

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

201277Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 201277

Agency receipt date: 5/14/2010

Drug: gadobutrol

Applicant: Bayer Healthcare Pharmaceuticals Inc

Indication: gadolinium-based contrast agent (GBCA) to detect and visualize areas with disrupted blood brain barrier (BBB) and/or abnormal vascularity of the central nervous system

Reviewing Division: Division of Medical Imaging Products

Background:

The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of gadobutrol for the indication listed above.

Chronic nonclinical studies were not required for this application because of the acute use of the agent.

Pharmacologic class:

The Established Pharmacologic Class text phrase proposed for the highlights section of labeling is "gadolinium-based contrast agent". This phrase is not currently listed in the FDA list of Established Pharmacologic Class text phrases. Another phrase, paramagnetic contrast agent, is currently listed. However, recently approved PLR labeling for several other agents containing gadolinium and used for imaging have used the term "gadolinium-based contrast agent". Therefore, this may be the most appropriate term to use for gadobutrol under this application.

Conclusions:

The studies that were conducted are adequately summarized in the primary review. The findings appear to be consistent to those seen previously with this class of compounds with kidney being the primary target tissue. There are no outstanding nonclinical safety issues. I agree with the division pharm/tox conclusion that this application can be approved from a pharm/tox perspective.

I agree with the labeling changes proposed in the primary pharm/tox review with a minor possible additional change that I discussed with the division pharm/tox supervisor.

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

/s/

PAUL C BROWN
02/25/2011

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	201-277
Supporting document/s:	Original-1
Applicant's letter date:	May 14, 2010
CDER stamp date:	May 14, 2010
Product:	Gadovist 1.0 [®] (Gadobutrol)
Indication:	Diagnostic MRI to detect and visualize areas of the central nervous system
Applicant:	Bayer HealthCare Pharmaceuticals, Inc., P.O. Box 1000, Montville, NJ 07045-1000
Review Division:	Division of Medical Imaging Products (HFD-160)
Reviewer:	Olayinka A. Dina, Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Division Director:	Rafel Dwaine Rieves, M.D.
Project Manager:	James Moore, Pharm.D, M.A.

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 201-277 are owned by Bayer HealthCare Pharmaceuticals, Inc. or are data for which Bayer HealthCare Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 201-277 that Bayer HealthCare Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Bayer HealthCare Pharmaceuticals, Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 201-277.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	8
1.1	INTRODUCTION	8
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	8
2	DRUG INFORMATION	14
2.1	DRUG	14
2.2	RELEVANT INDs, NDAs, AND DMFs	16
2.3	DRUG FORMULATION	16
2.4	COMMENTS ON NOVEL EXCIPIENTS	18
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	18
3	STUDIES SUBMITTED	19
3.1	STUDIES REVIEWED	19
3.2	STUDIES NOT REVIEWED	22
3.3	PREVIOUS REVIEWS REFERENCED	25
4	PHARMACOLOGY	25
4.1	BRIEF SUMMARY	25
4.1	PRIMARY PHARMACOLOGY	25
4.2	SECONDARY PHARMACOLOGY	34
4.3	SAFETY PHARMACOLOGY	34
5	PHARMACOKINETICS/ADME/TOXICOKINETICS`	61
5.1	PK/ADME	61
5.3	TOXICOKINETICS	90
6	GENERAL TOXICOLOGY	90
6.1	SINGLE-DOSE TOXICITY	90
6.2	REPEAT-DOSE TOXICITY	106
7	GENETIC TOXICOLOGY	123
8	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	138
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT	138
9.2	EMBRYONIC FETAL DEVELOPMENT	145
9.3	PRENATAL AND POSTNATAL DEVELOPMENT	161
9	LOCAL TOLERANCE	169
10	SPECIAL TOXICOLOGY	175
10.1	NEPHROGENIC SYSTEMIC FIBROSIS (NSF)	175
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	228
12	APPENDIX/ATTACHMENTS	231

Table of Tables

Table 1: Composition of Gadobutrol formulation, SH L562BB	17
Table 2: Composition of other gadobutrol formulations used in nonclinical studies	17
Table 3: Relaxivity values r_1 and r_2 at 0.47 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]	27
Table 4: Relaxivity values r_1 and r_2 at 2.0 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]	27
Table 5: Relaxivity values r_1 and r_2 at 1.5 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]	27
Table 6: T_1 Shortening of gadolinium chelates at 1.5Tesla	27
Table 7: Liberation of histamine from mast cells by gadobutrol.....	36
Table 8: Irwin test - Effect of Gadobutrol and Magnevist.....	39
Table 9: Effects of Gadobutrol or Magnevist on maximal electroshock-induced convulsions in mice	41
Table 10: Effect of pretreatment with gadobutrol or Magnevist on maximal electroshock- induced convulsions in mice.....	43
Table 11: Effect of GBCAs on hERG-mediated potassium current	50
Table 12: Study Design (Report A08428).....	55
Table 13: Treatment groups in Report 21000.....	57
Table 14: Summary of single-dose Pharmacokinetic studies in different animal models	62
Table 15: Concentration of ^{153}Gd (nmol-eq/mL) in plasma after intravenous administration of ^{153}Gd ZK 135079 at 0.1 or 0.5 mmol/0.5 MBq/kg to rats.....	65
Table 16: Pharmacokinetic parameters of ^{153}Gd -ZK 135079 after intravenous administration at 0.1 or 0.5 mmol/0.5 MBq/kg to rats	65
Table 17: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in selected tissues after intravenous administration of ^{153}Gd -ZK 135079 at 0.1 mmolGd/0.5 MBq/kg.....	66
Table 18: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in tissues after intravenous administration of ^{153}Gd -ZK 135079 at 0.5 mmolGd/0.5 MBq/kg.....	66
Table 19: Cumulative excretion of the radioactivity into urine and feces after single intravenous administration of ^{153}Gd -ZK 135079 at 0.1 or 0.5 mmol/0.5 MBq/kg to rats	67
Table 20: Cumulative excretion of the radioactivity into bile, urine and feces after intravenous administration of ^{153}Gd -ZK 135079 at 0.5 mmol/0.1 MBq/kg to bile- fistula rats	67
Table 21: Radioactivity in plasma after intravenous administration of ^{153}Gd -ZK 135079 at 0.5 mmol/0.1 MBq/kg to maternal rabbits.....	69
Table 22: Pharmacokinetic parameters of ^{153}Gd -ZK 135079 after intravenous administration at 0.5	70
Table 23: Radioactivity in tissues after intravenous administration of ^{153}Gd -ZK 135079 at 0.5 mmol/0.1 MBq/kg to rabbits.....	71
Table 24: Plasma concentration of ^{153}Gd labeled ZK-135079 after a single intravenous administration of a low and high dose of gadobutrol in 5 female dogs	74
Table 25: Pharmacokinetic parameters of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low or high dose in 5 female beagle dogs	74
Table 26: Urinary amounts of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low and high dose in 5 female beagle dogs	75
Table 27: Fecal amounts of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low and high dose in 5 female beagle dogs	75

Table 28: Dosing schedule used in Study A31073	77
Table 29: Mean Gd plasma concentration versus time curves obtained upon single intravenous.....	79
Table 30: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after five daily intravenous .	82
Table 31: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in tissues at 2 days after five daily intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1, 0.5 and 2.5 mmol/0.5 MBq/kg/day to rats	83
Table 32: Cumulative excretion of the radioactivity into urine and feces after five daily intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1 mmol/0.02 MBq/kg/day, 0.5 mmol/0.1 MBq/kg/day and 2.5 mmol/0.5 MBq/kg/day to rats □: urine ■: feces	84
Table 33: Cumulative excretion of the radioactivity into urine and feces after five daily intravenous administration of $^{153}\text{Gd-ZK135079}$ (Gadobutrol) at 0.1 mmol/0.002 MBq/kg/day, 0.5 mmolGd/0.1 MBq/kg/day and 2.5 mmol/0.5 MBq/kg/day to rats.....	84
Table 34: Transfer of radioactivity to neonates via milk after intravenous administration of $^{153}\text{Gd-Gadobutrol}$ (0.5 mmol/0.5 MBq/kg) to lactating rats	89
Table 35: Study Design (Report A28309).....	91
• Injection site vasculitis/perivasculitis was observed in animal of both sexes in all treatment groups. Perivascular hemorrhage was observed in all study groups including controls	
Table 36: Predominant histopathological findings on sacrifice day 3	93
Table 37: Predominant histopathological findings on sacrifice day 22	94
Table 38: Pharmacokinetic parameters determined in study A28309	95
Table 39: Study Design (Report PH-36304).....	97
Table 40: Study groups (A41318)	103
Table 41: Summary of repeat-dose intravenous studies in the rat	106
Table 42: Some statistically significant hematology values (Report 9658)	109
Table 43: Macroscopic findings in controls and gadobutrol (SH L562 A)-treated animals sacrificed after 4 weeks or at the end of the recovery period	110
Table 44: Mean Gd levels in serum [$\mu\text{mol/L}$] rats after daily intravenous administration of gadobutrol over 4 weeks	117
Table 45: Mean pharmacokinetic parameters of Gd in rats after daily intravenous administration of gadobutrol over 4 weeks	117
Table 46: Treatment schedule (A10548).....	119
Table 47: Mean serum Gd concentrations (+/-SD) after i.v administration of gadobutrol at the low, mid and high dose levels	121
Table 48: Report 9972 - Chromosome aberration test performed without metabolic activation	131
Table 49: Report 9972 - Chromosome aberration test performed with metabolic activation	132
Table 50: Mammalian cell gene mutation test (HGPRT/V79 cell line) - Assay 1	134
Table 51: Mammalian cell gene mutation test (HGPRT/V79 cell line)- Assay 2	135
Table 52: Results - In vivo mouse bone marrow micronucleus assay	137
Table 53: Doses in range-finding studies	140
Table 54: Study Design (main and satellite groups) – Report A39049	140
Table 55: Summary of AUC(0-24) and Cmax values (Report A39049).....	142
Table 56: Mating Data (Report A39049).....	143
Table 57: Reproduction Parameters (Study No. A39049)	144

Table 58: Study Design (main and satellite groups) - Report A34150.....	147
Table 59: Toxicokinetic parameters	148
Table 60: Mean PK parameters in female Wistar rats after daily intravenous treatment with 5, 7.5 and 10 mmolGd/kg gadobutrol on days 6 -17 of gestation.....	149
Table 61: Summary of Maternal Survival	150
Table 62: Fertility indices (Report A34150)	150
Table 63: Implantation sites and losses, and fetal data.....	151
Table 64: Study Design (A36661)	153
Table 65: Dose selection (Report A36661)	154
Table 66: Maternal Survival (A36661)	154
Table 67: Mean pharmacokinetic parameters in female rabbits after daily intravenous	155
Table 68: Implantation sites and losses, and fetal data (Report A36661)	156
Table 69: Treatment scheme (Report No. A894)	159
Table 70: Dose selection (Report No. A894).....	159
Table 71: Study groups (Report No. PH-35738)	162
Table 72: Dose selection (Report PH-35738).....	163
Table 73: Survival data - F0 dams (Report PH-35738)	163
Table 74: Reproduction data in F0 dams (Report PH-35738)	165
Table 75: Pup live birth and survival indices (PH-35738).....	167
Table 76: F ₁ Reproductive performance.....	167
Table 77: Treatment schedule (Report No. 9622)	170
Table 78: Local findings at injection site following administration of Gadobutrol SH L 562 B in the rabbit.....	170
Table 79: Gadolinium-Based Contrast Agents (GBCAs).....	176
Table 80: Classification of GBCAs Based on Structure and Charge	177
Table 81: Objectives of Reports A39927, A47234, A47235, A47233 and A42715.....	178
Table 82: Microscopic skin effects (Report A40180)	182
Table 83: Gd concentrations in skin, femur and liver after GBCA injection in rats	185
Table 84: Study groups for Report A40160	189
Table 85: Skin Gd levels following treatment with different GBCAs	196
Table 86: Serum Gadolinium levels ($\mu\text{mol Gd/L}$) after administration of 2.5mmol/kg of GBCAs in rats	198
Table 87: Pharmacokinetic parameters in blood after the administration of 2.5 mmol/kg of GBCAs in rats	199
Table 88: Study Design (Report No. A42496).....	201
Table 89: Serum chemistry markers in Nephrectomized and Non-nephrectomized Rats (Mean \pm SD).....	203
Table 90: PK parameters in 5/6 nephrectomized vs. non-nephrectomized rats	204
Table 91: Gadolinium values in the serum after injection of a single i.v. Omniscan injection of 2.5 mmol/kg in 5/6 nephrectomized and non-nephrectomized rats (Mean \pm SD).....	204
Table 92: Serum chemistry markers in Nephrectomized and Non-nephrectomized Rats (Mean \pm SD).....	205
Table 93: Exposure of the skin to Gadolinium in 5/6 nephrectomized and non-nephrectomized rats.....	206

Table 94: Treatment Schedule (Report A39927).....	209
Table 95: Serum Chemistry data (Report A39927)	211
Table 96: Gadolinium concentration in different tissues (Report A47234).....	215
Table 97: Ionic radius and stability in Log of DTPA-Lanthanoid complexes used in Report A47233	221
Table 98: Macroscopic/Microscopic lesion Grading	222
Table 99: NOAELs, Safety Margins and prominent findings in Toxicity Studies.....	230

Table of Figures

Figure 1: Structural formula of unlabeled Gadobutrol.....	16
Figure 2: Structural formula of labeled (^{153}Gd)-gadobutrol	16
Figure 3: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after intravenous administration of $^{153}\text{Gd-ZK135079}$ at 0.1 mmol/0.5 MBq/kg (\square) or 0.5 mmol/0.5 MBq/kg (+) to rats (Symbols and bars are mean \pm S.D. (n=4)).....	65
Figure 4: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after five daily administration of $^{153}\text{Gd-ZK135079}$ at 2.5 mmol/0.5 MBq/kg/day to rats	82
Figure 5:	192
Figure 6: Gd concentration in skin biopsies taken from animals treated with different GBCAs	196
Figure 7: Comparison of skin Gd concentration in biopsies taken on day 35 (a), day 63 (b), day 168 (c) and day 364 (d).....	197
Figure 8: Serum clearance of Omniscan in 5/6 nephrectomized (blue) vs. non-nephrectomized (green) rats	205
Figure 9: Gd concentration in different organs	206
Figure 10: Gadolinium content in tissues after treatment of rats with 2.5 mmol/kg gadodiamide at different time points.....	212
Figure 11: Gd concentration in different tissues 56 days after the last of 3 injection of Gd-DTPA-BMA.....	216
Figure 12: Lanthanoid concentration in the skin before and 3, 7, 14 and 21 days after administration of Lanthanoids	224
Figure 13: Lanthanoid concentration in the different tissues 21 days after administration	224

1 Executive Summary

1.1 Introduction

Gadobutrol, is a paramagnetic, macrocyclic contrast agent for magnetic resonance imaging (MRI). It is a low osmolality and low viscosity gadolinium-based contrast agent (GBCA) in which the gadolinium ion (Gd^{3+}) is bound in a neutral and stable complex. Gadobutrol is intended for use in adults and children ages two years and older at a recommended dose of 0.1 mmol/kg (or 3.7 mmol/m²) for the detection and visualization of disrupted blood brain barrier (BBB) and/or abnormal vascularity of the central nervous system. Following administration, Gadobutrol rapidly distributes to the extracellular fluid. Similar to other MRI contrast agents, Gadobutrol is water-soluble, does not cross the intact blood-brain barrier and is excreted unchanged in the urine.

1.2 Brief Discussion Of Nonclinical Findings

1.2.1 Pharmacology

Primary pharmacodynamics: The efficacy of Gadobutrol is based on its paramagnetic (relaxivity) effect. Gadobutrol acts by shortening both the T_1 (spin-lattice) and T_2 (spin-spin) relaxation times of surrounding water protons to produce its signal-enhancing effect. Based on the findings of *in-vitro* and *in-vivo* studies in rodents, the T_1 -weighted effect was more dominant. In *in-vitro* studies, the relaxivity of Gadobutrol was comparable to other GBCAs. *In-vivo*, Gadobutrol enhanced visualization of liver and well-perfused tumors in rodent models.

Safety pharmacology: Gadobutrol produced a slight concentration-dependent inhibition of hERG-mediated potassium current (K^+ I_{Kr}).

At doses 0.5, 2.7 and 13.5 multiples of the intended human dose, intravenously administered Gadobutrol did not appear to have remarkable effects on blood pressure, PR-interval and the QRS duration in conscious dogs. However, based on an increase in QT and QTcQ intervals at the administered high dose, a NOAEL of 2.7x-MHD was established. At 2x, 4x and 8x MHD, Gadobutrol produced decreases in locomotion, twitching and respiration in mice. A NOAEL of 2.5 mmol/kg (2x MHD) was determined. At 2.5 mmol/kg, Gadobutrol produced an increase in respiration and a decrease in tidal volume in propofol-anesthetized rabbits. Evaluation of the effect of Gadobutrol on renal function conducted in rats and rabbits revealed no alteration in renal function at up to 2 mmol/kg.

1.2.2 Pharmacokinetics

Nonclinical single-dose pharmacokinetic (PK) iv studies were conducted using unlabeled or labeled (^{153}Gd)-Gadobutrol. A linear PK was observed across species (rat, rabbit, dog and monkey) with elimination half-life ($t_{1/2}$) ranging from 13 to 59 minutes indicative of a rapid elimination. Similarly, a short $t_{1/2}$ of 1.82 h was obtained at the clinical dose of 0.1 mmol/kg in human adults. Drug exposure, measured by the area under the plasma concentration versus time curve (AUC) increased dose proportional after a single-dose administration of Gadobutrol in the species studied. Gadobutrol has a low plasma protein binding with less than 5% bound to plasma proteins. Gadobutrol did not appear to penetrate the blood-brain barrier and radioactivity was lowest in the CNS (brain and spinal cord).

Biodistribution: Rat tissue and whole-body distribution studies suggested a low-level uptake of Gd in bone following a single intravenous dose of Gadobutrol. The bone Gd is then slowly released and excreted within 24 hours. Measured radioactivity at 0.25 hours was highest in the kidneys but less in the plasma where labeled Gd concentration decreased rapidly to below the lower limit of quantification 3-6 hours post-dose. After repeated administration of (^{153}Gd)-Gadobutrol using 0.1, 0.5 and 2.5 mmol/kg once daily for 5 days, there was a dose-proportional accumulation of radioactivity in rats sacrificed two days after the last dose at which time the highest radioactivity concentration was obtained in the kidneys.

Metabolism: Gadobutrol is not metabolized and only the unchanged drug was present in urine samples of rats and dogs.

Excretion: In rats, over 90% Gadobutrol was excreted primarily via the renal route while fecal excretion accounted for about 1- 2%. Less than 0.03% of the maternal dose was excreted in milk.

1.2.3 Toxicology

Prominent findings included a dose-related, reversible vacuolation of the proximal tubules of the kidneys, and an increase in relative and absolute kidney weight.

Single-dose toxicity: Expanded single-dose intravenous toxicity studies were conducted in rats and dogs. Studies in both species were accompanied by a 21-day recovery period. In the rat single-dose study, animals were administered Gadobutrol at doses 3x, 10x and 32x MHD. Vacuolation of renal proximal tubules occurred at all doses tested. Respiratory difficulty, vocalization or clonic-tonic seizures preceded death in animals that died. Mortality occurred in 2/10 rats per sex one day following treatment at 20 mmol Gd/kg (32x MHD). Based on the results, NOAEL was not established. In dogs administered a single dose of Gadobutrol at 2x, 9x or 54x MHD, kidney vacuolation was observed at 2x MHD and higher. Nausea, vomiting and cardiovascular effects (decreased blood pressure, increased heart rate and RR and QT

intervals) occurred at 9x MHD. NOAEL was not established in the dog single-dose study.

Repeat-dose toxicity: Repeat-dose intravenous toxicity studies were conducted in rats or dogs with a 10- or 8-week recovery period, respectively. In the rat repeat-dose study, 1x, 2x or 5x MHD were administered 7 times per week over 4 weeks. Increased kidney weight and renal vacuolation, observed in both sexes, occurred at 1x MHD and higher; kidney discoloration, enlargement and tubular necrosis occurred at higher doses. There was an increase in absolute and relative kidney weight in males administered the 5-fold human dose. NOAEL was not established in this study.

Dogs were administered 2x, 5x and 16x dose multiples of the intended human dose once per day for 4 weeks. Dose-related tubular vacuolation of the kidneys not reversible at recovery was observed. Nausea/vomiting, discoloration of kidneys occurred at 5x MHD. Increase in heart rate was observed in animals of both sexes at 16x MHD.

Genetic toxicology: Genotoxicity assessment of gadobutrol was performed using *in-vitro* bacterial reverse mutation test, chromosomal aberration test in human peripheral blood lymphocytes, the hypoxanthine phosphoribosylguanine transferase (HGPRT) locus in cultured Chinese hamster V79 cells, and in the *in-vivo* bone marrow mouse. Gadobutrol was negative for all assays conducted.

Carcinogenicity: Not required for single use diagnostic agents. Sponsor submitted a request for waiver that was granted by agency.

Reproductive toxicity:

i). Fertility and early embryonic development: In the GLP study reviewed, no treatment-related effect on mating performance was observed in animals of both sexes. However, there was a dose-dependent and treatment-related enlargement of the kidney at ≥ 2.2 mmolGd/kg. Based on findings of this study, the NOAEL for systemic toxicity was 2.2 (or 3.6x-MHD mmolGd/kg/day while the NOAEL for reproductive performance was 7.5 mmolGd/kg/day (or 12.2x-MHD), the highest dose studied.

ii) Embryofetal development: Embryofetal toxicity was evaluated in rats, rabbits and monkeys. In the rat, there was evidence of embryotoxicity at 7.5 mmol Gd/kg (or 12.2x MHD). While there were skeletal variations, no malformations were observed in fetuses at up to 10 mmol Gd/kg (16.2x MHD). In rabbits, a NOAEL was not established for maternal toxicity and a low incidence of embryotoxicity occurred at the low dose (2.5 mmol Gd/kg or 8.1x MHD). In monkeys, incidence of abortions was observed when gadobutrol was administered at the high dose of 2.5 mmol Gd/kg/day (or 8.1x MHD). The low dose of 0.75 mmol Gd/kg did not elicit maternal toxicity or teratogenicity.

iii) Pre- and postnatal development in rat: Except for enlarged kidneys that were observed from the mid dose (2.2 mmol Gd/kg (or 3.6 x MHD) and 2 mortalities that occurred at the high dose (7.5 mmol Gd/kg or 12.2x MHD), there were no other remarkable treatment-related findings in maternal (F₀) animals. Besides one male death

occurring at 2.2 mmol Gd/kg (3.6x MHD), there were no treatment-related effects on reproductive indices in F₁ pups, at all the tested doses. No treatment-related effects were observed in F₂ pups. Based on the results, NOAELs were determined as 0.6, 2.2 and 7.5 mmolGd/kg (or 1x, 3.6x and 12.2x MHD) for maternal (F₀) effects, pre- and postnatal development in F₁ generation and for late effects including fertility testing in F₁ animals, respectively.

Studies in Juvenile animals: In an extended single-dose toxicity study, Gadobutrol was administered intravenously to neonate rats at 0.6, 2.0 and 6.0 mmol Gd/kg (or 1x, 3x and 10x MHD), there was mortality in two pups administered the high dose. All other survived to scheduled sacrifice. Renal tubular vacuolation, a consistent histopathological finding in the gadolinium class of contrast agents was observed as from the mid dose (2 mmol Gd/kg) and higher. Using the von Kossa stain, there was no evidence of tissue mineralization. Based on the toxicokinetic study, Gadolinium was present in the plasma, skin, liver, kidney, heart, and sternum. The systemic exposure and the exposure to organs and tissues was dose-proportional and the fractions of the dose in organs 24 h after administration was low and ranged between 0.01 and 0.9%.

1.2.4 Special toxicology

i). Nephrogenic Systemic Fibrosis (NSF): NSF is a recently described, serious, fibrotic and highly debilitating disorder most frequently described in patients with end-stage renal disease or acute renal failure. While NSF primarily affects the skin, other organ compartments may also be involved. Although the etiology of NSF is not fully understood, exposure to gadolinium-containing contrast agents has been associated with the onset of symptoms. Considerable progress towards a better understanding of this disease has occurred since the first association between the clinical use of GBCAs and NSF was made (Grobner et al., 2006). Consequently, there are several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate <30 mL/min/1.73m²). The use of GBCAs is currently contraindicated in such individuals. The commonly reported hypothesis for the involvement of gadolinium in the pathomechanism of NSF is the in vivo dechelation of GBCAs.

