayo Lanionu
5/19/2008 07:28:38 AM
PHARMACOLOGIST

NDA: 22090 _____

45 Day Filing Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

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		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	x		
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		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during presubmission communications or discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA.	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 some	For example, Study DERA 1004 used 2 dogs/sex/group only.
6) If the formulation to be marketed is not identical to the formulation used in 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	x		

<p>the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?</p>			
<p>8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.57? Is information available to express human dose multiples in either mg/m² or comparative serum/plasma AUC levels?</p>	<p>x</p>		
<p>9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item #10 below why it is not.</p>	<p>x</p>		<p>Some studies were not adequately designed. For example, Study DERA 1004 used 2 dogs/sex/group only. In addition, non-English certificate has been identified. However, considering the fact that similar studies were conducted using both formulations (SHL569A and SHL569B) and significant safety information has been accumulated for this class drug, the totality of safety information derived from the studies are probably sufficient. Therefore, the study deficiencies will be treated as review issues rather than fileable issues.</p>
<p>10) Reasons for refusal to file: N/A</p>			

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this page is the manifestation of the electronic signature.**

/s/

Yanli Ouyang
9/13/2007 10:31:13 AM
PHARMACOLOGIST

Adebayo Lanionu
9/13/2007 10:41:56 AM
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