In a series of exploratory, non-GLP studies, potential skin lesions and changes in skin and tissue concentrations of gadolinium were evaluated in animals administered Gadobutrol or other gadolinium-based contrast agents (GBCAs) having different structural and ionic formations.

Since NSF frequently occurred in association with renal impairment, a rodent model of surgically induced renal impairment (5/6 nephrectomy) was used to evaluate the role of severely diminished renal function on the levels of Gd concentration and its long-term retention in the skin of rats following 5-day repeated administration Gadovist, Magnevist, OptiMARK and Omniscan. Findings showed a prolonged presence of Gd in the serum of nephrectomized rats compared with intact controls rats.

It was concluded from preclinical studies that there was a potential for Gd skin deposition following the use of GBCAs; that the propensity for skin deposition appeared highest with the linear, nonionic Gd agents and lesser with the linear, ionic and the macrocyclic compounds and that accumulation of Gd in skin and tissues appeared higher in the 5/6 nephrectomized model compared to non-nephrectomized rats

ii). Antigenicity: In a GLP study to determine the antigenicity of gadobutrol (SH L562 BB) in the dog, there were no changes in the immunological parameters observed pre-challenge compared to the challenge phase.

iii). Impurities: Impurities were within acceptable limits. According to the sponsor, the level of 2 impurities, (b) (4) and (b) (4), was determined as (b) (4). The level is lower than the threshold requiring confirmation of the safety.

1.2.5 Local tolerance:

There were no significant findings in local tolerance studies performed to evaluate administration of gadobutrol by the intravenous, intraarterial, paravenous and intramuscular routes.

1.3.1 Approvability

The preclinical studies conducted support safety and efficacy. No additional studies are required. This reviewer recommends Gadobutrol be approved.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following changes in the label would more appropriately reflect findings from preclinical studies

(Section 8 of the proposed label) **USE IN SPECIFIC POPULATIONS**

8.1 Pregnancy

Pregnancy Category C:

Label proposed by sponsor:

There are no adequate and well-controlled studies of Gadovist 1.0 in pregnant women. While it is unknown if Gadovist 1.0 crosses the human placenta, other gadolinium based products do cross the placenta in humans and result in fetal exposure. Limited published human data on exposure to other gadolinium-based products during pregnancy did not show adverse effects in exposed neonates. Retardation of the embryonic development and embryoletality occurred in pregnant rats receiving maternally toxic doses of Gadovist 1.0 (≥ 7.5 mmol/kg body weight) that were 12.2 times the human equivalent dose and in pregnant rabbits receiving doses (≥ 2.5 mmol/kg body weight) that were 8 times the recommended human dose (based on body surface area). In the rabbits, this occurred without evidence of maternal toxicity. Gadovist 1.0 should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal reproductive and developmental toxicity studies were conducted in rats, rabbits, and monkeys. Gadovist 1.0 was not teratogenic when given intravenously during organogenesis at doses up to 16.2 times (rats), 31.9 times (rabbits), and 8.1 times (monkeys) the recommended single human dose (based on body surface area). Because pregnant animals received repeated daily doses of Gadovist 1.0, their overall exposure was significantly higher than that achieved with the standard single dose administered to humans.

Recommended label:

Pregnancy Category C:

There are no adequate and well-controlled studies of (b) (4) GADOVIST in pregnant women. While it is unknown if (b) (4) crosses the human placenta, other gadolinium based products do cross the placenta in humans and result in fetal exposure. Limited published human data on exposure to other gadolinium based products during pregnancy did not show adverse effects in exposed neonates. (b) (4)

Gadovist 1.0 should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

(b) (4)

Because pregnant animals received repeated daily doses of (b) (4) Gadobutrol, their overall exposure was significantly higher than that achieved with the standard single dose administered to humans.

8.3 Nursing mothers

Label proposed by sponsor:

It is not known whether gadobutrol is excreted in human milk. (b) (4)

A large rectangular area of the document is redacted with a solid grey box, covering several lines of text.

Recommended label:

(b) (4)

A large rectangular area of the document is redacted with a solid grey box, covering the majority of the lower half of the page.

2 Drug Information

2.1 Drug

Introduction: Gadovist injection is a contrast agent for magnetic resonance imaging (MRI). In this review, the product is referred to as Gadobutrol (ZK 135079) except in instances where the proposed Trade name, Gadovist was used. Similar to other extracellular magnetic resonance contrast agents, gadobutrol contains the paramagnetic rare earth metal gadolinium. In gadobutrol, the gadolinium ion is chelated by butrol in a firmly bound, electrically neutral, macrocyclic complex. The

thermodynamic stability constant ($\log k_{\text{GdL}}$) of gadobutrol at 25°C in 0.1N KCl is 21.75 ± 0.3 (where, k_{GdL} equals $[\text{GdL}] / [\text{Gd}^{3+}] [\text{L}^{3-}]$). Gadobutrol produces contrast enhancement by shortening T_1 (spin-lattice) and T_2 (spin-spin) relaxation times of water protons in body tissues where it is present. On magnetic resonance sequences, A significant increase in T_1 -weighted MRI signal was observed in tissues in the presence of gadobutrol. The product is presented in a 1.0 mol Gd/l concentration, which is twice the concentration of other approved gadolinium-based contrast agents (GBCAs).

CAS Registry Number (Optional)

138071-82-6

Generic Name

Gadobutrol (I.N.N.)

Code Name

BAY-86-4875 (as listed in Form 356h-0000); Code: ZK001135079 or ZK135079 (Bayer Schering Pharma AG).

Chemical Name

[10-[(1RS,2SR)-2,3-Dihydroxy-1-(hydroxymethyl)propyl]-1,4,7,10 tetraazacyclododecane-1,4,7-triacetato(3-)] gadolinium

Molecular Formula/Molecular Weight $\text{C}_{18}\text{H}_{31}\text{GdN}_4\text{O}_9$ / 604.72

Structure or Biochemical Description

Figure 1: Structural formula of unlabeled Gadobutrol

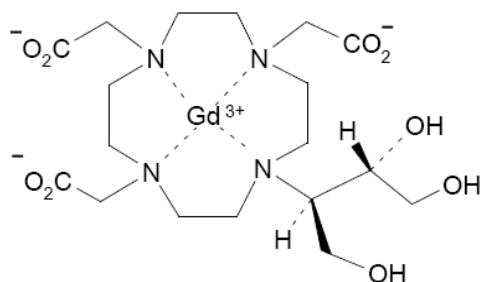
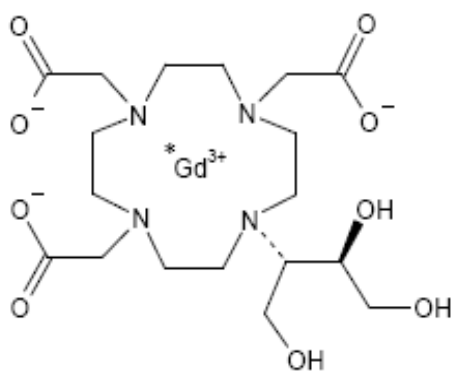


Figure 2: Structural formula of labeled (^{153}Gd)-gadobutrol



Racemate

*: ^{153}Gd (radioactive gadolinium isotope)

Pharmacologic class

Diagnostic contrast agent with MRI

2.2 Relevant INDs, NDAs, and DMFs

IND 056410 (Gadobutrol)

DMF 13061 (Gadobutrol)

DMF 13063 (Calcobutrol Sodium)

2.3 Drug Formulation

Composition of the to-be-marketed Gadobutrol formulation (SH L 562 BB):

Gadobutrol is a clear, colorless to pale yellow aqueous solution (pH 7.0 to 7.4) containing 1 mmol gadobutrol (equivalent to 604.72 mg gadobutrol per ml) as the active ingredient in addition to the excipients calcobutrol sodium, trometamol, hydrochloric acid (for pH adjustment) and water for injection. Gadobutrol does not contain preservatives. Drug formulation details are shown in the following Table:

Table 1: Composition of Gadobutrol formulation, SH L562BB

Name of Ingredient	Amount	Function
Active substance(s):		
Gadobutrol (active ingredient)	604.720 mg	Active ingredient
Excipients:		
*Calcium sodium butrol	(b) (4)	
Calcobutrol sodium (Ca-Na-butrol)	(b) (4)	
Trometamol		
Hydrochloric acid (1N)		pH adjustment
Water		Water for injection/Solvent

Reviewer's Table adapted from sponsor's Table; * According to the sponsor, during the production of the solution the excess (b) (4) calcium sodium butrol is formed by the reaction with (b) (4)

Other gadobutrol formulations used in nonclinical studies:

In addition to SH L 562BB formulation, the sponsor used three other formulations of gadobutrol in nonclinical studies namely, SH L 562 A, SH 562 AA and SH L 562 B. The composition of the three formulations is described below:

Table 2: Composition of other gadobutrol formulations used in nonclinical studies

Ingredient	Amount			Function
	SH L562A	SH L 562 AA	SH 562 B	
Gadobutrol (active ingredient)	(b) (4)			(b) (4)
Calcobutrol sodium (Ca-Na-butrol)				
GdNa ₂ DTPA				
Trometamol				
Hydrochloric acid (0.1 M)	(b) (4)			pH adjustment
Water	(b) (4)			Water for injection

Reviewer's Table adapted from sponsor's Table

Formulations SH L562A and SH L562B (0.5 and 1.0 mmol/ml, respectively) contain (b) (4) as excess chelator. According to the sponsor, (b) (4)

The sponsor claimed that the compositions of the AA and BB formulations were comparable with the earlier A and B formulations, even though they contain higher amount of excipients relative to the active ingredient gadobutrol. Consequently, four formulations were used in the safety pharmacology and toxicology studies of gadobutrol. The 0.5 mmol/ml formulations (SH L562A and SH L562AA) were used in toxicology studies conducted during the early stage of development.

Route of administration: Intravenous injection

2.4 Comments on Novel Excipients

2.5 Comments on Impurities/Degradants of Concern

2.6 Proposed Clinical Population and Dosing Regimen

Gadobutrol is intended for use in contrast-enhanced MRI in adults and children (2 years and older) to detect and visualize areas with disrupted blood brain barrier and/or abnormal vascularity of the central nervous system (CNS). For MRI of the CNS in humans, Gadobutrol is intended for use in the recommended adult single intravenous dose of 0.1 mmol/kg (or 0.1 ml/kg / 3.7 mmol/m², based on body surface area).

2.7 Regulatory Background

Introduction: Gadobutrol was initially approved in 1998 for “Contrast enhancement in cranial and spinal MRI” in Switzerland and additional approvals in Australia, Canada, the European Union (EU), South Africa, Mexico, New Zealand, Turkey, Asia and Eastern Europe. Gadobutrol was subsequently approved for contrast enhanced magnetic resonance angiography (MRA) in 2003 (EU and other countries) and contrast enhancement MRI of the liver and kidneys in 2007 (EU and other countries). IND 56410 for gadobutrol was submitted to the FDA by Berlex on July 15, 1998.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- Report No. 8932: Relaxivity of ZK 135079
- Report No. 9294: MR-Imaging of different lesions (cerebral infarct, brain tumor, liver tumor and intramuscular tumor) in the rat after i.v. injection of ZK 135079
- Report No. A698: Dose dependency of enhancement after intravenous administration of gadobutrol and gadopentetic acid, dimeglumine in an intramuscular tumor in the rat
- Report No. A42715: Stability of Gadolinium-based contrast agents in human serum

Safety Pharmacology

- Report No. 9139/II: Pharmacological and Biochemical Characteristics of Gadobutrol (ZK 135079)
- Report No. A19994: Neurotropic effects of gadobutrol in the Irwin test in mice after single intravenous administration
- Report No. A19996: Effect of gadobutrol on maximal electroshock-induced convulsions after single intravenous administration to mice in comparison with Magnevist®
- Report No. A22448: Effect of gadobutrol on maximal electroshock-induced convulsions in mice after single intravenous administration in comparison with gadopentetic acid
- Report No. A22977: Effect of gadobutrol on maximal electroshock-induced convulsions in mice after single intravenous administration in comparison with gadopentetic acid
- Report No. A19995: Effect of gadobutrol on pentylenetetrazole-induced convulsions after single intravenous administration to mice
- Report No. A711: Tolerance (ED₅₀) of Gadobutrol and ZK 92242 after intracisternal injection in the rat (M+F)
- Report No. A08412: Electrophysiological examination of Gadovist, Omniscan, Prohance, and Imeron® on the HERG-mediated potassium current
- Report No. A32843: Effects of hyperosmotic conditions induced with D-mannitol on HERG-mediated potassium current recorded from stably transfected CHO cells
- Report No. A08161: Effects of Gadovist® 1.0 mmol/ml on cardiac action potential in isolated guinea pig papillary muscle in comparison to Imeron® 400
- Report No. A08428: Cardiovascular Effects of Gadovist in Conscious, Telemetered Beagle Dogs
- Report No. A21000: Effect of gadobutrol (Gadovist®) on respiratory function after single intravenous infusion to propofol-anesthetized rabbits
- Report No. A20704: Effect of gadobutrol (Gadovist®) on renal function after single intravenous administration to rats

Pharmacokinetics

- Report No. 9585: Pharmacokinetics of ¹⁵³Gd-ZK 135079 after single intravenous administration to rats
- Report No. 9586: Placental transfer and distribution of ¹⁵³Gd-ZK 135079 after single intravenous administration to rabbits
- Report No. 9615: Pharmacokinetics and biotransformation of ¹⁵³Gd-labeled ZK 135079 after single intravenous administration in the dog

- Report No. 9587: Pharmacokinetics of ^{153}Gd -ZK 135079 after five daily intravenous administrations to rats
- Report No. A31073: Pharmacokinetics of gadolinium administered as ZK 135079 after single intravenous administration in female Cynomolgus monkeys and determination of gadolinium (ZK 264672) in monkey plasma samples by ICP-MS
- Report No. A475: Whole body autoradiographic study after intravenous administration of 0.5 mmol (3.7 MBq) ^{153}Gd -Gadobutrol/kg to male and pregnant Wistar rats
- Report No. 9584: Transfer of ^{153}Gd -ZK 135079 to milk and neonate via milk in rats

Single-Dose Toxicity

- Report No. A28309: Gadovist 1.0 mmol/ml - Systemic toxicity study in rats (M+F) with single intravenous administration and observation periods of 3 and 22 days
- Report No. PH-36304: SH L 562 BB (Gadovist® I1 mol/L) - Extended single dose toxicity study in neonatal rats after intravenous administration on PND 4 with a following recovery period up to day 28
- Report No. A41318: Gadovist 1.0 mmol/ml - Systemic toxicity study in dogs (M+F) with single intravenous administration and observation periods of 3 or 22 days

Repeat-Dose Toxicity

- Report No. 9658: ZK 135079; SH L562A- Systemic tolerance study in rats after daily intravenous administration over about 4 weeks (16-18 application days) including a reversibility study
- Report No. A08936: Gadobutrol (SH L562BB) - Systemic toxicity study with daily intravenous administration to rats (M+F) followed by a subsequent observation period of approximately 10 weeks
- Report No. A10548: Gadobutrol (SH L562BB) - Systemic toxicity study with daily intravenous administration to dogs (M+F) followed by a subsequent observation period of 8 weeks

Genotoxicity

- Report No. 9503: Evaluation of SH L562A (ZK 135079) in the Ames Salmonella/Microsome mutagenicity test
- Report No. 9501: Evaluation of SH L562A (ZK 135079) in the Ames Salmonella/Microsome mutagenicity test with preincubation
- Report No. 9502: Evaluation of SH L562A (ZK 135079) in a bacterial mutagenicity test with Escherichia coli, strain WP2uvrA
- Report No. 9616: Evaluation of the clastogenic potential of SH L562A in human peripheral blood lymphocytes
- Report No. 9972: Evaluation of the clastogenic potential of SH L562A in human peripheral blood lymphocytes
- Report No. 9592: SH L562A (ZK 135079) – Evaluation of gene mutations in mammalian cells in culture: HGPRT-test with V79 Cells
- Report No. 9823: Studies on the mutagenic potential of SH L562A in the mouse Microsome test

Reproductive and Developmental Toxicity

Segment I: Fertility and early embryonic development to implantation studies

- Report No. A39049: Gadobutrol (SH L562BB) - Study of fertility and early embryonic development to implantation in the rat after daily intravenous (i.v.) administration

Segment II: Studies of the effects on embryo-fetal development

- Report No. A34150: Gadobutrol (SH L562BB) - Study of effects on embryo-fetal development in rats after daily intravenous administration from days 6 to 17 of gestation
- Report No. A36661: Gadobutrol (SH L562BB) - Study for effects on embryo-fetal development in rabbits after daily intravenous administration from day 6 to day 18 of gestation
- Report No. A894: ZK 135079 - Embryotoxicity including teratogenicity study in Cynomolgus monkeys after daily intravenous administration from day 20 to day 50 of gestation

Segment III- Studies of the effects on embryo-fetal development

- Report No. PH35738: SH L562BB – Study of the effects on pre- and postnatal development in rats including maternal function and toxicokinetic Investigation after Intravenous Administration.

Local Tolerance

- Report No. 9622: SH L562B - Local Tolerance test in the rabbit (M + F) after a single injection into the congested and uncongested marginal vein of the ear
- Report No. 9736: SH L562B – Local tolerance test in the dog after a single injection into the vena cephalica antebrachii
- Report No. 9569: SH 562B – Local tolerance test in the rabbit (M + F) after a single injection into the central artery of the ear
- Report No. 9566: SH L562B – Local tolerance test in the rabbit (M+F) after a single paravenous injection
- Report No. 9599: SH L 562B – Local irritation test in rabbits after a single intramuscular injection

Other Toxicity Studies**Nephrogenic Systemic Fibrosis (NSF)**

- Report No. A40180: Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs on occurrence of NSF-like skin lesions in rats
- Report No. A40160: Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs and zinc depletion on occurrence of NSF-like skin lesions in rats
- Report No. A42495: Potential long-term retention of Gadolinium based contrast agents after intravenous administration in rats
- Report No. A42496: Potential long-term retention of Gadolinium in renally impaired rats (5/6 nephrectomized) after intravenous administration of Gadolinium based contrast agents
- Report No. A39927: A systemic toxicity study in rats (M) with daily i.v. administration of gadodiamide (ZK 117439) over periods of 1 to 8 days to investigate the pathomechanism of skin lesions
- Report No. A47234: The role of residual Gadolinium in the induction of Nephrogenic Systemic Fibrosis-like lesions in rats
- Report No. A47235: Determination of cytokines after the single intravenous administration of Gd-DTPA-BMA in rats

- Report No. A47233: Potential toxic effects of lanthanoids complexes after multiple intravenous administration in rats

Antigenicity

- Report No. A20948: Gadobutrol (ZK 135079 / SH L562BB) - Antigenicity study in female dogs with once daily intravenous administration over 4 weeks followed by a 7 day treatment free period and subsequent intravenous challenge

3.2 Studies Not Reviewed**Primary Pharmacology**

- Report No. A211: Relaxivity of Gadobutrol (ZK 133 079) at 2 Tesla
- Report No. 9627: Relaxivity of ZK 139 834

Safety Pharmacology

- Report No. A711: Tolerance (ED₅₀) of Gadobutrol and ZK 92 242 after intracisternal Injection in the rat (m + f)
- Report No. A431: Hemodynamic Effects of ZK-135079 and Magnevist® injection in the closed-chest anesthetized dog
- Report No. AN71: Effects of Gadobutrol on respiratory and cardiovascular system in rabbits
- Report No. 9660: Investigation of the effect on renal function after a single intravenous application of Gadobutrol in comparison with Magnevist® in rabbits (White New-Zealand)
- Report No. A262: Duration of bleeding after single intravenous administration of ZK 135079 and ZK 139834 with Magnevist® in the rat
- Report No. 9659: Influence of ZK 135079 in comparison with dimeglumine gadopentetate on erythrocyte morphology (in-vitro investigation with canine blood).
-

Pharmacokinetics**Analytical Methods and Validation Reports**

- Report No. A18122: Validation of an ICP-MS method for quantitative determination of gadolinium in different matrices of different species
- Report No. 9702/M: Measurement of Gadolinium concentration in different matrices by inductively coupled plasma atomic emission spectrometry – method evaluation

Other Pharmacokinetic studies

- Report No. Pharmacokinetics of ZK 135079 in rats
- Report No. 9669: Dose proportionality and biotransformation of ZK 135079 after single intravenous administration in rats
- Report No. 8998: Biodistribution and elimination of ZK 135079 in rats 24 hours and 7 days after single intravenous administration

Single-Dose Toxicity

- Report No. A28309: Gadovist 1.0 mmol/mL Systemic toxicity study in rats (M+F) with single intravenous administration and observation periods of 3 and 22 days
- Report No. 9330: Acute toxicity of SH L 562 A in male rats after a single i.v. application with approximate LD₅₀ determination

- Report No. B600: A single intravenous-dose toxicity study of gadobutrol (SH L 562 AA) in male and female rats
- Report No. 9344: Acute toxicity of SH L562 A in weaned male rats after a single i.v. application with approximate LD₅₀ determination
- Report No. 9344: ZK 135079- Dose range finding study in dogs (M) after single i. v. administration
- Report No. 9883: SH L 562 A – Systemic tolerance in Beagle dogs after a single i.v. administration (acute toxicity study)
- Report No. 9345: Acute toxicity of SH L562 A in male mice after a single i.v. application with approximate LD₅₀ determination
- Report No. 9346: Acute toxicity of SH L562 A in female mice after a single i.v. application with approximate LD₅₀ determination
- Report No. 9331: Acute toxicity of SH L562 A in male mice after a single i.g. application with approximate LD₅₀ determination
- Report No. 9329: Acute toxicity of SH L562 A in male rats after a single i.g. application with approximate LD₅₀ determination

Repeat-Dose Toxicity

- Report No. SG069: Preliminary intravenous repeated dose toxicity study of Gd-butriol in rats (English translation of Japanese DRF 9-days repeated dose toxicity study in rats)
- Report No. A03528: A 4-week (7 times/week) repeat-dose toxicity study of gadobutrol (SH L 562 A) in rats
- Report No. 9926: SH L562 A – Systemic tolerance study in rats following once daily i.v. administration on two subsequent days
- Report No. A031: SH L 562 A – Systemic tolerance study in Beagle dogs after daily intravenous administration over about 4 weeks (5 times per week) for a total of 16-18 administrations
- Report No. SG135: Evaluation of the Report entitled “Toxicity study of SH L562 A in dogs after daily intravenous administration for 4 weeks, including a 4-week reversibility study” of Anpyo Center Dated 21 Aug. 1996 (translation of Japanese Report)

Reproductive and Developmental Toxicity**Segment I: Fertility and early embryonic development to implantation studies**

- Report No. SG124/AK42: Reproductive and Developmental Toxicity study of SH 562 A in rats after administration from Pregestation through early gestation (Segment I)

Segment II: Studies of the effects on embryo-fetal development

- Report No. A30997: Gadobutrol (SH L562BB)- Dose-range finding study in pregnant rats after daily intravenous administration from days 6 to 17 of gestation
- Report No. A32272: Gadobutrol (SH L 562BB) -Dose-range finding study in pregnant rabbits after daily intravenous administration from days 6 to 18 of gestation
- Report No. A576: Gadobutrol: Embryotoxicity including teratogenicity study in the rat after daily intravenous administration from day 6 to 15 of gestation
- Report No. 9927: Orientating embryotoxicity including teratogenicity study in rabbits after intravenous administration (dose-finding study)
- Report No. A420: Gadobutrol: Embryotoxicity including teratogenicity study in the rabbit after daily intravenous administration from day 6 to 18 of gestation

Segment III- Studies of the effects on embryo-fetal development

- Report No. PH 35165: Feasibility study for the study for effects on pre- and postnatal development in rats including maternal function after intravenous administration
- Report No. SG127: Reproductive and developmental toxicity study with SH L 562 A administration during the perinatal and lactation period in rats (Segment III)

Local Tolerance

- Report No. 9690: SH L562A – Local tolerance test in the rabbit (M + F) after a single Injection into the congested and uncongested vein of the ear
- Report No. 9736: SH L562B – Local tolerance test in the dog after a single injection into the vena cephalica antebrachii
- Report No. 9569: SH 562B – Local tolerance test in the rabbit (M + F) after a single injection into the central artery of the ear
- Report No. 9566: SH L562B – Local tolerance test in the rabbit (M+F) after a single paravenous injection
- Report No. 9599: SH L 562B – Local irritation test in rabbits after a single intramuscular injection

Serum hormone Levels

- Report No. A010: SH L 451AA, SH L542A, SH L562A – Serial investigation of changes in serum hormone concentrations (serum, FSH, LH and testosterone concentrations) in the rat following once daily i.v. administration of 5.0 mmol gadopentetic acid, dimeglumine, gadopenamide and gadobutrol/kg on two subsequent days

Antigenicity

- Report No. SG143: Determination of the Antigenicity of Gadobutrol (1) Active Systemic Anaphylaxis (2) Guinea Pig Homologous Passive Cutaneous Anaphylaxis Test
- Report No. SG144: Determination of the Antigenicity of Gadobutrol by the Passive Cutaneous Anaphylaxis (PCA) Test in the Mouse-Rat System
- Report No. 9642: SH L562A – Optimization test in guinea pigs to determine a potential sensitizing effect (delayed hypersensitivity)
- Report No. 9728: ZK 135079 – Optimization test in guinea pigs to determine a potential sensitizing effect (delayed hypersensitivity)

Impurities

- Report No. AK84: (b) (4) – Systemic tolerance study in rats after daily intravenous administration over ca. 2 weeks
- Report No. AL74: Evaluation of (b) (4) in the Ames Salmonella/Microsome mutagenicity test
- Report No. AM22: Evaluation of (b) (4) in the Ames Salmonella/Microsome test with preincubation
- Report No. AM72: Chromosome aberration assay in human lymphocytes *in-vitro* with (b) (4) Project 530800
- Report No. AL58: Evaluation of (b) (4) in the Ames Salmonella/Microsome mutagenicity test
- Report No. AM21: Evaluation of (b) (4) in the Ames Salmonella/Microsome mutagenicity test with preincubation
- Report No. AM73: Chromosome aberration assay in human lymphocytes in vitro with (b) (4); (b) (4) Project 530900

- Report No. SG130: Single- dose toxicity of CaNaDO3A-butrol after intravenous administration to mice

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Brief summary

Gadobutrol (ZK 135079 / SH L562BB) is a hydrophilic macrocyclic gadolinium compound that elicits a low binding to plasma proteins. The amount of unbound gadobutrol after 1 $\mu\text{mol/ml}$ gadobutrol was added to human plasma was >94%. Gadobutrol appears stable in human serum at normal and elevated phosphate levels. The gadobutrol complex has a high thermodynamic and kinetic stability indicating a stable binding of gadolinium, which is essential for the inertness of the complex. Gadobutrol produces contrast enhancement by shortening T_1 and T_2 relaxation times in areas where it is present. Its relaxivity was shown to be comparable to gadopentetate dimeglumine (Magnevist).

4.1 Primary Pharmacology

Primary pharmacodynamic studies were conducted to characterize the pharmacological and biochemical properties of gadobutrol, determine its shortening effects on relaxation time (relaxivity) in-vitro and evaluate its in-vitro stability in human serum. The diagnostic efficacy of gadobutrol was investigated in rat models of brain infarction, liver, brain and intramuscular tumor. The pharmacological and biochemical properties of gadobutrol (Study report 9139/II) was described in in-vitro screening experiments.

In-vitro measurements of T_1 and T_2 relaxation times in water and plasma revealed a notable T_1 -shortening effect of gadobutrol on proton relaxation times. Gadobutrol produced a good contrast enhancement in animals with experimentally induced cerebral infarcts and brain, liver or intramuscular tumors. Gadobutrol was stable in-vitro following incubation at 1 mmol/l, pH 7.4 and 37°C for 15 days in human serum containing normal levels or elevated phosphate levels similar to those observed in patients with end-stage renal disease.

4.2.1 In-vitro pharmacology studies

Gadobutrol and Relaxivity: Shortening effects of Gadobutrol (ZK135079) on relaxation time *in-vitro*

Introduction: Gadobutrol caused a shortening effect of the T_1 (spin-lattice) and T_2 (spin-spin) relaxation times of water protons (hydrogen nuclei) because of its

paramagnetic properties. T_1 and T_2 shortening effects are concentration-dependent and their proportionality factor, r_1 and r_2 , are known as T_1 and T_2 relaxivity, respectively. Relaxivity, measured as a change in the relaxation rate per unit concentration, is the slope of the linear plot (linear regression) of concentration (mmol/l) versus relaxation rates (s^{-1}). T_1 and T_2 relaxivity of gadobutrol were measured *in-vitro* studies. Using NMR pulse spectrometer (Minispec PC 20) r_1 and r_2 were measured at 0.47 Tesla (T) at 40°C (Reports 8932 and 9627) or with an MRI scanner at 2.0 T (Report A211) at room temperature (21-25 °C). The objectives and methods used in studies 8932, A211 and 9627 are similar and the following evaluation reflects the findings, conclusions and comments on the three studies.

4.2.1.1 Report No. 8932:

Study title: Relaxivity of ZK135079

Volume #, and Page #:	Module 4.2.1.1.1, 9 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Study #:	KM 88175, KM 89075, KM 89366, KM90075
Date of study initiation:	Not provided. Report date: July 31, 1990
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (lot #, % purity – not provided) (ZK 135079, p1/9)
Substance Batch numbers:	AZ 40, PLA 303, PLA 370, PLA 411
Formulation Batch numbers:	653, 731, 836, 890
Animal species/strain/sex per dose:	N/A; in-vitro study
Age:	N/A
Weight:	N/A
Doses/Vehicle:	0.25, 0.5 and 1.0 mmol/l
Duration/route:	N/A; in-vitro study

Key study findings: In this and related *in-vitro* studies, gadobutrol decreased T_1 and T_2 relaxation times in both water and plasma samples - an indication of its paramagnetic effect and contrast-enhancing properties. The shortening effect of gadobutrol was comparable to gadopentetate and other gadolinium-based contrast agents (GBCAs).

Study Design: Due to its paramagnetic effect, gadobutrol decreases the relaxation times (relaxivity) of protons in water molecules. This study was conducted to determine the paramagnetic effect of gadobutrol by measuring the relaxation time of water and plasma (swine and bovine) samples containing 0 (demineralized water), 0.25, 0.5 and 1.0 mmol/l of gadobutrol. Relaxation time was measured at 40°C using an NMR pulse spectrometer) running at 0.47 Tesla. Relaxivity is the slope of the linear plot of concentration (mmol/L) against the relaxation rates (sec^{-1}).

Results: Gadobutrol decreased T_1 and T_2 relaxation times at 0.47 T. The data summarized in Tables 3 and 4 incorporate the findings of Reports 9627 (relaxivity of Gd-EOB-DTPA measured at 0.47T) and Report A211 (relaxivity of gadobutrol at 2.0 T). In reports 9627 and A211, the relaxivity of gadopentetate dimeglumine (reference

compound) was also measured in water and plasma. In reports 9627 and A211, the relaxivity of gadopentetate dimeglumine was determined at the same concentrations used in report 8932 namely 0, 0.25, 0.5 and 1.0 mmol/l.

Table 3: Relaxivity values r_1 and r_2 at 0.47 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]

Test article	Water		Plasma	
	$r_1 (\pm \text{SD})$	$r_2 (\pm \text{SD})$	$r_1 (\pm \text{SD})$	$r_2 (\pm \text{SD})$
Gadobutrol (Report 8932)	3.58 ± 0.25	3.99 ± 0.13	5.61 ± 0.55	6.50 ± 0.59
Gadopentetate dimeglumine (Report 9627)	3.67 ± 0.04	4.12 ± 0.04	4.95 ± 0.12	5.65 ± 0.22

Reviewer's Table adapted from sponsor's Data (Reports 8932 and 9627)

Table 4: Relaxivity values r_1 and r_2 at 2.0 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]

Test article	Water		Plasma	
	$r_1 (\pm \text{SD})$	$r_2 (\pm \text{SD})$	$r_1 (\pm \text{SD})$	$r_2 (\pm \text{SD})$
Gadobutrol	4.26 ± 0.01	5.05 ± 0.08	6.68 ± 0.01	9.15 ± 0.25
Gadopentetate dimeglumine	3.73 ± 0.05	4.54 ± 0.09	5.31 ± 0.03	6.80 ± 0.17

Reviewer's Table adapted from sponsor's data (Report A211)

Table 5: Relaxivity values r_1 and r_2 at 1.5 Tesla [$\text{sec}^{-1} \cdot (\text{mmol/l})^{-1}$]

Test article	Water		Plasma	
	r_1	r_2	r_1	r_2
Gadobutrol	3.3	3.9	5.2	6.1
Gadopentetate dimeglumine	3.3	3.9	4.1	4.6

Reviewer's Table adapted from sponsor's Table. Data is based on Rohrer et al.(2005) as cited by sponsor.

From the data of Rohrer et al (2005), T_1 shortening time for gadobutrol (1.0 M) was the highest when compared with some other gadolinium chelates as shown below:

Table 6: T_1 Shortening of gadolinium chelates at 1.5Tesla

Gd-chelate	T_1 Shortening (s)
Gadobutrol (1.0 M)	1.034
Gadopentetate dimeglumine (0.5 M)	0.853

Gadobenate (0.5 M)	0.949
Gadodiamide (0.5 M)	0.865

* Sponsor's Table based on Rohrer et al (2005) cited by the sponsor; T_1 was calculated from r_1 relaxivity in plasma at 37°C

Conclusions: Gadobutrol decreased both T_1 and T_2 relaxation times. The relaxivity values for gadobutrol were similar to values obtained for gadopentetate dimeglumine in reports 9627 and A211 and both compounds appear to have similar imaging properties.

Reviewer's comments: Agreed

4.2.2 In-vivo pharmacology studies

4.2.2.1 Report No. 9294:

Study title: MR Imaging of different lesions (cerebral infarct, brain tumor, liver tumor and intramuscular tumor) in the rat after i.v. injection of ZK 135079

Volume #, and Page #:	Module 4.2.1.1.1, 19 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Germany
Study #:	KM 90400
Date of study initiation:	Not provided; Studies were conducted June 13-20, 1990
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol, lot #: N/A, %purity: N/A
Animal species/strain/sex per dose:	Female rats (Han Wistar SPF (b) (4))
Age:	N/A
Weight:	160-240 g
Doses/Vehicle:	0.1 and 0.3 mmol Gd/kg

Key study findings: In four *in-vivo* rat models of cerebral infarct and tumors in the brain, liver and muscle, 0.1 and 0.3 mmol Gd/kg gadobutrol (ZK 135079) were shown to exhibit good diagnostic efficacy. Similar to Magnevist, gadobutrol enhanced the liver parenchyma and well-perfused tumors.

Study Design: In a study performed to determine the suitability of gadobutrol for MRI and to assess the effect of increasing the dose of gadobutrol from 0.1 to 0.3 mmol Gd/kg, lesions including a cerebral infarct and tumors in the brain, liver and muscle were visualized using T_1 -weighted MRI at 2 Tesla. Gadobutrol (0.1 mmolGd/kg) was administered intravenously to female Wistar rats followed by a second dose (0.3 mmolGd/kg) after 20 min. Images were produced before, and 1 min after the administration of gadobutrol.

Results: Similar to Magnevist, gadobutrol at 0.1 or 0.3 mmol Gd/kg enhanced the liver parenchyma and well-perfused tumors. An improved delineation of lesions was observed at 0.3 compared to 0.1 mmol Gd/kg.

Conclusion: The sponsor concluded that increased lesion visualization was achieved by increasing the dose of gadobutrol from 0.1 to 0.3 mmol Gd/kg.

Reviewer's comment: While I agree with the sponsor, the possibility of increased toxicity should be weighed against a potential gain in diagnostic outcome due to an increase in dose.

4.2.2.2 Report No. A698:

Study title: Dose dependency of enhancement after intravenous administration of gadobutrol (SH L562 A) and gadopentetic acid (SH L451 AA), dimeglumine in an intramuscular tumor in the rat

Volume #, and Page #:	Module 4.2.1.1.1; 14 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Germany
Study #:	KM 93027
Date of study initiation:	Not provided; Studies were conducted in February 1993
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (lot #: N/A %purity: N/A (ZK 135079/Formulation SH L 562 A, p 1/14) Gadopentetic acid (Magnevist) - ZK 93035/Formulation No. H L452 AA
Animal species/strain/sex per dose:	Rat (Schering SPF Wistar Han, Schering); 5 females/group
Age:	N/A
Weight:	195-215 g
Doses/Vehicle:	Gadobutrol or Magnevist: 0.1, 0.3 and 0.5 mmol/kg; vehicle: saline
Duration/route:	Intravenous

Key study findings: Intravenous gadobutrol or Magnevist at 0.1- 0.5 mmol Gd/kg dose range increased the signal intensity of tumors in a dose-dependent manner. There appeared to be no difference between the two compounds in signal intensity. It was concluded that the signal intensity of an intramuscular tumor in the rat was a function of dose.

Study Design: Enhancement of Novikoff hepatomas in the right hind legs of female Wistar Han rats was investigated as a function of dose following an intravenous administration of gadobutrol and gadopentetate administered as cumulative doses of

0.1, 0.3 and 0.5 mmol/kg. Using a 2T imager (SISCO SIS 85), T₁-weighted images were obtained before and 1 minute after application of the contrast agent.

Results: A dose-dependent increase in signal intensity of the tumors was observed for both gadobutrol and gadopentetate (dimeglumine). No difference in dose-dependent signal intensity was observed between the two contrast agents. At doses of gadobutrol or gadopentetic acid up to 0.5 mmol/kg, the T₁-signal intensity of visualizing an intramuscular tumor in the rat increased as a function of dose.

Conclusions: Using gadobutrol or Magnevist in doses up to 0.5 mmol Gd/kg, a dose-dependent increase in tumor signal intensity was observed in Novikoff hepatomas in the right hind legs of female Wistar rats.

Reviewer's comment: I agree with the conclusion of the sponsor. The result also confirms the findings from in-vitro study 9294 showing a dose-dependent increase in MR signal intensity. Similar to the comments on the in-vitro studies, a pertinent note is made of the possibility of a dose-dependent toxic effect arising from the use of contrast enhancing gadolinium-based products. This caution is especially relevant in view of the recently recognized severe complication of nephrogenic systemic fibrosis (NSF) associated with gadolinium-based contrast agents affecting primarily patients with renal disease, such as stage 4 or 5 chronic kidney disease (CKD).

4.2.3 In-vitro stability of ZK 135079

Introduction: In study number KM07269 (Report No. A42715), the kinetic profiles of Gd³⁺ dissociation from gadolinium-based contrast agents (1 mmol/l) were determined using serum from healthy human volunteers under physiological conditions.

The influence of 10 mmol/L phosphate on Gd³⁺ dissociation was investigated in an attempt to simulate the elevated serum phosphate levels often seen in patients with end-stage renal disease.

4.2.2.3 Report No. A42715:

Study title - Stability of Gadolinium-based contrast agents in human serum

Volume # and Page #:	Module 4.2.1.1.1, No. of pages: 32
Conducting laboratory and location:	(b) (4) (Contrast Media Research on behalf of Bayer Schering Pharma/Bayer HealthCare)
Study #:	KM 07269
Date of study initiation:	June 2007
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (lot #, %purity, N/A)

	(ZK 135079/SH L562B)
	Magnevist
	ZK00093035/SH L451A, SH L569B
	Gadoteric acid, Gadodiamide, Gadobenic acid, Gadoteridol, Gadoversetamide, 'gadofosveset trisodium
Animal species/strain/sex per dose:	N/A; in-vitro study
Doses/Vehicle:	1 mmol/l for all GBCAs tested
Duration/route:	In-vitro study

Key study findings:

- After incubation for 15 days, the release of Gd^{3+} from non-ionic GBCAs in human serum was about 10-fold higher than from ionic linear gadolinium contrast agents.
- Elevated levels of serum phosphate enhanced Gd^{3+} release from non-ionic and to a lesser extent from the ionic linear GBCAs.
- Macrocyclic GBCAs appeared stable in both normal and elevated phosphate serum levels.

Objective: The study was performed to assess the stability of the gadolinium complex and the dissociation rate of gadolinium-based contrast agents (GBCAs) in human serum. In order to simulate the situation in patients with end-stage renal disease who often have elevated phosphate levels, the influence of 10 mmol/L phosphate was also studied.

Study Design:

- The kinetics of Gd^{3+} dissociation of commercially available GBCAs (Gadobutrol, Omniscan, Optimark, Magnevist, Primovist, Vasovist, Prohance and Dotarem) were determined in pooled human serum obtained from three healthy volunteers and stored frozen at $-20^{\circ}C$. Each contrast agent was tested at a concentration of 1 mmol/l (pH 7.4, $37^{\circ}C$).
- Initial Gd^{3+} release and Gd^{3+} release after 15 days were determined using the ICP-MS method.

Results:

1. GBCAs: Initial Gd release and release after 15 days: In these results, GBCAs were grouped based on their stabilities in human serum (pH 7.4; $37^{\circ}C$) as non-ionic linear, ionic linear and macrocyclic.

a). Non-ionic, linear GBCAs (Omniscan and Optimark):

TT 1: Non-ionic linear GBCAs. Summary of the results.

	Omniscan®	Optimark®	Gadodiamide	Gadoversetamide
Gd ³⁺ release after 15 d [%]	20 [17; 20]	21 [19; 22]	25 [22; 26]	29 [26; 32]
Initial Gd ³⁺ release rate [%/d]	0.16 [0.15; 0.17]	0.44 [0.40; 0.51]	24 [12; 31]	17 [12; 30]

As shown in the above Table (TT1), there was a greater initial release of Gd ion with Gadodiamide and Gadoversetamide (the active substances of Omniscan and Optimark). The active substances do not contain excess ligand. Gd release after 15 days was still higher with the active compounds.

b). ionic, linear GBCAs (Magnevist, Multihance, Primovist (Eovist) and Vasovist (Ablavar) :

TT 2: Ionic linear GBCAs. Summary of the results

	Magnevist®	Multihance®	Primovist®	Vasovist®
Gd ³⁺ release after 15 d [%]	1.9 [1.2; 2.0]	1.9 [1.3; 2.1]	1.1 [0.76; 1.2]	1.8 [1.4; 1.9]
Initial Gd ³⁺ release rate [%/d]	0.16 [0.12; 0.36]	0.18 [0.13; 0.38]	0.07 [0.05; 0.08]	0.12 [0.11; 0.18]

The amounts Gd at initial release from the ionic, linear GBCAs were similar to values obtained for Omniscan and Optimark, both of which contain significant amounts of excess ligand. Magnevist, Multihance, Primovist and Vasovist, compounds released significantly lower amounts of Gd after 15 days when compared to the non-ionic linear compounds.

c). Macrocyclic GBCAs (Gadovist, Prohance and Dotarem):

TT 3: Macrocyclic GBCAs. Summary of the results

	Gadovist®	Prohance®	Dotarem®
Gd ³⁺ release after 15 d [%]	< 0.1	< 0.1	< 0.1
Initial Gd ³⁺ release rate [%/d]	< 0.007	< 0.007	< 0.007

Compared to the non-ionic linear and the ionic, linear GBCAs, macrocyclic compounds released negligible Gd both at initial measurement and after 15 days incubation.

2. Effect of elevated Phosphate on Gd release from GBCAs

With the non-ionic, linear GBCAs, the presence of additional phosphate resulted in a 100-fold increase in initial Gd release and 75-fold increase after 15 days.

The initial rates determined for the ionic, linear compounds was 12-30 fold. The release after 15 days was not comparable to amounts in the native serum.

Elevated phosphate did not result in any measurable release of Gd in the macrocyclic compounds.

Conclusions:

- While high amount of Gd^{3+} were released into the serum by all linear Gd compounds, macrocyclic GBCAs appeared stable in human serum at 37°C for 15 days under physiological conditions and in the presence of elevated phosphate levels.
- After 15 days, the amount of Gd^{3+} released from the non-ionic linear compounds was 10-fold greater than with the ionic linear GBCAs. The presence of excess ligand appeared to stabilize the nonionic formulations.
- While elevation in serum phosphate levels resulted in accelerated release of Gd^{3+} , the rate of release was faster for non-ionic than for ionic linear Gd-based complexes.

Reviewer's comments: After intravenous administration, GBCAs distribute into the extracellular space. The distribution is enhanced by the chelation of gadolinium to organic ligands which enhances the renal filtration of GBCAs for subsequent excretion by the kidneys. With normal functional kidneys, the elimination half-life is about 90 minutes with greater than 90% of the injected dose eliminated within the first 24 hours. The macrocyclic GBCAs appear generally more stable than their linear counterparts (Tweedle et al., 1991; Frenzel et al., 2008), a conclusion confirmed by the finding of this study in which little or no Gd^{3+} was released from the macrocyclic GBCAs into the serum with or without elevated phosphate levels.

Thermodynamic and kinetic stability are critical in predicting the amount of free gadolinium, which may result from dechelation of chelates in physiological and pathological situations. The high kinetic stability of the macrocyclic structure combined with a high thermodynamic stability minimizes the amount of free gadolinium released in tissue parenchyma's (Port et al., 2008). The overarching concern regarding the stability of all gadolinium chelates therefore relates to the risk of transmetallation, where gadolinium has the potential to be exchanged between ligands or be released as free

Gd³⁺ and retained in the body. As indicated from the findings of this study and published data, the GBCAs differ significantly with respect to transmetallation and kinetic and thermodynamic stability and their propensity to release free gadolinium, which is hypothesized to induce NSF (Kuo, 2008). Consequently, considerable emphasis has been placed on gadolinium chelate stability in-vivo and its impact on patient safety in recent years, given the possible association of gadolinium stability and the occurrence of Nephrogenic systemic fibrosis (NSF). It has been shown that patients with reduced kidney function have prolonged GBCA elimination half-life that may impact further the occurrence of NSF.

4.2 Secondary Pharmacology

No secondary pharmacology studies were performed

4.3 Safety Pharmacology

4.3.1 The pharmacological and biochemical properties of Gadobutrol

Introduction: In study report 9139/II, the sponsor described the pharmacological and biochemical properties of gadobutrol in *in-vitro* screening experiments using 0.5 mmol Gd/ml formulations of gadobutrol containing 0.5 mg/ml DTPA and diluted to various concentrations.

4.3.1.1 Report 9139/II:

Study title: Pharmacological and Biochemical Characteristics of Gadobutrol (ZK135079).

Volume # and Page #:	Module 4.2.1.3.1; 23 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Germany
Study #s:	KM 89174, KM 90017 and KM 90075
Date of study initiation:	October 1989
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (lot #, %purity – N/A) (ZK 135079/SH L562A) Gadopentetate dimeglumine (Magnevist/ZK 93035) was used in study KM 90075 (Batch Nos. 82004 and 12015)
Animal species/strain/sex per dose:	N/A; in-vitro study
Age:	N/A
Weight:	N/A
Duration/route:	N/A; in-vitro study

Key study findings:

- The partition coefficient of 0.1 mmol/l gadobutrol in 1-butanol and Tris-HCl buffer was negligible.
- The binding of gadobutrol to plasma proteins using ultrafiltration or steady state dialysis was 2.7% and 5.4%, respectively.
- At the highest concentration tested, gadobutrol inhibited lysozyme by 17%. There was a minimal activation of serum complement when gadobutrol was incubated with dog serum.
- Although gadobutrol inhibited hemolysis, the extent of inhibition as indicated by the IC_{50} was small ($IC_{50} = 23$ mmol/l) but higher than the value obtained with gadopentetate dimeglumine (12 mmol/l).
- Gadobutrol caused a minimal liberation of histamine from mast cells relative to the positive control (compound 48/80).

Study Design:

The partition coefficient of gadobutrol, its plasma protein binding, the extent to which it inhibits lysozyme or release of histamine, its ability to activate serum complement or inhibit hemolysis were determined in this study.

Complement activation: To determine serum complement activation by contrast agents, aqueous solutions containing increasing concentrations of gadobutrol in gelatin-HEPES-buffer (GHB) were incubated with 100 μ l of serum from the beagle dog.

Inhibition of hemolysis: The method for determining inhibition of hemolysis was similar to activation of serum complement.

Results:

- The partition coefficient of gadobutrol (final concentration of 0.1 mmol/l) using 1-butanol and Tris-HCl buffer at pH 7.6 was 0.006 compared to 0.0001 for gadopentetate dimeglumine (data from a previous study).
- Relative protein binding of gadobutrol using the ultrafiltration method was 2.7%, in contrast to 1.0 % for dimeglumine.
- Protein binding was similarly higher for gadobutrol (5.4%) compared to dimeglumine (-4.4 %) in the steady-state method.
- There was only a minimal inhibition of lysozyme (17%) by Gadobutrol.

Assay	Gadobutrol	Dimeglumine gadopentetate
Partition coefficient (1-butanol/buffer, pH 7.6)	0.006	0.0001 **
Relative binding to human plasma protein * • by ultrafiltration • by steady state dialysis	2.7 % 5.4 %	1.0 % -4.4 %
Inhibition of lysozyme (I ₅₀ : mmol/L)	> 300	77
Liberation of histamine from mast cells (I ₅₀ : mmol/L)	> 250	140 **
Activation of serum complement (I ₅₀ : mmol/L)	316	448 **
Inhibition of hemolysis (I ₅₀ : mmol/L)	23	12

* negative values can be considered to be zero. Binding was calculated relative to Yb-DTPA.

** results obtained in previous examinations

Serum complement was only slightly activated when beagle dog serum was incubated with gadobutrol concentrations ranging 0 – 353 mmol/l. The IC₅₀ was for gadobutrol (316 mmol/l) was lower than for dimeglumine (448 mmol/l). Gadobutrol also minimally inhibited hemolysis when mixed with Beagle dog serum and antibody-coated red blood cells at concentrations between 0 and 187 mmol/L. The IC₅₀ determined was higher (23 mmol/l) for gadobutrol when compared to dimeglumine (12 mmol/l). The data also indicated that gadobutrol had almost no effect on histamine release (0.6 -1.1nmol/l liberated histamine. The amount of histamine liberated by gadobutrol was comparable to that by dimeglumine which released 0.6 – 12.3 nmol/L. The positive control, compound 48/80 released a significantly higher amount of histamine (7.6 nmol/L). A range was not given for the positive control (Compound 48.80) since it was tested only at 5 ug/mL. However, as shown in the attached sponsor table, both gadobutrol and dimeglumine were tested over a dose range

Table 7: Liberation of histamine from mast cells by gadobutrol

Experiment No.	compound	liberated histamine nmol/mL	relative liberation, I ₅₀
KM 90 075	48/80 (5 µg/mL)	7.6	100 %
	none, spontaneous	0.5	0 %
	62-250 mmol/L Gadobutrol	0.6 - 1.1	I ₅₀ > 250 mmol/L
**	48/80 (5 µg/mL)	16.1	100 %
	none, spontaneous	0.2	0 %
	40-225 mmol/L Dimeglumine gadopentetate	0.6 -12.3	I ₅₀ = 140 mmol/L

** results obtained in previous examination

Conclusions: The results of the protein binding study confirm a predominantly hydrophilic nature of gadobutrol. The results also indicated that overall, the effect of gadobutrol on inhibition of lysozyme, activation of complement, inhibition of hemolysis and release of mast cell histamine was considerably minimal. Where there was an effect, such occurred at doses much higher than could be achieved after intravenous administration.

Reviewer's comments: It appeared from the results of this set of in-vitro studies that that gadobutrol did not bind to plasma proteins to any significant extent. The degree of plasma protein binding is important for evaluating the distribution of drugs. In-vitro studies with plasma are therefore valuable for predicting in-vivo protein binding. Plasma protein binding of drugs has also been shown to have significant effects on clinical pharmacokinetics and pharmacodynamics. It was shown that gadobutrol has a minimal inhibitory effect on lysozyme, an enzyme important in the immune system. Taken together with its little or no effect on complement activation and release of histamine, it appears that application of gadobutrol, as a contrast agent for MRI will not play a significant role in immune depression. Therefore, based on the findings of this study, administered the clinical dose gadobutrol could be considered relatively safe from a pharmacological or biochemical perspective.

4.3.2 Central nervous system

The effect of gadobutrol on the CNS was evaluated in mice and rats. The effects on general behavior following a single intravenous administration of gadobutrol formulation SH L562A (0.5 mmol/L) were studied using the Irwin test in mice (Report A19994). SH L562A administered as a single dose was used to determine if gadobutrol caused a depressive effect on electroshock convulsion in mice (A19996, A22448, A22977 and A19995). Two confirmatory studies using SH L562BB formulation were performed to determine the proconvulsive effect of gadobutrol (Report No. A22977). The effect of gadobutrol on pentylenetetrazole-induced convulsions was studied in mice (Report A19995). CNS function was also studied in rats following an intracisternal administration of gadobutrol (Report No. A711).

4.3.2.1 Report No. A19994:

Study title: Neurotropic effects of gadobutrol in the Irwin test in mice after single intravenous administration

Volume #, and Page #:

Module 4.2.1.3.1. No. of pages: 14
(b) (4)

Conducting laboratory and location:

(b) (4) study #:

92222

Date of study initiation:

May 12, 1992

GLP compliance:

Yes (), No (x)

QA report: Yes (), No (x)
Drug, lot #, and % purity: Gadobutrol
(SH L 562 BB / ZK 135079), Batch number - 31045416; lot # -N/A; % purity – N/A
Magnevist
(SH L451A 0 ZK 93035/Dimeglumine); Batch Number – 14020; lot # -N/A; % purity – N/A
Animal species/strain/sex per dose: Male ICR mice (b) (4)
6 animals/group
Age: 5 weeks old at purchase
Weight: 27-32 g
Doses/Vehicle: Gadobutrol or Magnevist (Reference compound) each administered in 3 doses, namely: 2.5, 5 and 5 mmol Gd/kg
Saline vehicle (control); 20 ml/kg
Dose volume: 20 ml/kg (for each test compound)
Duration/route: Single dose / intravenous

Dose (mmol Gd/kg)	2.5	5	10
Dose multiple (Based on BSA)	2.02x	4.05x	8.11x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Key study findings:

- At 2.5, 5 and 10 mmol Gd/kg (respectively, 2.02x, 4.05x and 8.11x the intended human dose), intravenously administered gadobutrol had neurotropic effects in male mice.
- The observed effects were behavioral, neurological and autonomic.
- No mortality was reported at any dose of gadobutrol.
- Magnevist (reference compound) showed similar signs however, there were mortalities at 10 mmol Gd/kg.

Study Design: The purpose of the Irwin test was to determine the acute neurotropic effects in mice following a single intravenous administration of gadobutrol. Six groups of mice were administered 2.5, 5 and 10 mmol Gd/kg of Gadobutrol or Magnevist (reference compound) intravenously. One group of control animals received a corresponding volume of saline. Animals were observed at 30 min, 4h and 24h following administration of the test compounds for signs and symptoms of vegetative, neurological and behavioral effects.

Results:

- The results summarized in the Table below indicate that behavioral effects (increased or decreased locomotion), neurological symptoms (twitchings) and autonomic effects (decreased respiration, hypothermia, and hyperthermia) were observed 30 min after administration.
- In all animals, recovery had occurred by 4 h post injection.

- Effects were observed at all doses of gadobutrol tested and the NOAEL was determined as <2.5 mmol Gd/kg
-

Table 8: Irwin test - Effect of Gadobutrol and Magnevist

Parameter	Saline	Gadobutrol (mmol Gd/kg)			Magnevist® (mmol Gd/kg)		
		2.5	5	10	2.5	5	10
		6 mice/group			6 mice/group		
↓ Respiration				1			
↑ Locomotion		1					
↓ Locomotion			1	1			1
Hypotonic gait							
						1	
Twitchings				1			
Increased Latency, tail pinch					1		1
Hypothermia				2			
Hyperthermia	2	2			1	1	
Death						2	4
Swelling of inferior limb				3			

Reviewer's Table adapted from sponsor's Table; Numbers represent animals showing symptoms

Conclusions:

- Behavioral effects, neurological symptoms and autonomic effects were observed following single intravenous administration of Gadobutrol (2.5-10mmol Gd/kg).
- The effects observed included including slowed respiration, increased or decreased locomotion, twitchings, hypothermia/hyperthermia and swelling of inferior feet.
- There was no mortality at any of the doses of gadobutrol tested. However 2/6 and 4/6 deaths occurred at 5 and 10 mmol Gd/kg respectively in mice treated with the reference compound, Magnevist
- In general, similar effects were observed in mice that received Magnevist?
- Hyperthermia also occurred in 2/6 control mice compound Magnevist, which unlike Gadobutrol, was lethal at the 10 mmol Gd/kg.
- NOAEL (<2.5 mmol Gd/kg) was not established for gadobutrol or Magnevist in this test

Reviewer's comments: Since symptoms were observed at all doses tested, this reviewer does not agree with the sponsor that gadobutrol caused 'only few, unspecific and transient effects' and that the effects were 'unsustained'. Based on the findings, the

effects were well defined and similar findings were observed in mice treated with the reference compound, Magnevist administered in the same doses and in the same study. NOAEL was not established.

Reviewer's comments: The effects do not appear to be dose-dependent but rather seemed to be isolated occurrences. The observed effects occurred at 30 min post-injection, were resolved by the 4h observation time point and no lethality was reported within a 24 h period in doses up to 10 mmol Gd/kg (8x the HD).

4.3.2.2 Report No. A19996:

Study title: Effect of gadobutrol on maximal electroshock-induced convulsions after single intravenous administration to mice in comparison with Magnevist®

Volume #, and Page #:	Module 4.2.1.3.1, 11 pages
Conducting laboratory and location:	(b) (4)
(b) (4) study #:	92231
Date of study initiation:	June 23, 1992
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (SH L 562 BB / ZK 135079), Batch number - 3104 5416; lot # -N/A; % purity – N/A, Magnevist (SH L451A 0 ZK 93035/Dimeglumine); Batch No. – 14020; lot # -N/A; % purity – N/A
Animal species/strain/sex per dose:	Male ICR mice (b) (4) 5-6 animals/group; 5 treatment groups
Age:	5-7 weeks old
Weight:	27.3-32.2 g
Doses/Vehicle:	Gadobutrol or Magnevist (Reference compound) each administered in two doses (2.5 and 5 mmol Gd/kg; Saline vehicle (control); 20ml/kg
Dose volume:	20 ml/kg (for each test compound)
Duration/route:	Single dose / intravenous (tail vein)
Infusion rate:	1 ml/min
Protocol deviation:	One animal each in the saline and 5 mmol Gd/kg gadobutrol-treated group had to be excluded due to failure of electrical stimulation.

Dose (mmol Gd/kg)	2.5	5
Dose multiple (Based on BSA)	2.02x	4.05x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Key study findings: Gadobutrol at 2.5 mmol Gd/kg, intravenous did not affect maximal electroshock-induced convulsions. There was an increased incidence of death at 5 mmol Gd/kg Gadobutrol.

Study Design: 5 groups of mice were administered Gadobutrol or Magnevist (reference compound) intravenously in doses of 2.5 and 5 mmol Gd/kg. Control mice received saline. Each animal received a current of 16 mA with 800 V for 0.2 sec through corneal electrodes 5 minutes after receiving gadobutrol, Magnevist or saline. Animals were observed for occurrence of tonic and clonic convulsions or death immediately after applying the electroshock.

Results:

- When administered at 2.5 mmol Gd/kg (or 2.02x the human dose), Gadobutrol did not prevent maximal electroshock-induced convulsions
- As a sign of proconvulsive activity, gadobutrol caused death in 1/6 mice at 2.5 mmol Gd/kg with a significant increase in death (4 animals) at 5 mmol Gd/kg.
- Magnevist did not affect the incidence of tonic/clonic convulsions but had a proconvulsive effect resulting in death of 2/6 mice at each dose.
- The results are summarized in the following sponsor's Table:

Table 9: Effects of Gadobutrol or Magnevist on maximal electroshock-induced convulsions in mice

Treatment	n	Number of animals with		
		convulsions	lethality	
		tonic	clonic	
Saline	5	5	5	0
Gadobutrol 2.5 mmol Gd/kg	6	5	5	1
Gadobutrol 5 mmol Gd/kg	5	5	2	4 *
Magnevist® 2.5 mmol Gd/kg	6	6	4	2
Magnevist® 5 mmol Gd/kg	6	5	4	2

20 mL/kg i.v.

n = number of animals per group

*: p<0.05 vs. saline-treated group by Fisher's exact probability test

Conclusions: Gadobutrol did not produce anticonvulsive effects at 2.5 mmol Gd/kg. It demonstrated a proconvulsive effect in one animal at 2.5 mmol Gd/kg. This animal was the single mortality reported in this study. The incidence of mortality was significant increased at 5 mmol Gd/kg gadobutrol. Magnevist appeared to have a slight anticonvulsive effect at 2.5mmol Gd/kg. Magnevist demonstrated a proconvulsive effect at both dose tested.

Reviewer's comments: Based on the findings, both Gadobutrol and Magnevist demonstrated proconvulsive effects. Anticonvulsive effect was not pronounced with both compounds at the doses tested.

4.3.2.3 Report No. A22448:

Study title: Effect of gadobutrol on maximal electroshock-induced convulsions in mice after single intravenous administration in comparison with gadopentetic acid

Volume #, and Page #:	Module 4.2.1.3.1; 15 pages
Conducting laboratory and location:	Bayer Schering Pharma, Germany
(b) (4) study #:	SP20040017
Date of study initiation:	April 27, 2004
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562 BB / ZK 135079), Batch number - 13008C; lot # -N/A; % purity – N/A
	Magnevist (SH L451A 0 ZK 93035/Dimeglumine); Batch Number – 21508A; lot # -N/A; % purity – N/A
Animal species/strain/sex per dose:	Male NMRI mice (b) (4) 40 mice; 8 animals/group in 5 treatment groups
Age:	N/A
Weight:	28-30 g
Doses/Vehicle:	Gadobutrol or Magnevist (Reference compound) each administered in 2 doses, namely: 2.5 and 5 mmolGd/ml
	Saline vehicle (control); 20 ml/kg
Dose volume:	20 ml/kg (for each test compound)
Infusion time:	30s
Duration/route:	Single dose / intravenous (tail vein)
Protocol deviation:	Statistics was performed by nonclinical statistics department

Dose (mmol Gd/kg)	2.5	5
Dose multiple (Based on BSA)	2.02x	4.05x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Key study findings: While Gadobutrol, administered at 2.5 mmol Gd/kg or 5 mmol Gd/kg (2.02x and 4.05x the intended human dose), did not prevent electroshock-induced convulsions, the findings demonstrated that, similar to Magnevist, gadobutrol caused a proconvulsive effect that resulted in mortalities in mice treated with both doses.

Unlike Gadobutrol, Magnevist dose-dependently caused an increase in death rate in mice that received electric currents.

Study objective: In the maximal electroshock (MES) test to determine possible anticonvulsive or proconvulsive effects properties of gadobutrol, (Study 92231/Report No. A19996), the incidence of death was increased in animals treated with 5 mmol Gd/kg. The aim of the present study was to verify the findings of the earlier study.

Study Design: Gadobutrol and Magnevist were each tested at 2.5 and 5 mmol Gd/kg administered intravenously to groups of male NMRI mice. Control animals received saline. Maximal electroshock was delivered as previously described (A19996) and incidence of tonic or clonic convulsions, and death determined 1 min after maximal electroshock stimulation.

Results: The results are described in the sponsor's Table below:

Table 10: Effect of pretreatment with gadobutrol or Magnevist on maximal electroshock-induced convulsions in mice

Treatment	Dose (mmol Gd/kg)	n	Number of animals with convulsions		lethality
			tonic	clonic	
Vehicle ^{a)}	—	8	7	6 ^{D)}	2
Gadobutrol	2.5	8	8	7 ^{D)}	1
“	5.0	8	7	6 ^{D)}	2
Gadopentetic acid	2.5	8	8	2 ^{D)}	5 ^{IV)}
“	5.0	8	7	1 ^{II)}	6 ^{III) IV) V)}
“					

a): 0.9 % sodium chloride in purified water

n: number of animals per group

At 2.5 or 5 mmol Gd/kg (respectively, 2.02x and 4.05x the intended human dose), Gadobutrol did caused in 1/8 and 2/8 mice, respectively however, gadobutrol did not appear to have anticonvulsive effects.

At both 2.5 and 5 mmol Gd/kg, Magnevist had proconvulsive effect and caused death in 5/8 and 6/8, respectively.

This mortality rate was significant when compared to the effect of Gadobutrol administered at the same dose of 2.5 mmol Gd/kg. At 5 mmol Gd/kg, the Magnevist-induced mortality increased significantly when compared to the vehicle-treated group,

with one animal death occurring after administering Magnevist and 5 deaths occurring during the tonic seizure.

Conclusions: Although the sponsor concluded that, “Gadobutrol in doses of 2.5 mmol Gd/kg and 5 mmol Gd/kg has no effect on electroshock-induced convulsions and death rate in mice’, the findings indicated that while Gadobutrol, like Magnevist, did not appear to have anticonvulsive effects, both compounds were proconvulsive and were lethal at the two doses tested.

Reviewer’s comments: I do not agree with the conclusion of the sponsor that Gadobutrol was not lethal in mice. The proconvulsive effect of gadobutrol was greater in study report A19996 compared to the findings of A22448 where fewer deaths were reported. Taken together with the findings of a previous study, the reviewer disagrees with the sponsor that gadobutrol was not proconvulsive in mice.

4.3.2.4 Report No. A22977:

Study title: Effect of gadobutrol on maximal electroshock-induced convulsions in mice after single intravenous administration in comparison with gadopentetic acid

Volume #, and Page #:	Module 4.2.1.3.1, 16 pages
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	SP20040021
Date of study initiation:	June 7, 2004
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (lot #: N/A, %purity –N/A) (ZK 135079/SH L 562BB, p 6/13) Gadopentetate dimeglumine, (lot #: N/A, %purity –N/A), ZK 93035/SH L451 AA Saline (0.9% NaCl) was used as vehicle/control substance
Substance Batch numbers:	Gadobutrol – 14009A Gadopentetate dimeglumine – 21508A
Formulation Batch numbers:	N/A
Animal species/strain/sex per dose:	Male NMRI Mice (b) (4) 8/group; 6 groups
Age:	N/A
Weight:	27-30 g
Doses/Vehicle:	Gadobutrol; 3 doses (2.5, 5 and 10 mmolGd/kg) Gadopentetate: 2 doses (2.5 and 5 mmol Gd/kg)
Duration/route:	intravenous route
Infusion time:	15 sec
Injection volume:	20 ml/kg

Protocol deviations:

The statistical analysis of the incidences of tonic and clonic convulsions was not performed because animals died immediately after treatment or tonic seizures.

Dose (mmol Gd/kg)	2.5	5	10
Dose multiple (Based on BSA)	2.02x	4.05x	8.11x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Key study findings: At 2.5, 5 and 10 mmol Gd/kg, Gadobutrol did not reduce the incidence of tonic and/or clonic convulsions thus did not show any anticonvulsive activity. In addition, no gadobutrol-treated animals died indicating absence of proconvulsive activity.

Study objective: The purpose of this study was to clarify conflicting reports obtained in two previous studies, A19996 and A22448, conducted to determine possible anticonvulsive properties or proconvulsive effects of Gadobutrol. While Gadobutrol enhanced the death rate in report A19996, it did result in less mortality in Report A22448. To address this conflict, study report A22977 investigated the effect of Gadobutrol at 10 mmol Gd/kg, which is 2-fold higher than the maximal gadobutrol dose in the two previous studies. Magnevist was tested a reference compound.

Study Design: Three doses of Gadobutrol (2.5, 5 and 10 mmol Gd/kg) and 2 doses of Magnevist (2.5 and 5 mmol Gd/kg) were injected intravenously to male NMRI mice using 5 dose groups. Group 6 consisted of control animals treated with the saline vehicle. Test and control articles were injected in an infusion volume of 20 ml/kg. Control animals received 20 ml/kg of the vehicle. Maximal electroshock was applied via the cornea 5 min after application of test compound/vehicle. Parameters determined for each treatment group included presence of tonic seizure; presence of clonic seizure and occurrence of death.

Results: Two mice in the vehicle-treated group died during the tonic seizure phase. At the administered doses of 2.5, 5 and 10 mmol Gd/kg, Gadobutrol did not reduce the incidence of tonic and/or clonic convulsions thus did not show any anticonvulsive activity. In addition, no gadobutrol-treated animals died indicating absence of proconvulsive activity. At 2.5 mmol Gd/kg, gadopentetate caused a 12.5% higher death rate in mice compared to corresponding vehicle-treated mice following electroshock treatment. A 50% incidence of death (4 of 8 mice) was reported following 5 mmol Gd/kg gadopentetate and 25% mortality (2/8 mice) occurring during tonic seizures at 5 mmol Gd/kg gadopentetate.

Conclusions: The sponsor concluded that gadobutrol had no effect on maximal electroshock-induced convulsions and death rate in mice. This results added to the finding of a similar study (Report No.A22448) indicate that the enhanced death rate attributable to 5 mmol Gd/kg gadobutrol observed in Report No. A19996 was an

accidental finding, a conclusion supported by the finding in Report A22977 that a 2-fold higher amount of gadobutrol (10 mmol Gd/kg) had no effect on maximum electroshock-induced convulsions and death rate in mice.

Reviewer's comments: I agree with the sponsor's conclusion on the summation of data from the 3 reports A22448, A19996 and A22977 that gadobutrol did not have any effect on maximal electroshock convulsions and did not cause deaths due to proconvulsive effects.

4.3.2.5 Report No. A19995:

Study title: Effect of gadobutrol on pentylenetetrazole-induced convulsions after single intravenous administration to mice.

Volume # and Page #:	Module 4.2.1.3.1; No. of pages: 10
Conducting laboratory and location:	(b) (4) for Bayer Schering Pharma AG, Germany
Study #:	94236
Date of study initiation:	May 30, 1994
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol ZK135079/SH L562 A (lot #, N/A %purity –N/A) Pentylenetetrazole
Substance Batch numbers:	N/A
Formulation Batch numbers:	31021
Animal species/strain/sex per dose:	6-7 Male ICR mice (b) (4)/group
Age:	4 weeks
Weight:	26-31 g
Doses/Vehicle:	1, 2.5 and 5 mmol Gd/kg gadobutrol/Saline vehicle
Duration/route:	Single/intravenous via tail vein
Infusion rate:	1 ml per min

Dose (mmol Gd/kg)	1	2.5	5
Dose multiple (Based on BSA)	0.8x	2.1x	4.1x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Key study findings: At 1, 2.5 and 5 mmol Gd/kg (or 0.8x, 2.1x and 4.1x the intended human dose, respectively), gadobutrol did not affect pentylenetetrazole-induced convulsions in mice.

Study objective: The purpose of this study was to determine the effect(s) of single intravenous doses of gadobutrol on the threshold dose for induction of clonic seizures by pentylenetetrazole.

Study Design: Four groups (n=6-7 mice/treatment group) were used for this study. Gadobutrol at 1, 2.5 and 5 mmol Gd/kg (or 0.8x, 2.1x and 4.1x the intended human dose, respectively) was intravenously infused at 1ml/min via the tail vein. Saline was used as volume control. Immediately after pretreatment with test substance or saline, animals were intravenously administered pentylenetetrazole (PTZ; 15 mg/ml) at a rate of 0.2 ml/min. The PTZ infusion time (sec) elapsing from the start of the infusion to the occurrence of the first clonic seizure was recorded for each animal. The infused PTZ dose required to induce the clonic seizures i.e., the PTZ threshold dose, was calculated for each animal. The mean PTZ threshold \pm S.D. was determined for each group. The data was statistically analyzed by one-way ANOVA and compared with the saline-treatment group with the Dunnett's test. Statistical significance was established at 5% level.

Results: Gadobutrol at doses up to 5 mmol Gd/kg had no significant effect on the PTZ threshold dose for triggering clonic seizures in mice.

Conclusions: Gadobutrol in single administration up to 5 mmol Gd/kg did not affect PTZ-induced convulsions.

Reviewer's comments: I agree with the sponsor's conclusions.

Neural tolerance of Gadobutrol administered by the intracisternal route

Report No. A711: Tolerance (ED₅₀) of Gadobutrol and ZK 92242 after intracisternal injection in the rat (M+F)

Volume #, and Page #:	Module 4.2.1.3.1, pages 1- 9
Conducting laboratory and location:	Bayer Schering Pharma AG, Germany
Study #:	KM 82066; KM 90075
Date of study initiation:	circa November – December 1982
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (ZK135079); formulation No. 000 890; Control article: ZK 92042, Lot #: 0918
Substance Batch numbers:	PLA 411
Animal species/strain/sex per dose:	Rat/Han: WIST (Schering), 5/sex/dose (per compound)
Age:	N/A
Weight:	130-170 g
Doses/Vehicle:	Gadobutrol (0.5 mol Gd/l diluted with Ringer's solution to 15, 45, 75, and 135 μ mol Gd/ml); ZK 92 242 (0.5 mol Gd/l diluted with Ringer's solution to 6.3, 7.8, 10.4, 15.6 and 31.3 μ mol/l Gd/l)
Route:	Intracisternal administration

Key findings: Gadobutrol was well tolerated when it was administered via the intracisternal route.

Study objective: The purpose of this study was to compare the neural tolerance of Gadobutrol (ZK 135079) with a formulation containing the ionic gadolinium chloride. The effective dose of the compounds was determined.

Study design: Lightly anesthetized rats were prepared for suboccipital puncture. An early formulation of gadobutrol diluted to concentrations of 15, 45, 75, and 135 mmol/l with Ringer solution as administered into the cisterna magna via the occipital puncture in a dose volume of 80 μ l. In order to evaluate an ED₅₀ the animals were observed for the presence of neurofunctional deficits and tested by grips and righting reflex, lack of motor coordination or epileptogenic cramps occurring at 15 min, 3 h and 24 h post-injection following individual animal stress from tossing the animal. Data was statistically evaluated by probit analysis.

Results: 50% of animals studied had neurofunctional deficits at 18 or 6 μ mol /kg respectively for gadobutrol or ZK 92242. The difference between the two formulations was statistically significant. Results indicate a 3-fold greater neural tolerance of gadobutrol than gadolinium chloride formulation in rats.

Conclusions: The sponsor concluded that an intracisternal dose of 18 μ mol Gd/kg was better tolerated than ZK 92242 in the rat.

Reviewer's comments: The proposed indication for gadobutrol includes MRI of disrupted BBB. The intracisternal route of administration is a means of drug delivery used experimentally to by-pass the blood-brain barrier and serves as an unconventional route of administration to determine the neural effects of a drug when administered in such a way as to mimic a non-functional or disrupted BBB. The result of the study might provide evidence of the safety of gadobutrol when used as a contrast agent in MR imaging of disrupted blood-brain barrier.

4.3.3 Cardiovascular system

Introduction: Gadobutrol was evaluated for its effects of on the cardiovascular system using both *in-vitro* (human hERG potassium channel and cardiac action potential in isolated guinea pig papillary muscle) and *in-vivo* tests performed in conscious, telemetered Beagle dogs, anesthetized dogs and rabbits.

4.3.3.1 In-vitro cardiovascular studies

The effects of gadobutrol (SH L 562 BB) on the human potassium channel were evaluated using Chinese Hamster Ovary (CHO) cells stably transfected with the hERG (human Ether-a-go-go) potassium channel using the whole cell patch clamp technique. Report A08412 compared the electrophysiological effects of Gadobutrol, Omniscan, Prohance and Imeron on the hERG-mediated potassium current. In another non-GLP study (Report 32843); the effects caused by mannitol-induced hyperosmolar solutions on inhibition of the hERG-mediated potassium current were examined in order to determine the contribution of osmolality on the outcomes of drug effect on in-vitro tests. The effect of gadobutrol on the action potential in isolated papillary muscle in comparison to Imeron[®] 400 was evaluated using guinea-pig papillary muscle specimens prepared from male guinea pigs Report A08161).

4.3.3.1.1 Report No. A08412:

Study title: Electrophysiological examination of Gadovist, Omniscan, Prohance, and Imeron[®] on the hERG-mediated potassium current

Volume # and Page #:	Module 4.2.1.3.1; 28 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Germany
Study #:	GEP_SCH_210_00_00_00
Date of study initiation:	Jan 4, 2002
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (ZK135079/SH L562 BB); Gadodiamide (ZK 117439), Gadoteridol (ZK 138947) and Iomeprol (ZK 32308); lot #: N/A, and % purity – N/A
Animal species/strain/sex per dose:	In-vitro study with CHO cells
Doses/Vehicle:	10, 30 and 100 mmol/l of each test article.
Duration/route:	N/A

Key study findings: Application of Gadobutrol, Omniscan, Prohance and Imeron caused a slight concentration-dependent inhibition of hERG-mediated potassium current. The effects of gadobutrol on hERG-mediated currents were not different from those observed with Omniscan, Prohance and Imeron and all four agents only slightly inhibited hERG-mediated current amplitude with threshold at 30 mmol Gd/L.

Study objective: The purpose of this study was to investigate the effects of Gadobutrol, Omniscan, Prohance and Imeron on the human hERG potassium channel. The experiments were performed in Chinese hamster ovary (CHO) cells stably expressing this channel.

Study Design:

- The patch-clamp technique in CHO cells was used to determine whole cell potassium currents activated at a frequency of 0.1 Hz.
- Gadobutrol, Omniscan, Prohance or Imeron were tested at 10, 30 and 100 mmol/L
- In separate studies, each concentration of test compound was added to the extracellular buffer solution for incubating the CHO cells for 6 min.
- Terfenadine was used as positive control for inhibition of the hERG channel. The positive control experiments were performed in November 2001 and January 2002. According to the sponsor, the positive control results had a good correlation with cited current literature (Rampe et al., 1997). It was however not stated that positive control experiments were conducted on the same day as test and negative control studies.

Results: The mean remaining relative current amplitudes (\pm s.d) re shown in the following Table:

Table 11: Effect of GBCAs on hERG-mediated potassium current

Test compounds	Test concentration (mmol/l)		
	10	30	100
Gadobutrol	0.89 ± 0.02	0.80 ± 0.09	0.55 ± 0.15
Omniscan	0.91 ± 0.05	0.83 ± 0.06	0.66 ± 0.06
Prohance	0.84 ± 0.05	0.73 ± 0.10	0.65 ± 0.12
Imeron	0.98 ± 0.10	0.89 ± 0.09	0.60 ± 0.10

Mean remaining relative current \pm s.d

- All four compounds tested (Gadobutrol, Omniscan, Prohance and Imeron) slightly inhibited the amplitude of the hERG-mediated current in a concentration-dependent manner with a threshold around 30 mmol/l.
- However, substantial effects were observed at maximal evaluated concentration of 100 mmol/L, which caused a reduction of about 40%.
- The effects on the current were not reversible at the maximal concentration.
- Half-maximal inhibition concentrations (IC_{50}) were not estimated.
- There were no significant changes in the current in saline control experiments performed at the same experimental days and under identical experimental conditions.

Reviewer's comment: Based on the findings of this study, the sponsor concluded that the four contrast agents - Gadobutrol, Omniscan, Prohance and Imeron, slightly inhibited hERG-mediated potassium current dose-dependently with the threshold at 30 mmol/L. There was however, a significant and irreversible block of the hERG current at 100 mmol/l. Although at 100 mmol/L the inhibition was significant, the dose at which it was achieved was non-physiological. Results obtained from the positive control terfenadine experiments acceptably validated the study data.

The reviewer agrees with the conclusion of the study that gadobutrol did not substantially inhibit the hERG-mediated potassium current. It is known that gadolinium-

based contrast agents affected the hERG-mediated potassium current, an effect commonly ascribed to osmolarity. For this reason, the effect of GBCAs on hERG channel is usually compared with the effect of an isoosmotic mannitol solution. This comparison was made by the sponsor in the study report A32843 described below:

4.3.3.1.2 Report No. A32843

Study title: Effects of hyperosmotic conditions induced with D-mannitol on hERG-mediated potassium current recorded from stably transfected CHO cells

Volume # and Page #:	Module 4.2.1.3.1 20 pages
Conducting Laboratory and location:	Bayer Schering Pharma AG, Germany
Study #:	SP200550053
Date of Study initiation:	November 30, 2005
GLP Compliance:	Yes (), No (x)
QA Report:	Yes (), No (x)
Drug, lot #, and % purity:	D- Mannitol (ZK25805); lot #: N/A; % purity – 99%
	E-4031 (ZK344354); Negative control); lot #:
	N/A; % purity – 98%
Substance Batch numbers:	ZK25805 - 025K0090
	ZK344354 - B55300
Animal species/strain/sex per dose:	N/A; In-vitro study

Key study findings: Hyperosmolar external solution with D-mannitol (400, 500 or 600 mOsm/L) markedly decreased hERG potassium current in an osmolarity-dependent manner. At 600 mOsm/L, there was an inhibition of hERG potassium current similar to the block induced by E-4031, the specific hERG channel blocker.

Study objective: The purpose of this study was to investigate the effect of hyperosmolar external conditions induced with D-mannitol (ZK 25805) on the hERG potassium channel mediated current recorded from CHO cells that have been stably transfected with hERG cDNA.

Study design: Whole cell patch-clamp technique was used in this study using CHO cells, which stably express the hERG gene. Potassium current in the floating CHO cells was activated at a frequency of 0.1 Hz and was recorded at room temperature in the presence of vehicle (bath solution) for < 10 minutes (pre-treatment phase) followed by the observation with vehicle or hyperosmolar solution prepared by addition of D-mannitol to the bath solution for < 15 minutes (treatment phase). After washing out the vehicle or D-mannitol (ZK 25805), current was recorded for <10 minutes to test for reversibility of the treatment effect.

D-mannitol was added to the bath solution at the concentration of 38, 108, 208 or 308 mmol/L corresponding to the nominal osmolality of 330, 400, 500 or 600 mOsm/L, respectively. The standard bath solution (at nominal osmolality of 292 mOsm/L) was used as a negative control in order to account for spontaneous changes in current amplitude during the measurement. The specific HERG channel blocker ZK 344354 (E-4031) at 1 μ mol/L dissolved in bath solution was applied as a positive control article at the end of an experiment to confirm that the outward current had been due to HERG-related potassium channel.

Results: Hyperosmolar external solution with D-mannitol (400, 500 or 600 mOsm/l) markedly decreased hERG potassium current in an osmolality-dependent manner with IC₅₀ of 401 mOsm/l. At 600 mOsm/L, the potassium current was almost completely inhibited and comparable to the block induced by 1 μ M E-4031, a specific blocker of hERG channels.

Conclusions: Hyperosmolar external conditions induced by D-mannitol reduced the hERG-related potassium currents. The sponsor concluded that when hyperosmolar drug formulations (e.g. contrast agents) are used, control substances with comparable osmolality should be tested in parallel in order to distinguish between specific drug effects and non-specific effects of hyperosmolality.

Reviewer's comments: Inhibition of the rectifier current (I_{Kr}), the major mechanism for drug-induced QT prolongation of the ECG, is associated with increased risk of fatal arrhythmia. Therefore, absence of the hERG potassium current inhibition at therapeutic drug concentrations is a positive endpoint in nonclinical drug development. This study was conducted to determine the role of hyperosmolar solution on the hERG potassium channel mediated current recorded from CHO cells expressing the hERG current and it was shown that hERG-related potassium currents could be inhibited by hyperosmolar concentrations.

According to the sponsor, when clinical formulations of gadolinium containing contrast agents were used in patch-clamp studies, inhibition of HERG potassium current was seen at high concentrations. In studies with bath solutions containing > 5 % of the contrast agent's clinical formulation, all agents showed concentration-dependent inhibition of HERG. It was therefore unclear whether inhibition of HERG current was due to a compound specific effect or due to the unspecific hyperosmolar condition of the bath solution mixed with the formulation of the gadolinium contrast agent. I agree with the sponsor's conclusion that since hyperosmolar external conditions reduce hERG-related potassium currents, investigations of hyperosmolar drug formulations seen with contrast agents need to be tested with control solutions of comparable osmolality in order to distinguish between specific drug effects and the non-specific effects of osmolality. This study would be more meaningful if was performed concurrently with the Gd compound.

4.3.3.1.3 Report No. A08161:**Study title: Effects of Gadovist® 1.0 mmol/ml on cardiac action potential in isolated guinea pig papillary muscle in comparison to Imeron® 400**

Volume # and Page #: Module 4.2.1.3.1; 32 pages
Conducting laboratory and location: Bayer Schering Pharma AG, Germany
Study #: SP20010257 / SP20020004
Date of study initiation: November 28, 2001 and July 2, 2002
GLP compliance: Yes (), No (x)
QA report: Yes (), No (x)
Drug, lot #, and % purity: Gadobutrol ZK135079/SH L562BB
Substance Batch numbers: Gadobutrol - 13008C
Iomeprol (comparator) - ZK 32308 (Batch #: 30028)
Animal species/strain/sex per dose: N/A; In-vitro study
Doses/Vehicle: Gadobutrol or Iomeprol were each tested at 0.5, 5.0 and 50 mmol/l
Duration/route: In-vitro test

Key study findings: Gadobutrol at up to 50 mmol/L did not significantly affect repolarization of action potential at 30, 60 or 90% using the isolated guinea pig papillary muscle. There were no effects on membrane potential, action potential amplitude and upstroke velocity. Unlike, gadobutrol, Imeron had a marked effect on APD₃₀ comparable to dL-Sotalol in the control group.

Study objective: The objective of this study was to assess the effects of Gadobutrol on intracellularly recorded action potential parameters in the guinea pig isolated papillary muscle preparation and to compare the effects with those of the non-ionic contrast agent Imeron® 400.

Study Design: Papillary muscles removed from freshly excised male guinea pig hearts were superfused at 5ml/min with Tyrode solution gassed in 95% O₂:5% CO₂ at 35 °C. The muscles were electrically paced at 0.3 Hz, 1 Hz, and 3 Hz (via silver electrodes) and impaled with 3 M KCl-filled glass micropipettes to monitor membrane potential. For each experiment, test substances were studied at 0.5, 5.0 and 50 mmol/l and test performed after an incubation of 30 min. Corresponding volumes of saline were added to the superfusion in the controls. The muscles were exposed to each concentration of Gadobutrol (1 mmol/L) or Imeron 400 (1.05 mmol/L) for 30-35 min. DL-Sotalol (100 µmol/l; 15 min exposure) was used as positive control. Test parameters measured included 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). Muscles (n=6) per group were evaluated.

Results: Up to 50 mmol/L, gadobutrol did not significantly affect repolarization of action potential at 30, 60 or 90%. Correspondingly, there were no effects on membrane potential, action potential amplitude and upstroke velocity. On the contrary, Imeron had a marked effect on APD₃₀ comparable to dl-Sotalol in the positive control group. APD₆₀,

and APD₉₀ effects were not different from controls. Imeron significantly increased membrane potential at 0.3 and 1 Hz by about -4 mV. This hyperpolarization was not observed at 3 Hz. 100umol/l dl-Sotalol produced the anticipated effects on action potential duration namely, a prolonged APD₃₀, APD₆₀, and APD₉₀ values relative to pre-application values. The membrane potential and action potential amplitude as well as upstroke velocity were unaffected.

Conclusions: There were no significant changes in action potential attributable to Gadobutrol in this study, although a prolongation in action potential duration was observed with in sotalol-treated muscles. Overall, the results of this study do not appear to indicate that gadobutrol presents any evidence of a potential to prolong repolarization of action potential in cardiac muscle.

Reviewer's comments: I agree with the sponsor's conclusions.

4.3.3.2 In-vivo cardiovascular studies

The *in-vivo* effect of gadobutrol formulation SH L 562 BB on the cardiovascular system was determined in conscious, telemetered Beagle dogs (GLP Study Report A08428). The non-GLP study (Report A431) using SH L562A compared the hemodynamic effects of gadobutrol and Magnevist in the closed-chest anesthetized dog. The cardiohemodynamic effects of gadobutrol (SH L562A) were examined using Japanese White rabbits anesthetized with subcutaneous or intravenous urethane (Report AN71).

4.3.3.2.1 Report No. A08428:

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

Volume #, and Page #:	Module 4.2.1.3; 100 pages
Conducting laboratory and location:	(b) (4)
(b) (4) study #:	DERA1005
Date of study initiation:	January 29, 2002
GLP compliance:	Yes (x) No ()
QA report:	Yes (x) No ()
Drug, lot #, and % purity:	Gadobutrol. The vehicle was 0.9% saline. There was no separate control group. (SH L 562 BB / ZK 135079, p1/100)
Batch number:	13008C
Animal species/strain/sex per dose:	Beagle dogs/4 males, 0 females/ 1 treatment group
Age:	13–16 months (on dosing Day 1)
Weight:	13.89-16.87 kg (on dosing Day 1)

Doses/Vehicle: 0.1, 0.5 and 2.5 mmol/kg of Gadobutrol (SH L 562BB). Vehicle was 0.9% w/v saline (Batch nos. 01I13G50 and 01K27G50; Baxter Healthcare Ltd)

Duration/route: Single intravenous administration

Key study findings: Intravenous administration of Gadobutrol at 0.1, 0.5 and 2.5 mmol/kg (or 0.54x, 2.70x, and 13.5x the intended human dose) had no notable effects on systolic, diastolic and mean blood pressure, PR interval and QRS duration. A transient elevation of the heart rate occurred at 2.5 min following the administration of 0.1 and 0.5 mmol Gd/kg. The increased heart rate was more pronounced at the high dose (2.5 mmolGd/kg). There was a significant transient increase in QT and QTcQ intervals in the high dose (2.5 mmol Gd/kg) group occurring, respectively, at 0.5 and 0.5-2.5 minutes.

Study objectives: To assess the potential effects of gadobutrol on arterial blood pressure, heart rate and lead II ECG in conscious telemetered dogs

Study Design: There was one treatment group of 4 male telemetered dogs. Using the restraint technique only, the dogs were acclimatized to the dosing procedure prior to the commencement of study. Each animal served as its own control. Animals were dosed intravenously in ascending doses by manual infusion and each dog received the vehicle or Gadobutrol on treatment days 1, 2, 6 and 9 as follows:

Table 12: Study Design (Report A08428)

Day	Gadobutrol Dose (mmol/kg)	Dose volume (ml/kg)	Infusion time (s)	Dose (mmol/m ²)	Human dose Multiple (mmol/m ²)
1	0	2.5	50	0	0
2	0.1	0.1	10	2	0.54
6	0.5	0.5	10	10	2.70
9	2.5	2.5	50	5	13.5

Dose (mmol Gd/kg)	0.1	0.5	2.5
Dose multiple (Based on BSA)	0.54x	2.7x	13.5x

BSA = Body surface area; Constructed by reviewer from sponsor's data

Control animals received 2.5 ml/kg saline. Control and test articles were administered by manual infusion via an intravenous catheter at an infusion rate of 0.1- 2.5 ml/kg as shown in the table above. Parameters, measured continuously before and after dosing, were systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and lead II ECG variables namely, PR interval, RR interval, QRS duration and QT interval. Mean arterial blood pressure (MAP), QTcF, QTcQ were calculated as described. Statistical analysis was by one-way ANOVA with repeated measures followed by Dunnett's post-hoc test with statistical significance at p<0.05. The time points used for

comparison were -15, -1, 0.5, 1, 2.5, 5, 10, 20, 30, 45, 60, 120, 240 and 360 min Data generated from the lead II ECG at these time points was the average of at least 15 complexes of data at that time point.

Results: There were no notable dose-related effects of Gadobutrol on arterial blood pressure. However, there was a transient increase in heart rate at 2.5 min after the injection of 0.1 and 0.5 mmol Gd/kg. This increase was more pronounced at the high dose of 2.5 mmolGd/kg. At 2.5 mmolGd/kg, a significant but transient increase was observed in QT and QTcQ intervals at 0.5 and 0.5-2.5 min, respectively.

Reviewer's comments: Overall, I agree with the sponsor's conclusion that gadobutrol administered intravenously at 0.1, 0.5 and 2.5 mmol Gd/kg did not appear to have any significant effects on systolic, diastolic and mean blood pressure, the PR interval and the duration of QRS.

4.3.3.3 Cardiovascular and Respiratory Systems

Two studies were submitted to evaluate the effect of gadobutrol on respiratory and/or cardiovascular function. Report A21000 described a GLP-compliant study of intravenously administered gadobutrol in propofol-anesthetized, spontaneously breathing New Zealand White rabbits. The second study (Report AN71) examined the effect of intravenous gadobutrol in Japanese White rabbits anesthetized with urethane in comparison with Magnevist. Study report A21000 was evaluated in this review.

4.3.3.3.1 Report No. A21000:

Study title: Effect of gadobutrol (Gadovist®) on respiratory function after single intravenous infusion to propofol-anesthetized rabbits

Volume #, and Page #:	Module 4.2.1.3.1, No. of pages: 71
Conducting laboratory and location:	(b) (4)
Study #:	SPJ200401
Date of study initiation:	February 17, 2004
GLP compliance:	Yes (x), No (); OECD Principles of Good Laboratory Practice and Japan, MHW Ordinate No. 21 (Mar 26, 1997); Signed
QA report:	Yes (x), No (); Signed
Drug, lot #, and % purity:	Gadobutrol: ZK135079/SH L 562B; lot #: N/A; Purity: N/A
Substance Batch numbers:	N/A
Formulation Batch numbers:	21003E
Animal species/strain/sex per dose:	Male rabbit, New Zealand White (NZW), 8/group

Age: N/A
 Weight: Arrival (2.44 - 2.79 kg)
 Day of drug administration (2.53 - 2.97 kg)
 Doses/Vehicle: Gadobutrol, 0.5 and 2.5 mmolGd/kg/saline
 Duration/route: intravenous by infusion (0.5 and 2.5 mmol/kg)
 and bolus injection (0.1 mmol/kg)
 Protocol deviations: None

Key findings: Saline or gadobutrol administered at 0.1 or 0.5 mmol Gd/kg did not have any effect on respiratory function, blood pressure or heart rate in spontaneously breathing anesthetized rabbits. At 2.5 mmolGd/kg gadobutrol, there was an increase in respiratory frequency and a decrease in tidal volume. There was also a decrease in esophageal pressure difference and resistance. A small but significant decrease in compliance as well as the heart rate was observed in the high dose group.

Study Design:

- Gadobutrol (or saline vehicle) was administered to propofol-anesthetized male NZW (n=8/treatment group) rabbits in four treatment groups comprising of three gadobutrol treatment groups and one vehicle control group.
- Two doses of gadobutrol (0.5 and 2.5 mmol Gd/kg) were infused at 30 ml/kg via the femoral vein while the 0.1 mmolGd/kg dose was injected as a bolus.
- Saline was used as control at volume of 2.5 mL/kg.

Table 13: Treatment groups in Report 21000

Treatment Group	Number of animals	Treatment	Dose (mmol Gd/kg)	Infusion volume (ml/kg)
1	8	saline	0	2.5
2	8	Gadobutrol	0.1	0.1
3	8		0.5	0.5
4	8		2.5	2.5
Adapted from sponsor's table				

Dose (mmol Gd/kg)	0.1	0.5	2.5
Dose multiple (Based on BSA)	0.32x	1.62x	8.1x
BSA = Body surface area; Constructed by reviewer from sponsor's data			

- A tracheal tube was connected to an amplifier via a differential pressure transducer and a pneumotachometer to record respiratory flow from which the respiratory frequency (RF) and tidal volume (TV) were derived.
- Esophageal pressure was taken as a measure of intrapleural pressure.

- Data collection was started with a pre-phase of 30 min during which the study parameters (respiratory flow, respiratory frequency, esophageal pressure and blood pressure) were recorded. .
- All data were collected for 60 min (immediately, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min) after the start of the infusion.
- Data were expressed as the mean \pm standard deviation or percent of mean pre-value. The pre-value in all treatment groups was compared by Tukey-Kramer's test.

Respiratory and cardiovascular parameters

Parameter	Abbreviation
Respiratory frequency	RF
Tidal volume	TV
Expiratory esophageal pressure	EEP
Inspiratory esophageal pressure	IEP
Esophageal pressure difference	EPD
Compliance	CO
Resistance	RE
Mean blood pressure	MBP
Systolic blood pressure	SBP
Diastolic blood pressure	DBP
Heart rate	HR

Results:

- Saline or gadobutrol administered at 0.1 and 0.5 mmol Gd/kg did not affect parameters of respiratory function, blood pressure or heart rate in spontaneously breathing anesthetized rabbits.
- At 2.5 mmolGd/kg gadobutrol, there was an 85% increase in respiratory frequency and a 14% decrease in tidal volume.
- The esophageal pressure difference (EPD) decreased by 15% and resistance decreased by 25%.
- The changes in RF, TV, EPD and RE all occurred at 0 to 35 min after the start of infusion.
- Each parameter was compared to pre-value data.
- There was a small but significant decrease in compliance also in the high dose group in comparison with the saline vehicle group.
- Gadobutrol caused a 14% decrease in heart rate at 2.5 mmol Gd/kg

Conclusions: Gadobutrol did not appear to have any effect on respiration at 0.1 and 0.5 mmol/kg. However, at 2.5 mmol/kg, gadobutrol caused an increase in respiratory frequency and decreased the tidal volume, esophageal pressure difference and resistance. The sponsor concluded that at the high dose, gadobutrol increased pulmonary ventilation and showed signs of airway dilation.

Reviewer's comments: From the results, it would appear that gadobutrol did not have significant effects on respiration at 0.1 and 0.5 mmol Gd/kg (or 0.3- and 1.6-fold the intended human dose). However, at 2.5 mmol Gd/kg (or 8-fold the intended human dose), there were significant effects observed in pulmonary ventilation, airway dilation and heart rate. The effects observed at the high dose were reversible.

4.3.4 Renal system

Introduction: The sponsor submitted three studies conducted in rats and rabbits (Reports A20219, A20704 and 9660) to evaluate the effect of gadobutrol on renal function. Reports A20219 and A20704 were similar in objective, design and conclusions except for the doses used. The findings of A20219 were described in the comments on Report A20704. Study report 9660 described on a comparative basis, the effect of intravenous administration of gadobutrol and Magnevist (used as a reference standard) on renal function using New Zealand White rabbits. In study 9660, intravenous administration of 2 mmol Gd/kg gadobutrol or Magnevist did not adversely affect renal function in rabbits.

4.3.4.1 Report No. A20704:

Study title: Effect of gadobutrol (Gadovist®) on renal function after single intravenous administration to rats.

Volume #, and Page #:	Module 4.2.1.3.1; 17 pages
Conducting laboratory and location:	(b) (4) (For Schering, A.G., Germany)
Study #:	SPJ200402
Date of study initiation:	February 19, 2004
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadobutrol (ZK135079/SH L 562 B Lot #: N/A; Purity: N/A
Substance Batch number:	N/A
Formulation Batch number:	21003E
Animal species/strain/sex per dose:	Male Jcl: Wistar rats (b) (4) 4 groups, 10 rats/group
Age:	7 weeks
Weight:	172-190 g (fasting weight)
Doses/Vehicle:	3 doses (0.1, 0.5 and 2.5 mmol Gd/kg), saline
Duration/route:	Single, intravenous

Key findings: Gadobutrol increased osmolal clearance and decreased NAG excretion at the highest dose of 2.5 mmol/kg. However, there were no significant effects on all other parameters of renal function evaluated at the lower doses administered.

Study Design:

- Gadobutrol (0.1, 0.5 and 2.5 mmol Gd/kg) was administered as single i.v doses to male rats to determine its effect on renal function.
- Urine volume, urinary excretion of electrolytes (Na^+ , K^+ and Cl^-), Na^+/K^+ ratio, osmolality in serum and urine, osmolal clearance, creatinine concentration in serum and urine, creatinine excretion, creatine clearance, N-acetyl- β -glucosamine (NAG) excretion, total protein and serum urea nitrogen (BUN) were determined.
- All data were expressed as mean \pm S.D. Statistical analysis was made by Dunnett's test with a 5% level of significance.

Test article	No. of animals	Intravenous dose (mmol Gd/kg)	Dose rate (ml/kg)	Human dose multiple
Saline	10	0	25	0
Gadobutrol		0.1		0.2x
		0.5		0.8x
		2.5		4.1x

Results: Gadobutrol up to 0.5 mmol Gd/kg (or 0.8x the human dose) did not affect the renal function in rats. There was however a slight increase in osmolal clearance at 2.5 mmol Gd/kg (or 4 times the human dose). There was no effect on any of the other parameters of renal function evaluated.

Conclusions: Gadobutrol did not affect renal function in rats in both the low and mid doses tested (0.1 and 0.5 mmol Gd/kg, respectively). The slight increase in osmolality and the decrease in NAG excretion observed at 4x the HD (2.5 mmol Gd/kg) did not appear to signal any untoward effect on renal function.

Reviewer's comments:

- Although the results appear to indicate that gadobutrol did not have any effects on the parameters of kidney function evaluated, the doses tested had a low safety margin. It is noted however, that low safety margin is not inconsistent among members of the gadolinium-based contrast agents.
- N-acetyl- β -glucosamine (NAG) excretion is a biomarker that is increased in subjects exposed to substances with toxic effects on renal tubular cells. In experimental studies of glomerular diseases, an elevated excretion of NAG is commonly associated with dysfunction of tubular excretion induced by an increased traffic of proteins in the tubular lumen. A decrease in this biomarker was demonstrated in study report A20704.
- The slight decrease in NAG is therefore regarded not to have an impact on renal function.

- The increased osmolal clearance observed at the high dose could be due to the osmolality of the test formulation.
 - Similar parameters of renal function were evaluated in A20219 and A20704 and it can be concluded from the aggregate negative findings of both studies that gadobutrol, at the doses tested in the rat, did not appear to signal any adverse effects on renal function in humans.
-

5 Pharmacokinetics/ADME/Toxicokinetics`

5.1 PK/ADME

5.1.1 Brief Summary of Pharmacokinetics, Distribution and Excretion

Nonclinical single-dose pharmacokinetic (PK) iv studies were conducted in rats, rabbits, dogs and monkeys using unlabeled or labeled (^{153}Gd)-Gadobutrol. Concentrations of Gadolinium (Gd) were determined by inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectrometry (ICP-AES). A linear PK was observed across species with elimination half-life ($t_{1/2}$) ranging from 13 to 59 minutes indicative of a rapid elimination. Similarly, a short $t_{1/2}$ of 1.82 h was obtained at the clinical dose of 0.1 mmol/kg in human adults. Drug exposure, measured by the area under the plasma concentration versus time curve (AUC) increased dose proportional after a single-dose administration of Gadobutrol in the species studied. Gadobutrol has a low plasma protein binding with less than 5% bound to plasma proteins. Gadobutrol did not appear to penetrate the blood-brain barrier and radioactivity was lowest in the CNS (brain and spinal cord).

The rat tissue and whole-body distribution studies suggested a low-level uptake of Gd in bone tissue following a single intravenous dose of Gadobutrol. The bone Gd is then slowly released and excreted within 24 hours. Measured radioactivity at 0.25 hours was highest in the kidneys but less in the plasma where labeled Gd concentration decreased rapidly to below the lower limit of quantification 3-6 hours post-dose.

Gadobutrol is not metabolized and only the unchanged drug was present in urine samples of rats and dogs. In rats, over 90% Gadobutrol was excreted primarily via the renal route while fecal excretion accounted for about 1- 2%.

There was a dose-proportional accumulation of radioactivity in rats after repeated administration of (^{153}Gd)-Gadobutrol using 0.1, 0.5 and 2.5 mmol/kg once daily for 5 days with sacrifice two days after the last dose. The highest radioactivity concentration was obtained in the kidneys.

5.1.2 Pharmacokinetics after single-dose administration

Table 14: Summary of single-dose Pharmacokinetic studies in different animal models

Species / gender	Report #	Test substance	Dose (mmolGd/kg)	No. of animals	Matrix analyzed
Rat (M)	9585	¹⁵³ Gd	0.1	4	Plasma
			0.5		
Rat (M)	9123 / 9669	¹⁵³ Gd	0.25	5	Blood
			2.5		
Rabbit / Pregnant (F)	9586	¹⁵³ Gd	0.5	4	Plasma
Dog (F)	9616	¹⁵³ Gd	0.05	5	Plasma
			0.25		
Monkeys (F)	A31073	Gd	0.5	5	Plasma

Reviewer's Table constructed from sponsor Table and data; M, F=male, female; Gd = unlabeled gadobutrol, ¹⁵³Gd = labeled gadobutrol; BSA = body surface area

5.1.2.1 Report No. 9585

Study title: Pharmacokinetics of ¹⁵³Gd-ZK 135079 after single intravenous administration to rats

Study no.: **91411, 91423**
 Report date: **July 27, 1991**
 Study report location: **Module 4.2.2.2, pages 1-38**
 Conducting laboratory and location: **Bayer Yakuhin Ltd, Japan**
 Date of study initiation: **N/A; Study was conducted between April and June, 1991**
 GLP compliance: **No**
 QA statement: **No**
 Drug, lot #, and % purity: **¹⁵³Gd-ZK 135079 (0.01M), 2376-4, % purity – N/A**

Key Study Findings:

- Plasma kinetics, tissue distribution and excretion of ¹⁵³Gd-gadobutrol were determined male rats administered single intravenous doses of 0.1 and 0.5 mmol/0.5MBq/kg.
- With both doses, radioactivity was highest at 5 min but was rapidly cleared from plasma. No radioactivity detected at 360 min.
- Pharmacokinetic parameters (half-life, distribution volume, systemic exposure and clearance) were linear and similar at both doses.

- Between 5 min and 1 h post administration, the half-life was 13 min at both doses and decreased to less than the lower limit of quantitation by 6 h.
- The volume of distribution (Vd) was 0.25 and 0.28 L/kg respectively for 0.1 and 0.5 mmol/kg doses.
- Total systemic clearance (CL_{total}) was 13.1 and 15.6 mL/min/kg following administration of 0.1 and 0.5 mmol/kg, respectively.
- PK parameters were not different when determined at each dose tested.
- AUC increased proportionally with dose, from 6.49 $\mu\text{mol}\cdot\text{min}/\text{mL}$ at 0.1 mmol/kg to 31.0 $\mu\text{mol}\cdot\text{min}/\text{mL}$ after dosing with 0.5 mmol/kg.
- Radioactivity was detected in all the organs examined 15 min (0.25h) following the administration of 0.1 or 0.5 mmolGd/kg of ^{153}Gd -labeled gadobutrol. Except in the kidney, tissue radioactivity was lower than in plasma. Radioactivity decreased more slowly in the tissues than in the plasma.
- There was no distribution of radioactivity to red blood cells
- 30 days the 0.5 mmolGd/kg, <0.1% radioactivity was detectable in the kidney and bone.
- Following treatment with either dose, excretion was primarily by the renal route. 3-6% was excreted via the feces and 0.1% in bile-cannulated rats. Less than 1% of the administered dose was excreted via feces.

Design: To determine the plasma pharmacokinetics (PK) of gadobutrol after a single i.v dose in rats, blood (circa 0.2 mL) was collected from anesthetized animals at 5, 10, 20, 30, 45 min, 1, 3 and 6 h p.a via the cannulated jugular vein. To assess the tissue distribution, tissues and organs, comprising blood, plasma, brain, pituitary, eye, thyroid, thymus, heart, lung, liver, kidney, adrenal, pancreas, spleen, muscle, skin, fat, bone, bone marrow, stomach, intestine, testis, prostate, seminal vesicle and the carcass were obtained after exsanguination. For plasma, heparinized venous blood was collected at 0.25, 1, 3, 6, 24 hrs, 15 and 30 days after rats were treated with 0.1 or 0.5 mmol/0.5 MBq/kg ^{153}Gd -gadobutrol. Amount excreted was determined in urine and feces collected from animals kept in metabolic cages. To determine the amounts excreted into bile, urine and gastrointestinal tract in bile duct-cannulated rats, the common bile duct and the urinary bladder were cannulated in anesthetized rats. Following recovery bile, urine, feces were collected for 48h after test compound administration. Radioactivity in samples was measured with a gamma counter.

Methods

Doses:	0.1 or 0.5mmolGd/kg / 0.5 Mbq/kg. Biliary excretion was studied at a dose of 0.5 mmol/0.1 MBq/kg
Frequency of dosing:	Once
Route of administration:	Bolus intravenous; tail vein
Dose volume:	N/A
Formulation/Vehicle:	¹⁵³ Gd-labeled ZK 135079 / saline vehicle
Matrix:	Plasma
Analyte:	Radioactivity (¹⁵³ Gd)
Assay:	Gamma (γ)-counting
Species/Strain:	Rat (Male) / Sprague-Dawley, (b) (4)
Number/Sex/Group:	4/group
Age:	N/A
Weight:	310g – biliary excretion studies 160 – 220g – all other studies
Satellite groups:	none
Unique study design:	none
Drug, lot #, and % purity:	¹⁵³ Gd-labeled ZK 135079, Radiochemical purity was 99%, lot #: N/A
PK parameters:	AUC (μmol x min/mL) Vd (L/kg) T _{1/2} (5 min-1h) (min) CL (mL/min/kg)
Deviation from study protocol:	Not provided

Results:

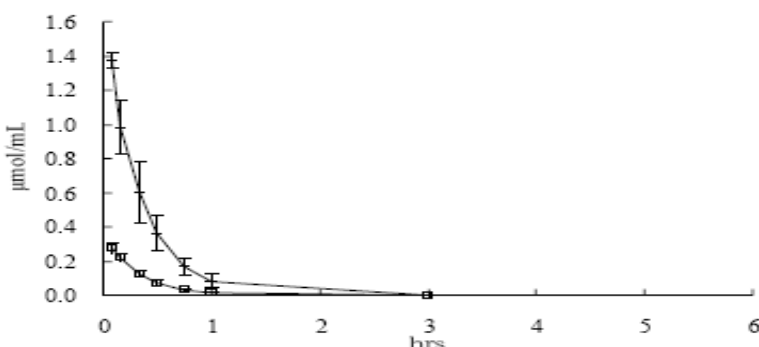
Summary: Following a single bolus intravenous administration of ¹⁵³Gd-gadobutrol (0.1 or 0.5 mmol/kg (0.5 MBq/kg)), radioactivity in plasma declined rapidly with a half-life of 13 minutes at both doses, and decreased to less than the lower limit of quantitation by 6 hours after administration. At the doses tested, the volume of distribution (Vd) was 0.25 and 0.28 L/kg while the total systemic clearance (CL_{total}) was 13.1 and 15.6 mL/min/kg after administration of 0.1 and 0.5 mmol/kg, respectively. The results showed no difference in these parameters between doses. AUC increased dose proportionally from 6.49 μmol·min/mL at 0.1 mmol/kg to 31.0 μmol·min/mL after dosing with 0.5 mmol/kg.

Plasma kinetics: Radioactivity was highest at 5 min for both doses test disappeared rapidly from plasma. The pharmacokinetic parameters were similar. No radioactivity was detected at 6h (360 min) after administration. Between 5 min and 1 h post administration, the half-life was 13 min.

Table 15: Concentration of ^{153}Gd (nmol-eq/mL) in plasma after intravenous administration of ^{153}Gd ZK 135079 at 0.1 or 0.5 mmol/0.5 MBq/kg to rats

Time p.a (min)	0.1 mmolGd/kg	0.5 mmolGd/kg
5	276.8 \pm 30.6	1373.1 \pm 42.6
10	220.6 \pm 26.8	984.7 \pm 157.2
20	126.8 \pm 19.2	601.5 \pm 178.6
30	72.7 \pm 17.7	367.0 \pm 101.6
45	32.4 \pm 7.6	168.1 \pm 52.8
60	17.3 \pm 4.2	82.9 \pm 40.6
180	0.5 \pm 0.4	1.6 \pm 1.8
360	n.d.	n.d.

Reviewer's Table reconstructed from sponsor's Table; p.a = post-administration; Values are mean \pm S.D; n.d = not determined.

Figure 3: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after intravenous administration of ^{153}Gd -ZK135079 at 0.1 mmol/0.5 MBq/kg (\square) or 0.5 mmol/0.5 MBq/kg (+) to rats (Symbols and bars are mean \pm S.D. (n=4)).**Table 16: Pharmacokinetic parameters of ^{153}Gd -ZK 135079 after intravenous administration at 0.1 or 0.5 mmol/0.5 MBq/kg to rats**

PK parameters	0.1 mmolGd/kg	0.5 mmolGd/kg
Elimination – [$t_{1/2}$ (min)]	13.4 \pm 2.8	13.0 \pm 3.1
Volume of distribution – [V_d (L/kg)]	0.25 \pm 0.04	0.28 \pm 0.03
Systemic exposure – [$\text{AUC}[0-\infty]$ ($\mu\text{mol} \cdot \text{min/mL}$)]	6.49 \pm 0.74	31.0 \pm 6.6
Total clearance - [CL_{tot} (mL/min/kg)]	13.1 \pm 1.6	15.6 \pm 2.8

Reviewer's Table adapted from sponsor's Table; AUC = Area under the concentration curve vs. time curve from zero to infinity; V_d = Volume of distribution in the central compartment; CL_{tot} = Total body clearance; Data were represented as mean \pm S.D.

Distribution to tissues and organs: Following the i.v administration of 0.1 or 0.5 mmolGd/kg of labeled (^{153}Gd)- gadobutrol, radioactivity was detected in all the organs examined at 0.25h. Levels of labeled gadobutrol in selected tissues and organs after 0.1 or 0.5 mmolGd/kg are shown in the Tables shown below. At the 0.25 h time point, the

radioactivity in all tissues examined was lower than in the plasma except the kidney. Thereafter, radioactivity decreased more slowly in the tissues than in the plasma. There was no distribution of radioactivity to blood cells. 30 days after administration (0.5 mmolGd/kg), radioactivity was still present in the kidney and bone at <0.1% of the injected dose.

Table 17: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in selected tissues after intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1 mmolGd/0.5 MBq/kg

Tissues	0.25h	1h	3h	6h	24h
Blood	126 ± 28	18 ± 1	n.d.	n.d.	n.d.
Plasma	183 ± 36	28 ± 1	0.3 ± 0.2	n.d.	n.d.
Brain	3 ± 1	1 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	n.d.
Heart	33 ± 8	6 ± 1	1 ± 0.3	1 ± 0.1	1 ± 0.1
Lung	61 ± 15	17 ± 4	2 ± 0.3	2 ± 0.2	2 ± 0.3
Kidney	556 ± 126	134 ± 31	55 ± 3	53 ± 8	69 ± 9
Stomach	41 ± 4	34 ± 25	2 ± 2	4 ± 2.2	2 ± 1
Liver	16 ± 3	7 ± 2	3 ± 0.2	3 ± 1	3 ± 0.1
Bone	11 ± 4	4 ± 1	1 ± 0.1	1 ± 1	1 ± 0.3
Skin	66 ± 34	13 ± 2	2 ± 0.4	3 ± 2	2 ± 0.3

Reviewer's Table was constructed from sponsor's Table; Values are means of 2 animals ± SD; n.d = not determined

Table 18: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in tissues after intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.5 mmolGd/0.5 MBq/kg

Tissues	0.25h	1h	6h	24h	30 days
Blood	596 ± 41	61 ± 12	n.d.	n.d.	n.d.
Plasma	905 ± 46	96 ± 21	n.d.	n.d.	n.d.
Brain	16 ± 4	3 ± 0.3	0.9 ± 0.2	n.d.	n.d.
Heart	162 ± 23	21 ± 4	4 ± 1	4 ± 0.3	n.d.
Lung	302 ± 66	45 ± 4	8 ± 1	7 ± 0.6	n.d.
Kidney	2738 ± 581	540 ± 158	299 ± 35	382 ± 31	8 ± 4
Stomach	172 ± 27	29 ± 5	32 ± 38	19 ± 23	n.d.
Liver	80 ± 19	27 ± 2	17 ± 2	15 ± 0.3	n.d.
Bone	25 ± 11	19 ± 1	7 ± 1	9 ± 8	2 ± 2
Skin	234 ± 44	47 ± 11	47 ± 11	12 ± 2	n.d.

Reviewer's Table was constructed from sponsor's Table; Values are means of 4 animals ± SD; not determined

Excretion into urine and feces: Absorbed radioactive gadobutrol was predominantly excreted into the urine as 82.7% or 93.7% after the administration of 0.1 or 0.5 mmolGd/kg. A comparatively lesser amount of gadobutrol was excreted via the feces. The Table below describes the cumulative excretion of the radioactivity into urine and feces over a period of 3 days following the administration of a single intravenous dose of labeled gadobutrol.

Table 19: Cumulative excretion of the radioactivity into urine and feces after single intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1 or 0.5 mmol/0.5 MBq/kg to rats

No. of Days post injection	% of dose			
	0.1 mmolGd/kg		0.5 mmolGd/kg	
	Urine (%)	Feces (%)	Urine (%)	Feces (%)
1	82.7 \pm 10.9	5.3 \pm 4.6	93.7 \pm 14.3	2.8 \pm 3.2
2	86.2 \pm 8.5	7.7 \pm 4.4	98.7 \pm 9.1	3.2 \pm 3.4
3	87.7 \pm 7.7	9.1 \pm 4.6	100.4 \pm 7.2	3.5 \pm 3.5

Reviewer's Table adapted from sponsor's Table; Values are mean \pm S.D. (n=4).

Cumulative excretion of radioactivity into bile, urine and feces over 48 hrs):

Table 20: Cumulative excretion of the radioactivity into bile, urine and feces after intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.5 mmol/0.1 MBq/kg to bile-fistula rats

No. of Hours post injection	% of dose		
	Bile	Urine	Feces and GI contents
1	0.06 \pm 0.02	-	-
3	0.08 \pm 0.03	-	-
6	0.09 \pm 0.04	-	-
24	0.10 \pm 0.03	91.33 \pm 6.03*	-
48	0.10 \pm 0.03	91.62 \pm 6.06*	0.88 \pm 1.08*

Reviewer's Table adapted from sponsor's Table; Values are mean \pm S.D. (n=4, *: n=3).

GI: gastrointestinal.

Following the administration of labeled gadobutrol ($^{153}\text{Gd-gadobutrol}$) to bile-cannulated at a dose of 0.5 mmol/0.1 MBq/kg, only 0.1% of the administered dose was excreted in the bile within 48h. 92% was recovered in urine. Fecal and GI excretion was less than 1% of the administered dose.

Conclusions: Pharmacokinetics (plasma kinetics, tissue distribution and excretion) of $^{153}\text{Gd-ZK 135079}$ (gadobutrol)) were studied after a single intravenous doses of 0.1 and 0.5 mmol/0.5MBq/kg to male SD rats. Radioactivity, highest at 5 min for both doses tested, disappeared rapidly from plasma; pharmacokinetic parameters (half-life, distribution volume, systemic exposure and clearance were similar at both doses tested. No radioactivity was detected at 6h (360 min) after administration.

Between 5 min and 1 h post administration, the half-life was 13 min at both doses, and decreased to less than the lower limit of quantitation by 6 hours after administration. The volume of distribution (Vd) was 0.25 and 0.28 L/kg respectively, an indication of distribution to the extracellular space, a finding common to other contrast media.

The total systemic clearance (CL_{total}) was 13.1 and 15.6 mL/min/kg after administration of 0.1 and 0.5 mmol/kg, respectively. There was no difference in these parameters between doses. AUC increased proportionally with dose, from 6.49 µmol·min/mL at 0.1 mmol/kg to 31.0 µmol·min/mL after dosing with 0.5mmolGd/kg.

At 0.25h following the administration of 0.1 or 0.5 mmolGd/kg of labeled gadobutrol, radioactivity was detected in all the organs examined. At the same time point, the radioactivity in all tissues examined was lower than the in the plasma except the kidney, thereafter radioactivity decreased more slowly in the tissues than in the plasma. There was no distribution of radioactivity to blood cells and radioactivity (<0.1%) was still detectable 30 days after administration of the higher dose of 0.5 mmolGd/kg in the kidney and bone.

After the administration of 0.1 or 0.5 mmolGd/kg, excretion of gadobutrol was predominantly urinary. A much lower amount (3-6%) was excreted via the feces. In bile cannulated rats, only 0.1% of the administered dose of 0.5 mmol/0.1 MBq/kg of labeled gadobutrol was excreted in the bile within 48h while 92% was recovered in urine. Fecal and GI excretion was less than 1% of the administered dose.

Reviewer's comments: Agree

5.1.2.2 Report No. 9586

Study title: Placental transfer and distribution of ¹⁵³Gd-ZK 135079 after single intravenous administration to rabbits

Study no.:	91413 (NO1A91L25)
Report date:	May 9, 1991; Revised May 8, 2008 (signed revision)
Study report location:	Module 4.2.2.2.1; pages 1-
Conducting laboratory and location:	(b) (4) / Schering A.G., Pharma, Germany
Date of study initiation:	N/A
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	¹⁵³ Gd-labeled ZK 135079 (0.001M); Lot. # 2376-4; % purity: N/A

Key Study Findings: The distribution volume (0.2 L/kg) of ¹⁵³Gd-gadobutrol indicated a distribution to the extracellular space similar to other contrast agents. There was rapid renal excretion of gadobutrol as shown by the apparent elimination half-life of 37 min. Only a slight placental transfer of gadobutrol was evident from this study and less than 0.01% of the administered dose transferred from the dam to the fetus. This will be reflected in the label.

Design: (^{153}Gd)-labeled product (ZK-135079) was intravenously administered to sixteen pregnant rabbits on day 18 of gestation at a dose of 0.5 mmol/0.1 MBq/kg.

Four anesthetized animals were each sacrificed at 10 min, 1, 3 and 24h. ^{153}Gd activity was determined in blood, plasma, uterus, placenta, fetus, amniotic fluid, ovary, liver and kidney. Blood samples for plasma kinetics, were collected via the ear vein at 5, 10, 15, 20, 30, 45 min, and 1, 2, 4, 6, 24 h post administration. Radioactivity was measured using a gamma counter.

Methods

Doses:	0.5mmolGd/kg / 0.1 MBq/kg
Frequency of dosing:	Once; Day 18 of pregnancy
Route of administration:	Intravenous; ear vein
Dose volume:	N/A
Formulation/Vehicle:	^{153}Gd -labeled ZK 135079; (0.01 mol/L, specific radioactivity: 700 GBq/mol) was added to an aqueous solution (0.5 mol/L) of unlabeled ZK 135079 containing 0.5 mg/mL CaNa_3DTPA to a final concentration of 0.5 mmol/0.1 MBq/mL of ^{153}Gd -ZK 135079.
Matrix:	Blood, Plasma, uterus, placenta, fetus, amniotic fluid, ovary, liver and kidney.
Analyte:	Radioactivity (^{153}Gd) was determined in the above-named matrices
Assay:	Gamma (γ)-counting
Species/Strain:	Rat, Japanese White strain; (b) (4)
Number/Sex/Group:	16 pregnant females; dosed on gestation day 18
Age:	N/A
Weight:	3.5 - 4.2g
Satellite groups:	none
Unique study design:	none
PK parameters:	Plasma radioactivity ($\mu\text{mol/mL}$ plasma) AUC ($\mu\text{mol} \times \text{min/mL}$) Vd (L/kg) $T_{1/2}$ (min) CLtotal (mL/min/kg)
Deviation from study protocol:	Not stated

Results: Radioactivity in plasma was highest at 5 min post administration, disappeared rapidly within 1h and was no longer detectable at 24h:

Table 21: Radioactivity in plasma after intravenous administration of ^{153}Gd -ZK 135079 at 0.5 mmol/0.1 MBq/kg to maternal rabbits

Time (min or h) p.a	Radioactivity ($\mu\text{mol/mL}$ plasma)	Radioactivity ($\mu\text{mol/mL}$ plasma)
5 min	2.56 ± 0.28	2.56 ± 0.28
10	2.17 ± 0.17	2.17 ± 0.17
15	1.80 ± 0.16	1.80 ± 0.16
20	1.57 ± 0.13	1.57 ± 0.13

30	1.28 ± 0.10	1.28 ± 0.10
45	0.96 ± 0.08	0.96 ± 0.08
1 h	0.70 ± 0.08	0.70 ± 0.08
2	0.22 ± 0.04	0.22 ± 0.04
4	0.03 ± 0.02	0.03 ± 0.02
6	N.D.	N.D.
24	N.D.	N.D.

Reviewer's Table constructed from Sponsor's data; p.a = post-administration

PK parameters determined for ^{153}Gd -gadobutrol included half-life ($T_{1/2}$), distribution volume (V_d), systemic exposure (AUC) and total clearance (CL_{tot}) are shown in the Table below:

Table 22: Pharmacokinetic parameters of ^{153}Gd -ZK 135079 after intravenous administration at 0.5

$T_{1/2}$ (min)	37.2 ± 7.4
Distribution volume (L/kg)	0.22 ± 0.04
AUC [0- ∞] ($\mu\text{mol}\cdot\text{min}/\text{mL}$)	120 ± 11.5
CL_{tot} (mL/min·kg)	4.11 ± 0.42

Values are mean \pm S.D. (n=4).

Distribution to tissues and organs: The radioactivity in tissues and organs is presented in the Table below. Maximal radioactivity was found at 10 min post administration. Radioactivity was highest in the kidney followed by the plasma. Comparatively less radioactivity was observed in the uterus, placenta, and liver 10 min after administration. The amount of radioactivity in the fetus and amniotic fluid was less than 1% that of plasma. All the radioactivity in tissues had disappeared between 3 and 24h after administration.

Table 23: Radioactivity in tissues after intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.5 mmol/0.1 MBq/kg to rabbits

Tissue\ p.a.	10 min	1 h	3 h	24 h
blood	1.48 ± 0.09	0.39 ± 0.06	0.05 ± 0.02	N.D.
plasma	2.20 ± 0.17	0.63 ± 0.11	0.09 ± 0.04	N.D.
liver	0.24 ± 0.02	0.09 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
kidney	3.56 ± 1.00	1.56 ± 0.27	0.74 ± 0.28	0.37 ± 0.08
uterus	0.59 ± 0.04	0.22 ± 0.04	0.07 ± 0.02	0.02 ± 0.00
ovary	0.50 ± 0.06	0.17 ± 0.02	0.05 ± 0.01	0.01 ± 0.00
placenta	0.24 ± 0.04	0.11 ± 0.02	0.07 ± 0.01	0.03 ± 0.01
foetus	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.00
amniotic fluid	N.D.	N.D.	0.01 ± 0.00	0.02 ± 0.00

(μmol/mL or g wet tissues)

Values are mean±S.D. (n=4).

N.D.: Not detectable

Conclusions: It was concluded from this study that the distribution volume (0.2 L/kg) of ^{153}Gd -gadobutrol indicated a distribution to the extracellular space similar to other contrast agents. There was rapid renal excretion of gadobutrol as shown by the apparent elimination half-life of 37 min. Only a slight placental transfer of gadobutrol was evident from this study and less than 0.01% of the administered dose transferred from the dam to the fetus.

Reviewer's comments: The Based on the finding that only a slight placental transfer of gadobutrol was evident and less than 0.01% of the administered dose transferred from the dam to the fetus might signify an indication for safe use of this product in pregnancy. This finding will be reflected in the label. Overall, I agree with the results and conclusions of this study.

5.1.2.3 Report No. 9615:

Study title: Pharmacokinetics and biotransformation of ^{153}Gd -labeled ZK 135079 after single intravenous administration in the dog

Study no.: **KM 91075**
Report date: **January 30, 1992**
Study report location: **Module 4.2.2.2.1; 1-24 pages**
Conducting laboratory and location: **Bayer Schering Pharma AG, Berlin, Germany**
Date of study initiation: **N/A; Study period: April, 1991 – December, 1991**
GLP compliance: **N/A**
QA statement: **N/A**
Drug, lot #, and % purity: **0.5 mol/L ZK-135079 (Gadobutrol) with specific radioactivity of 148 MBq/mol (0.25 mmolGd/kg) and 740 MBq/mol (0.05 mmolGd/kg); lot #: N/A; % purity: N/A**

Key Study Findings: ^{153}Gd -labeled ZK-135079 (Gadobutrol) distributed mainly to the extracellular fluid space. Excretion was negligible. Excreted by primarily by the renal route with negligible fecal route excretion. The renal clearance of labeled gadobutrol was similar to the clearance of ^3H -labeled inulin, an indication that the renal excretion of gadobutrol was essentially by glomerular filtration. Both total and renal clearance of gadobutrol were similar. Dose proportionality of the PK parameters of ^{153}Gd -labeled gadobutrol was established. No metabolites were found in the plasma or urine.

Methods

Doses: 2 doses of ^{153}Gd -gadobutrol: Low dose (0.05 mmolGd/kg); High dose (0.25 mmolGd/kg); ^3H -inulin was co-administered with 0.05 mmol/kg gadobutrol.
 Frequency of dosing: Once
 Route of administration: Intravenous bolus (cubital vein)
 Dose volume: 4 mL/min
 Formulation/Vehicle: Aqueous solution of ^{153}Gd -ZK-135079
 Matrix: Plasma
 Analyte: Radioactivity (^{153}Gd)
 Assay: Gamma (γ)-counting
 Analytical method: Radiometric and HPLC; subsequent detection by UV and atomic emission spectrometry
 Species/Strain: Dog / Beagle (Breeder: (b) (4))
 Number/Sex/Group: 5/Females/group (2 groups)
 Age: N/A
 Weight: 8.2 – 13.9 kg
 Satellite groups: None
 Unique study design: None
 PK parameters: AUC ($\mu\text{mol} \cdot \text{h/mL}$); V_{ss} (L/kg); $t_{1/2, p}$ (h); $t_{1/2, u}$ (h); CL (mL/min/kg); CL_R (mL/min/kg)
 Deviation from study protocol: None
 Notes: PK data were represented as mean \pm S.D.
 AUC: Area under the concentration vs. time curve from zero to infinity
 V_{ss} : Volume of distribution at steady state condition
 $t_{1/2, p}$: Terminal elimination half-life calculated from plasma data
 $t_{1/2, u}$: Terminal elimination half-life calculated from urine data
 CL: Total body clearance
 CL_R : Renal body clearance

Study Design: Described in Methods (see Table).

Results: Plasma concentrations, PK parameter determinations and urinary and fecal amounts of ^{153}Gd -labeled Gadobutrol are described in the following Tables.

Plasma ^{153}Gd levels: Plasma concentrations of ^{153}Gd -labeled Gadobutrol were highest at the 5 min (0.083h) time point for both doses tested (Plasma concentrations rapidly diminished to levels below detection by 24h post administration).

Table 24: Plasma concentration of ^{153}Gd labeled ZK-135079 after a single intravenous administration of a low and high dose of gadobutrol in 5 female dogs

Time (h)	Plasma concentration of ^{153}Gd -Gadobutrol			
	Low dose (0.05 mmolGd/kg / 37 KBq/kg or 148 MBq/kg)		High dose (0.25 mmolGd/kg / 37 KBq/kg or 740 MBq)	
	Mean (n=5)	±S.D.	Mean (n=5)	±S.D.
0.083	0.194	0.036	1.170	0.040
0.167	0.177	0.015	0.964	0.075
0.333	0.140	0.014	0.757	0.044
0.5	0.115	0.010	0.598	0.039
1.0	0.072	0.005	0.364	0.025
1.5	0.045	0.007	0.228	0.027
2.0	0.024	0.007	0.142	0.026
3.0	0.008	0.003	0.057	0.014
4.0*	0.002	0.001	n.d	n.d
6.0	0.001	0.001	0.004	0.001
24.0	b.d	b.d	b.d	b.d

Reviewer's Table reconstructed from sponsor's data; b.d = below detection limit; * 4h measurement was done only for the low dose

PK parameters: The mean values (±SD) for PK parameters are shown in the Table below:

Table 25: Pharmacokinetic parameters of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low or high dose in 5 female beagle dogs

PK Parameters	PK values (n=5)			
	Low dose (0.05 mmolGd/kg / 37 KBq/kg or 148 MBq/kg)		High dose (0.25 mmolGd/kg / 37 KBq/kg or 740 MBq)	
	Mean	±S.D.	Mean	±S.D.
AUC 0-∞ [μmol*h/mL]	0.19	0.02	1.12	0.09
t _{1/2} (p) [h]	0.62	0.07	0.75	0.06
t _{1/2} (u) [h]	1.30	0.03	1.02	0.48
V _{ss} [L/kg]	0.23	0.03	0.23	0.02
CL [mL/min*kg]	4.36	0.58	3.75	0.30
CL _R [mL/min*kg]	4.21	0.73	3.53	0.46
Amt. excreted (renal)	93.0	4.40	98.0	1.36
Amt. excreted (fecal)	0.36	0.18	0.28	0.09
Recovery in urine*	93.1	4.23	98.3	1.33

Reviewer's Table reconstructed from sponsor's data; * = The amount recovered in the urine was similar to the recovery of inulin co-administered with low dose of labeled Gadobutrol

Urinary and fecal excretion determined at the two doses tested is shown in the Tables below. Excretion of labeled Gd was primarily renal.

Table 26: Urinary amounts of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low and high dose in 5 female beagle dogs

Time p.a	Cumulative Urinary ^{153}Gd (% of dose) n=5			
	Low dose (0.05 mmolGd/kg / 37 KBq/kg or 148 MBq/kg)		High dose (0.25 mmolGd/kg / 37 KBq/kg or 740 MBq)	
	Mean (n=5)	±S.D.	Mean (n=5)	±S.D.
0.5h	24.2	14.6	16.1	16.2
1h	48.4	22.4	39.4	24.6
2h	58.8	23.9	77.6	13.2
3h	68.6	25.8	87.4	9.2
4h	73.2	26.8	n.d	n.d
6h	75.3	23.3	93.7	5.8
1d	92.0	4.4	97.5	1.4
2d	92.4	4.4	97.7	1.4
3d	92.5	4.4	97.8	1.4
4d	92.6	4.4	97.9	1.4
5d	92.6	4.4	97.9	1.4
6d	92.7	4.4	98.0	1.4
7d	92.7	4.4	98.0	1.4

Reviewer's Table reconstructed from sponsor's data

Table 27: Fecal amounts of ^{153}Gd -labeled ZK-135079 (Gadobutrol) after a single intravenous administration of a low and high dose in 5 female beagle dogs

Time p.a (days)	Cumulative Fecal ^{153}Gd (% of dose) n=5			
	Low dose (0.05 mmolGd/kg / 37 KBq/kg or 148 MBq/kg)		High dose (0.25 mmolGd/kg / 37 KBq/kg or 740 MBq)	
	Mean (n=5)	±S.D.	Mean (n=5)	±S.D.
1	0.21	0.20	0.11	0.07
2	0.24	0.17	0.11	0.06
3	0.17	0.03	0.13	0.05
4	0.29	0.16	0.22	0.09
5	0.31	0.18	0.26	0.09
6	0.31	0.18	0.28	0.09
7	0.36	0.18	0.28	0.09

Reviewer's Table reconstructed from sponsor's data

Conclusions:

- ^{153}Gd -labeled ZK-135079 (Gadobutrol) distributed primarily to the extracellular fluid space.
- A negligible amount of ^{153}Gd labeled gadobutrol was excreted by the fecal route
- The amount of gadobutrol cleared by the kidney was identical to the total amount cleared, an indication that gadobutrol was predominantly eliminated via the renal route.
- The renal clearance of labeled gadobutrol was similar to the clearance of ^3H -labeled inulin indicating that renal excretion of gadobutrol was by glomerular filtration.
- The total and renal clearance of gadobutrol were similar. The data evaluated indicated dose proportionality of the PK parameters of ^{153}Gd -labeled Gadobutrol.
- No metabolites were found in the plasma or urine.
-

Reviewer's comments: Agree

5.1.2.4 Report No. A31073:

Study title: Pharmacokinetics of gadolinium administered as ZK 135079 after single intravenous administration in female Cynomolgus monkeys and determination of gadolinium (ZK 264672) in monkey plasma samples by ICP-MS

Study no.:	KINE20050289 / DE04019-27608
Report date:	August 11, 2008
Study report location:	Module 4.2.2.2.1. pages 1-59
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	December 27, 2005
GLP compliance:	N/A
QA statement:	N/A
Drug, lot #, and % purity:	ZK 135079 (Gadobutrol); lot #: N/A; % purity N/A

Key Study Findings: Following an initial high plasma concentration, Gd concentration decreased in a biphasic manner. A two-compartment data analysis indicated an alpha $t_{1/2}$ of 0.99h and a beta phase $t_{1/2}$ of 8.83 h. AUC was 378.2 $\mu\text{mol}\cdot\text{min}/\text{mL}$ and a

systemic clearance of 1.3mL/min/kg. Volume of distribution, V_c and V_{ss} , were 0.115 and 0.122 L/kg, respectively.

Design: The objective of this study was two-fold, namely, a) to determine plasma concentrations of Gd and b) to evaluate the pharmacokinetics of a single administration of unlabeled Gadobutrol to female Cynomolgus monkeys. Using an open, non-randomized, single-dose intra-individual comparison study, five female Cynomolgus monkeys were each administered a single dose (0.5mmolGd/kg) of ZK 135079 (Gadobutrol) by the intravenous route.

Blood samples (in lithium-heparin syringes) were collected for the determination of gadolinium (ZK 264672) concentration at 2, 5, 10, 20, 30, and 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, 24, and 32 h after dosing.

Table 28: Dosing schedule used in Study A31073

Animal no.	Body weight [kg]	Dose ZK 135079 [mmol]	Volume administered [mL]
346*	5.60	2.80	2.80
44	5.90	2.95	2.95
286	6.50	3.25	3.25
60	5.20	2.60	2.60
266	5.70	2.85	2.85

* Animal 346 was excluded from individual pharmacokinetic analysis as not enough material was available for repetition of analysis at most of the time points.

Methods

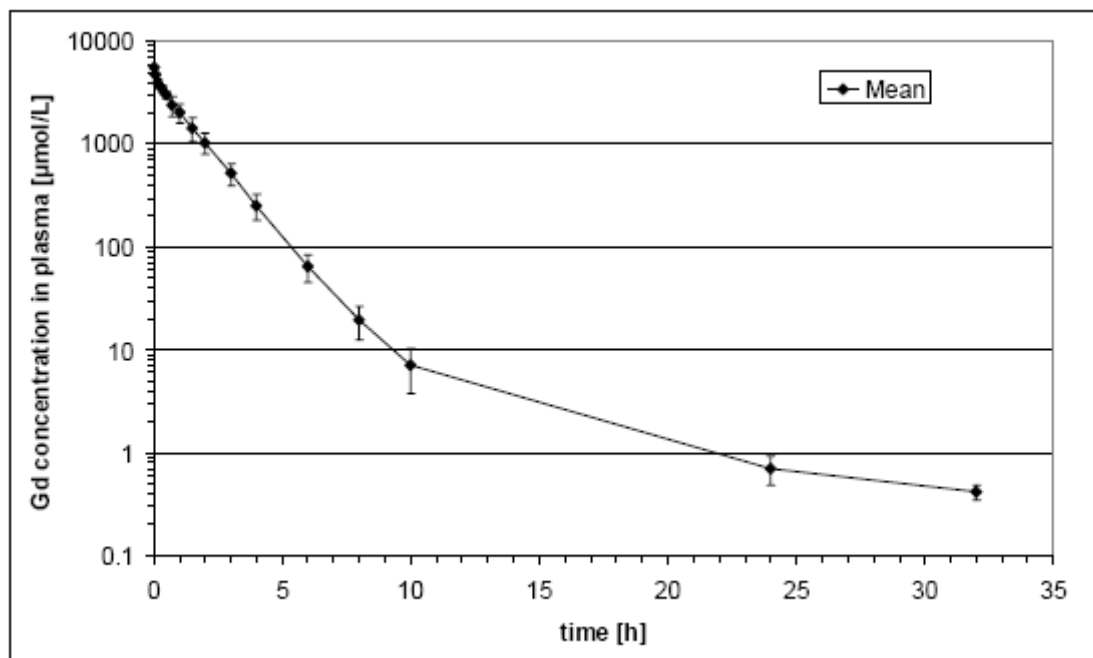
Doses:	0.5 mmolGd/kg
Frequency of dosing:	Once
Route of administration:	Intravenous bolus
Dose volume:	2.60-3.26 mL, based on body weight
Formulation/Vehicle:	Aqueous solution of Gadobutrol (ZK 135079/SH L562)
Matrix:	Monkey Li-Heparin Plasma
Analyte:	Gadolinium (ZK 264672)
Assay:	ICP-MS
Species/Strain:	Monkey/ Cynomolgus; Breeders: (b) (4)
Number/Sex/Group:	5/Female; 1 group
Age:	N/A
Weight:	5-7 kg (on day of dosing)
Satellite groups:	None
Unique study design:	None
PK parameters:	AUC ($\mu\text{mol} \cdot \text{h/L}$), V_z (L/kg), V_{ss} (L/kg), V_c (L/kg), V_3 (L/kg), $t_{1/2}$ (h), $t_{1/2\alpha}$ (h), $t_{1/2\beta}$ (h), $t_{1/2\text{eff}}$ (h), t_{max} (h), C_{max} ($\mu\text{mol/L}$), CL (mL/min/kg), MRT (h)
Deviation from study protocol:	One animal (#346) was excluded from individual pharmacokinetic analysis as not enough material was available for repetition of analysis at most of the time points.

AUC: Area under the concentration vs. time curve from zero to infinity; V_z : Volume of distribution during terminal phase; V_{ss} : Volume of distribution at steady state condition; V_c , V_3 : Volume of distribution in the central compartment or second compartment; $t_{1/2}$: Terminal elimination half-life; $t_{1/2\alpha}$, $t_{1/2\beta}$: Half-lives in the first (α) and second (β) compartment; $t_{1/2\text{eff}}$: Effective half-life; t_{max} : Time to reach maximum drug concentration; C_{max} : Maximum observed drug concentration; CL: Total body clearance; MRT: Mean residence time.

Results:Plasma kinetics:

Gadolinium was quantifiable in all animals during the 32h sampling period. After the sampling period, the Gd concentration was close to the lower limit of quantification of Gd in plasma in all cases.

Both noncompartmental and two-compartmental analysis were performed. Gadobutrol disappeared from plasma in a biphasic manner. The distribution phase occurred up to 10 h post administration followed by an elimination phase. A two-compartment analysis indicated that half-life during the alpha phase ($t_{1/2\alpha}$) was 0.99h while the beta phase half life ($t_{1/2\beta}$) was 8.83h. AUC was determined as 378.2 $\mu\text{mol min/mL}$ while systemic clearance was 1.33 mL/min/kg, a value slightly lower than the average GFR in the monkey (2 mL/min/kg). V_c and V_{ss} values were 0.115 and 0.122 L/kg, respectively indicating distribution to the extracellular space.

Table 29: Mean Gd plasma concentration versus time curves obtained upon single intravenous

Conclusions: Following a single intravenous administration of gadobutrol, a high initial plasma Gd concentration which decreased bi-exponentially with an effective half life of 1 h was observed. The clearance of 1.3 mL/min/kg was lower than the mean glomerular filtration rate (2 mL/min/kg) in the monkey. The volume of distribution ($V_{ss} = 0.12$ L/kg) was indicative of distribution into the extracellular space.

Reviewer's comments: Agree

5.1.3 Pharmacokinetics after repeat-dose administration

5.1.3.1 Report No. 9587:

Study title: Pharmacokinetics of $^{153}\text{Gd-ZK 135079}$ after five daily intravenous administrations to rats

Study no.: 91412
Report date: November 11, 1991
Study report location: Module 4.2.2.2.1 pages 1-24
Conducting laboratory and location: Bayer Yakuhin, Ltd., Japan
Date of study initiation: N/A
GLP compliance: Yes (); No (x)
QA statement: Yes (); No (x)
Drug, lot #, and % purity: $^{153}\text{Gd-gadobutrol}$; lot# and % purity: N/A

Key Study Findings: Plasma kinetics of $^{153}\text{Gd-Gadobutrol}$ were similar following single or repeated administrations. After 3h, Gadobutrol was no longer present in plasma. Two days after repeated (five days) administrations, the highest amount of Gd was found in the kidney, the amount being 2-3 times higher than was detected 24 h post single-dose administration. The radioactivity was mostly excreted by the renal route. There was no significant change in the excretion ratio into urine or feces when gadobutrol was administered by repeated administration.

Study Design: In addition to determining tissue distribution and excretion of intravenously administered labeled gadobutrol, the plasma kinetics, urinary and fecal excretion, and an analysis of radioactivity were also performed.

a). Plasma kinetics: $^{153}\text{Gd-Gadobutrol}$ was administered to i.v cannulated rats at a dose of 2.5 mmolGd/0.5 MBq/kg/day for five consecutive days. After the first and fifth (last) administrations, blood (ca. 0.2 mL) was collected at 0.25, 0.5, 1 and 3 h.

b). Tissue distribution: Rats were administered with $^{153}\text{Gd-ZK 135079}$ (0.1, 0.5 and 2.5 mmol/0.5 MBq/kg/day) for 5days. Two days after the last administration of $^{153}\text{Gd-ZK 135079}$, blood was collected from the vena cava with a heparinized syringe and was centrifuged to obtain plasma. Tissues and organs were collected after exsanguination and washed with physiological saline solution.

c). Urine and fecal excretion: Urine and feces were collected from rats administered 0.1, 0.5 or 2.5 mmolGd labeled Gadobutrol.

d). Analysis of radioactivity: Radioactivity in the samples was measured using a Gamma counter (MINAXI 5000 Series, 15 – 150 keV)

Methods

Doses: **Plasma kinetics:**
2.5 mmolGd/kg

Tissue Distribution:

0.1 mmolGd / 0.02 or 0.5 MBq/kg/day;
0.5 mmol/0.1 or 0.5 MBq/kg/day;
2.5 mmol/0.5 MBq/kg/day,
Duration: 5 days.

Frequency of dosing: Daily; Repeated x 5 days
Route of administration: Intravenous
Dose volume: Based on Body weight

Formulation/Vehicle: Aqueous; ¹⁵³Gd-labeled ZK 135079
(Gadobutrol (0.01 mol/L, specific radioactivity:
700 GBq/mol)

Matrix: Plasma

Analyte: Radioactivity

Assay: Gamma (γ)-counting

Species/Strain: Rat / Sprague-Dawley; Breeder: (b) (4)

Number/Sex/Group: 4/Males for Plasma PK or Tissue distribution

Age: N/A

Weight: 180-240 g

Satellite groups: None

Unique study design: N/A

PK parameters: Plasma concentration and tissue distribution

Deviation from study protocol: N/A

Results:

Plasma kinetics: The time course of plasma radioactivity during intravenous administration of 2.5 mmol/0.5 MBq/kg/day ¹⁵³Gd-Gadobutrol indicated no difference in plasma concentrations between the initial and last administration of the test article (¹⁵³Gd-Gadobutrol). This finding is shown in the Table and Figure below.

Table 30: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after five daily intravenous

p.a. (min)	first day	fifth day
15	5.05 ± 0.73	4.22 ± 0.46
30	2.63 ± 0.62	3.21 ± 0.46
60	0.83 ± 0.35	1.14 ± 0.13
180	n.d.	0.05 ± 0.02

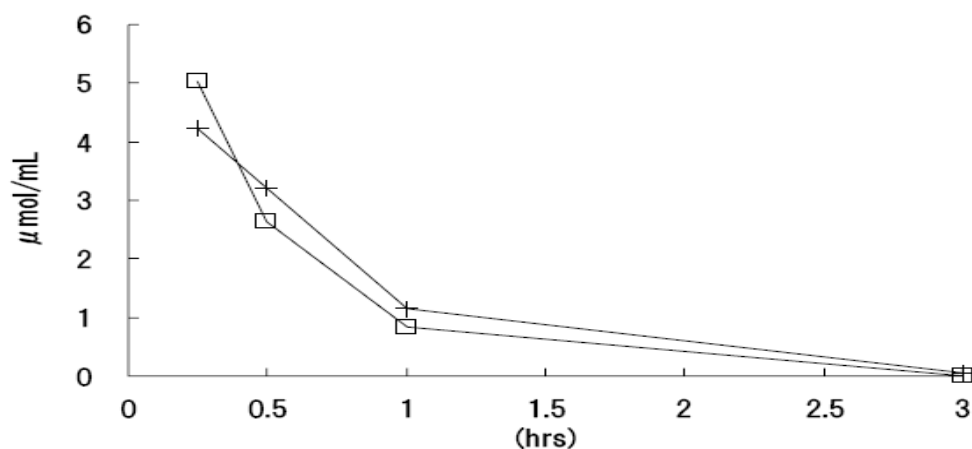
Values are mean \pm S.D.(n=4).

p.a.: post application

Figure 4: Concentration of ^{153}Gd ($\mu\text{mol-eq/mL}$) in plasma after five daily administration of $^{153}\text{Gd-ZK135079}$ at 2.5 mmol/0.5 MBq/kg/day to rats

□: first administration

+: fifth administration



Distribution to tissues and organs: Although radioactivity was no longer present in the blood and plasma 48h after the last (fifth) administration, detectable amounts of the radioactivity were found in the tissues two days after five daily intravenous administration of labeled gadobutrol. The radioactivity concentration were about 163, 1127, 6298 nmol/g wet weight in the kidney and 4.5, 31.8, 130.4 nmol/g wet weight in the liver at doses of 0.1, 0.5 and 2.5 mmol/kg, respectively. In almost all tissues, radioactivity increased dose-dependently. The amount of radioactivity present in the tissues 2 days after the last injection are shown in the Table below.

Table 31: Concentration of ^{153}Gd (nmol-eq/mL or g wet weight) in tissues at 2 days after five daily intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1, 0.5 and 2.5 mmol/0.5 MBq/kg/day to rats

issue/p.a.	0.1 mmol/kg	0.5 mmol/kg	2.5 mmol/kg
blood	n.d.	n.d.	n.d.
plasma	n.d.	n.d.	n.d.
brain	0.1 ± 0.0 (0.000)	0.7 ± 0.1 (0.000)	4.3 ± 1.0 (0.000)
pituitary	n.d.	n.d.	n.d.
eye	0.5 ± 0.2 (0.000)	4.0 ± 1.6 (0.000)	37.0 ± 7.4 (0.000)
thyroid	n.d.	n.d.	n.d.
thymus	1.5 ± 0.4 (0.001)	12.4 ± 2.0 (0.002)	88.3 ± 21.1 (0.002)
heart	1.4 ± 0.3 (0.002)	10.0 ± 1.0 (0.002)	57.9 ± 9.1 (0.002)
lung	3.4 ± 1.6 (0.006)	17.4 ± 1.4 (0.004)	99.5 ± 12.7 (0.005)
liver	4.5 ± 0.5 (0.076)	31.8 ± 2.2 (0.075)	130.4 ± 20.6 (0.060)
kidney	163.1 ± 21.2 (0.464)	1127 ± 234 (0.440)	6298 ± 2124 (0.442)
adrenal	3.1 ± 0.5 (0.000)	24.8 ± 5.2 (0.000)	126.3 ± 5.9 (0.000)
pancreas	2.2 ± 0.2 (0.002)	15.9 ± 2.5 (0.002)	125.6 ± 27.9 (0.003)
spleen	4.1 ± 0.5 (0.004)	31.3 ± 2.7 (0.005)	131.6 ± 30.4 (0.005)
muscle	0.9 ± 0.5	5.2 ± 0.9	34.6 ± 5.7
skin	2.2 ± 0.3	16.4 ± 2.0	122.2 ± 17.4
fat	0.7 ± 0.1	8.1 ± 6.7	37.6 ± 7.5
bone	6.8 ± 1.2 (0.324)	39.5 ± 4.2 (0.263)	73.3 ± 6.7 (0.091)
bone marrow	3.3 ± 0.9	28.5 ± 10.9	93.1 ± 25.1
stomach	1.9 ± 0.2 (0.004)	12.5 ± 1.5 (0.003)	58.5 ± 7.8 (0.003)
intestine	1.7 ± 0.2	12.1 ± 1.2	73.2 ± 7.3
testis	1.6 ± 0.1 (0.006)	10.8 ± 1.3 (0.005)	52.3 ± 3.8 (0.005)
prostate	1.7 ± 0.4 (0.001)	7.6 ± 2.7 (0.000)	56.8 ± 12.5 (0.001)
seminal vesicle	1.3 ± 0.4 (0.001)	8.9 ± 1.4 (0.001)	73.7 ± 15.0 (0.001)
carcass	2.3 ± 0.3 (0.483)	19.4 ± 2.9 (0.549)	74.6 ± 5.0 (0.462)

Excretion into urine and feces: More than 96% of the total radioactivity administered was excreted into urine within 2 days after the last administration (Data is shown in the Table below).

Table 32: Cumulative excretion of the radioactivity into urine and feces after five daily intravenous administration of $^{153}\text{Gd-ZK 135079}$ at 0.1 mmol/0.02 MBq/kg/day, 0.5 mmol/0.1 MBq/kg/day and 2.5 mmol/0.5 MBq/kg/day to rats □: urine ■: feces

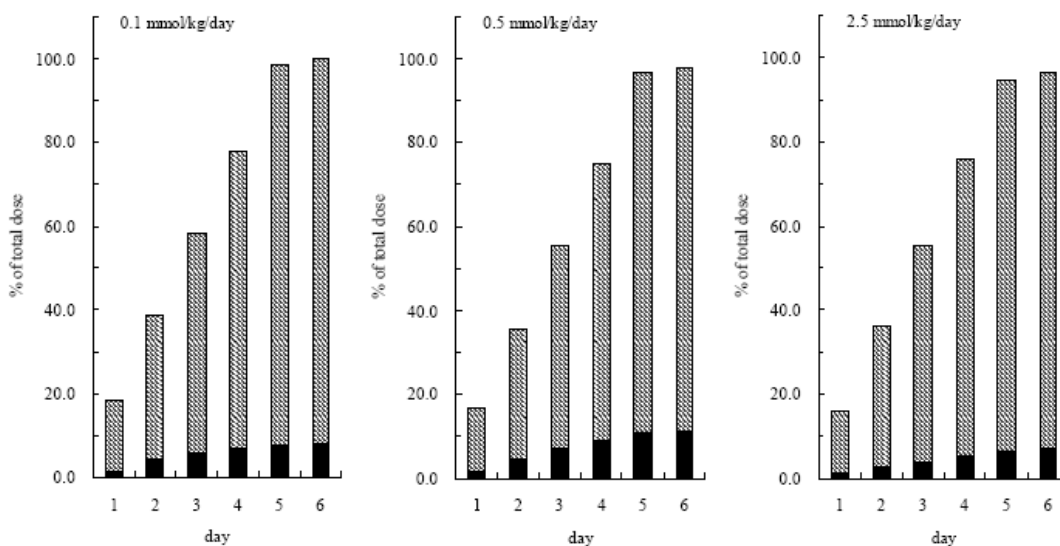


Table 33: Cumulative excretion of the radioactivity into urine and feces after five daily intravenous administration of $^{153}\text{Gd-ZK135079}$ (Gadobutrol) at 0.1 mmol/0.002 MBq/kg/day, 0.5 mmolGd/0.1 MBq/kg/day and 2.5 mmol/0.5 MBq/kg/day to rats

p.a. (day)	% of dose					
	0.1 mmol/kg		0.5 mmol/kg		2.5 mmol/kg	
	urine	feces	urine	feces	urine	feces
1	16.9 ± 0.6	1.3 ± 0.6	15.0 ± 1.2	1.8 ± 0.8	14.7 ± 1.5	1.3 ± 0.3
2	34.3 ± 1.8	4.2 ± 1.8	30.8 ± 3.6	4.9 ± 3.2	33.6 ± 2.5	2.8 ± 1.0
3	52.4 ± 2.5	5.8 ± 2.3	48.5 ± 2.6	7.1 ± 3.7	51.4 ± 1.6	3.9 ± 1.1
4	71.0 ± 2.6	6.9 ± 2.2	66.2 ± 4.5	8.9 ± 4.6	70.4 ± 2.5	5.3 ± 1.1
5	90.7 ± 2.8	7.8 ± 2.1	85.7 ± 3.3	10.8 ± 4.9	88.0 ± 2.3	6.5 ± 1.4
6	91.5 ± 2.9	8.3 ± 2.0	86.5 ± 3.2	11.2 ± 4.9	89.1 ± 2.4	7.1 ± 1.5
total	99.8 ± 3.3		97.7 ± 2.4		96.2 ± 1.9	

Values are mean±S.D.(n=4).

Conclusion: Plasma kinetics of $^{153}\text{Gd-Gadobutrol}$ were similar following single or repeated administrations. After 3h, Gadobutrol was no longer present in plasma.

Two days after repeated (five days) administrations, the highest amount of Gd was found in the kidney, the amount being 2-3 times higher than was detected 24 h post single-dose administration. The radioactivity was mostly excreted by the renal route. There was no significant change in the excretion ratio into urine or feces when gadobutrol was administered by repeated administration.

Reviewer's comments: It does not appear that the 5-day rat intravenous study was properly designed to determine drug accumulation in tissues. However, it can be concluded from this study that the accumulation of radioactivity in tissues was roughly dose-proportional in rats sacrificed two days after the last dose. However, this finding would not have a negative impact on the evaluation of this product since most patients would receive only a single dose of gadobutrol. In addition, the fact that radioactivity was detected in the small intestines in rats in tissue distribution studies suggested that small amounts of the intravenously injected dose was excreted into the bile

5.1.4 Distribution studies

5.14.1 Report No: A475

Study title: Whole body autoradiographic study after intravenous administration of 0.5 mmol (3.7 MBq)-¹⁵³Gd-Gadobutrol/kg to male and pregnant Wistar rats

Study no.:	KI 91.043
Report date:	March 05, 1993
Study report location:	Module 4.2.2.3; 24 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	N/A
GLP compliance:	Yes (); No (x)
QA statement:	Yes (); No (x)
Drug, lot #, and % purity:	¹⁵³ Gd-Gadobutrol, lot#: IS 2376-3, %purity: N/A

Key Study Findings:

- Following a single intravenous administration, ¹⁵³Gd-gadobutrol was rapidly distributed throughout the body except the brain, spinal cord and fetuses. Therefore, gadobutrol did not appear to penetrate the blood-brain and placental barriers, respectively.
- At 0.25 hours, radioactivity was highest in the kidneys but less in the plasma where labeled Gd concentration decreased rapidly to lower limit of quantification 3-6 hours post-dose.

- The rat tissue and whole-body distribution studies suggested a low-level uptake of Gd in bone tissue following a single intravenous dose of Gadobutrol. The bone Gd is then slowly released and excreted within 24 hours.
- The rat tissue and whole-body distribution suggested that there was a low level of Gd uptake in bone following a single intravenous dose. There was however no indication of a long-lasting retention of Gd-radioactivity within the rat.

Study Design: This study was designed to investigate the time course of distribution of the radiolabeled gadolinium following intravenous administration of 0.5 mmolGd (3.7 MBq) ^{153}Gd in male and pregnant rats by whole body autoradiography. At defined time points, rats were sacrificed and stored frozen (-20°C) until sectioning. Pregnant females were at 5 and 16 min, 2h and 1 day post-administration while males were sacrificed at 3 and 15 min, 2h, 1d and 7 days.

Methods

Doses:	0.5 mmolGd (3.7 MBq). Specific radioactivity: 12.kBq/mg (molecular wt-604.7 da)
Frequency of dosing:	Once
Route of administration:	Intravenous bolus
Dose volume:	Not provided
Formulation/Vehicle:	Aqueous solution of Gadobutrol (^{153}Gd -Gadobutrol)
Matrix:	Whole body autoradiographs
Analyte:	Radioactivity
Assay:	Autoradiography
Species/Strain:	Rat/Wistar-Han SPF, Breeder – Schering AG
Number/Sex/Group:	4 pregnant females (gestation day 18); 5 Males
Age:	N/A
Weight:	Females (c.a 250 g); Males (190-210 g)
Satellite groups:	None
Unique study design:	None
PK parameters:	Tissue radioactivity
Deviation from study protocol:	None

Results:

- Following administration, ^{153}Gd -gadobutrol was rapidly distributed throughout the body except the brain, spinal cord and fetuses.
- At 0.25h, radioactivity level was highest in the kidneys (the organ of excretion) followed by plasma and blood and lowest in the brain.
- Gadobutrol was almost completely eliminated within 24h and ^{153}Gd was detectable only in the kidney and the contents of the small intestines after 24h.
- No clear gender differences in distribution of radioactivity was observed.
- Although radioactivity decreased rapidly from plasma and blood to below the level of quantification within 3-6h post administration, a more gradual decrease

was observed in the tissues with radioactivity being present in several organ and in 24h.

- After 30 days, radioactivity was below the level of quantification except in the kidneys and bone.

Conclusions: Measured radioactivity 0.25 hours was highest in the kidneys but less in the plasma where labeled Gd concentration decreased rapidly to lower limit of quantification 3-6 hours post-dose. The rat tissue and whole-body distribution studies suggested a low-level uptake of Gd in bone tissue following a single intravenous dose of Gadobutrol. The bone Gd is then slowly released and excreted within 24 hours.

Reviewer's comments: The finding that there was no distribution of ^{153}Gd -radioactivity to the brain and spinal cord and fetuses indicated that the drug did not appear to penetrate the blood-brain and placental barriers, respectively. I agree with the sponsor's conclusion that the hydrophilic ^{153}Gd -gadobutrol is not capable of crossing the blood-brain barrier and the placental barrier in substantial amounts. A poor distribution of labeled Gadobutrol to fetuses was also observed after 0.5 mmolGd/kg of ^{153}Gd -gadobutrol was administered intravenously as a single-dose to pregnant rabbits on gestation day 18 (Report 9586). The rat tissue and whole-body distribution suggested that there was a low level of Gd uptake in bone following a single-dose intravenous administration. This bone Gd is then slowly released and excreted. However, there is no indication from the results of this study that there is a long-lasting retention of Gd-radioactivity within the rat.

5.1.5 Metabolism studies

Findings from the analysis of urine from rats and dogs identified only unchanged gadobutrol indicating that gadobutrol was not metabolized in the two species.

5.1.6 Excretion studies

To determine the potential of excretion of gadobutrol into milk, a study was conducted in neonate rats removed from lactating dams and injected intravenously with ^{153}Gd -gadobutrol as described in the following Report.

5.16.1 Report No: 9584**Study title: Transfer of ^{153}Gd -ZK 135079 to milk and neonate via milk in rats**

Study no.: **91414 (NO1A91 L42)**
 Report date: **July 19, 1991**
 Study report location: **Module 4.2.2.5; 12 pages**
 Conducting laboratory and location: **Schering AG, Berlin, Germany**
 Date of study initiation: **N/A**
 GLP compliance: **Yes (); No (x)**
 QA statement: **Yes (); No (x)**
 Drug, lot #, and % purity: **^{153}Gd -Gadobutrol, lot#: IS 2376-3, %purity: N/A**

Key Study Findings: The result of this study indicated that there was only a slight transfer of labeled gadobutrol to neonate rats via milk.

Study Design: In this study performed to investigate the transfer of ^{153}Gd -ZK 135079 (gadobutrol) to milk and neonate from mother rats ten neonate rats were separated from lactating dams 11-12 days after delivery. The neonates were isolated for 2 h from dam prior to the administering the dams with 0.5 mmol/0.5 MBq/kg labeled (^{153}Gd) gadobutrol intravenously. The neonates were returned to the dams immediately after the administration to freely suck milk. Maternal blood was sampled 10 min, 0.5h and 1h post-administration. One neonate per dam was sacrificed 3, 6 and 24h after administration and blood and stomach contents were collected for analysis of radioactivity.

Methods

Doses: 0.5 mmolGd (0.5 MBq/kg).
 Frequency of dosing: Once
 Route of administration: Intravenous bolus
 Dose volume: Not provided

 Formulation/Vehicle: Aqueous solution of Gadobutrol (^{153}Gd -Gadobutrol)
 Matrix: Blood (dam and neonates); stomach content (neonates)
 Analyte: Radioactivity
 Assay: Gamma radioactivity measurement in blood and milk
 Species/Strain: Rat/Sprague-Dawley,
 Number/Sex/Group: Lactating rats; 10 neonate rats
 Age: N/A
 Weight: Lactating rats (300-330 g); Neonate rats (20-30 g) at 11-12 day post-delivery
 Satellite groups: None
 Unique study design: None
 PK parameters: Tissue radioactivity
 Deviation from study protocol: None

Results: The radioactivity in the blood of dam and neonate and in the stomach milk are shown in the following Table:

Table 34: Transfer of radioactivity to neonates via milk after intravenous administration of ^{153}Gd -Gadobutrol (0.5 mmol/0.5 MBq/kg) to lactating rats

Time after administration	Maternal blood (nmol/mL)	Stomach milk			Neonatal Blood (nmol/mL)
		Milk wt. (g)*	nmol/g milk	% of dose	
10 min	563 \pm 63	-	-	-	-
30 min	282 \pm 38	-	-	-	-
1 hr	70 \pm 27	-	-	-	-
3 hr	-	0.39 \pm 0.08	59.0 \pm 58.4	0.014 \pm 0.010	6.0 \pm 8.6
6 hr	-	0.56 \pm 0.19	18.7 \pm 3.7	0.007 \pm 0.002	n.d.
24 hr	-	0.55 \pm 0.20	3.0 \pm 1.8	0.001 \pm 0.001	n.d.

Values are mean \pm S.D. (n=4), n.d.: not detected (< 30 cpm)

- : not measured, * : per 1 neonate

The radioactivity in maternal blood decreased from 563 nmol/mL at 10 min to 70 nmol/mL after 1h while the concentration in neonate stomach milk at 3h was 59 nmol/g milk or 0.01% of the administered dose to the dam and one-tenth of the radioactivity in the dam at 10 min. The radioactivity in neonate blood was 6 nmol/mL at 3h or one-hundredth the level in maternal blood measured at 10 min. The level in neonate blood fell below the limit of detection by 6h post administration.

Conclusions: It was concluded from this study that there was only a slight transfer of labeled gadobutrol to neonate rats via milk.

Reviewer's comments: I Agree with the conclusion from this study that there was a relatively small transfer of radioactivity into milk. This finding has labeling implication for nursing mothers. Since the issue is whether or not there was transfer of compound into milk, then amount transferred whether slight or not makes no difference to the fact that there was a transfer to milk. The label should therefore state the fact that gadobutrol is excreted into milk.

5.3 Toxicokinetics

Toxicokinetic studies where performed were included in the respective studies in which they were described.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose intravenous toxicity studies of gadobutrol (ZK 135079) were conducted in male and female rodents (mice; adult and juvenile rats) and dogs unless stated otherwise.

6.1.1 Single-dose toxicity studies in rats

6.1.1.1 Report No. A28309

Study title: Systemic toxicity study in male and female rats with single intravenous administration and observation periods of 3 and 22 days

Study no.:	TXST TXST20050112
Study report location:	4.2.3.1.1
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	June 07, 2005
GLP compliance:	Yes (x), ()
QA statement:	Yes (x), ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562BB) Batch I: 63711307 (99.99%) Batch II: 64712306 (98.8%) Batch III: 64712307 (94.4%)

Key Study Findings:

- Mortality occurred at the high dose (20 mmol Gd/kg) in four study animals, but necropsy did not reveal any specific organ damage.
- Vacuolation of the renal proximal epithelial cells accompanied by increased kidney weight was observed at all doses and persisted into recovery at 6 and 20 mmol Gd/kg.
-
- The sponsor determined the NOAEL as 6 mmolGd/kg. However, based on the finding of renal vacuolation beginning at the low dose in the day 3 sacrifice and persisted into recovery phase as from 6 mmol Gd/kg and higher, the reviewer

considered the NOAEL to be less than 2 mmolGd/kg. NOAEL could therefore not be determined

- Reversible local tissue reactions were observed at the site of drug application.
- Pharmacokinetic evaluation showed a linear, dose-dependent increase in mean systemic exposure (AUC 0-6h).
- No sex differences were observed in the pharmacokinetic profile

Methods

Doses: 0, 2.0, 6.0, and 20.0 mmol Gd/kg (or 3.2x, 9.7x and 32.4x the intended clinical dose based on BSA, respectively)

Frequency of dosing: Single dose

Route of administration: Intravenous; injection speed – 0.4ml/min

Dose volume: 0, 2.0, 6.0, and 20.0 ml/kg, corresponding to 0, 2.0, 6.0 and 20.0 mmol Gd/kg, respectively

Formulation/Vehicle: Gadobutrol - SH L562BB (aqueous solution) / 0.9% (w/v) saline

Species/Strain: Rat / Wistar Crl:WI (Han); (b) (4)

Number/Sex/Group: Main – 10/sex/group
Recovery – 6/sex/group
Satellite – 5/sex/group

Age (at initiation): Males (7 weeks), Females (8 weeks)

Weight: Males (165-217 g), Females (148-178g)

Satellite groups: Yes; 5/sex/group (for Toxicokinetic examination)

Unique study design: None

Deviation from study protocol: None

BSA = Body surface area

Table 35: Study Design (Report A28309)

Group	No. of animals/sex/group	Test/control compound	Dose per day (mmol Gd/kg)	Volume (ml/kg)
1 (control)	Ma: 10M+10F R: 6M+6F S: 5M+5F	0.9% NaCl	0	0
2		SH L562BB	2.0	2
3		SH L562BB	6.0	6
4		SH L562BB	20.0	20

Reviewer's Table; Ma, R, S = Main, Recovery, Satellite groups; M. F = Male, Female

Observations and Results: Single-dose toxicity was evaluated by clinical examination, mortality, food and water consumption, body weight and ophthalmoscopic examination.

Hematology, urinalysis, biochemistry and coagulation, necropsy and histopathologic examination were also performed.

Mortality: No treatment-related mortality was observed up to the mid-dose of 6 mmol Gd/kg (~10x HD). Two animals per sex at the high dose (20 mmol Gd/kg or 32x HD) died on day 1.

Clinical Signs:

- No treatment-related findings were observed at 2 and 6 mmol Gd/kg (3x and 10-fold the intended human dose).
- At 20 mmol Gd/kg (32x HD), clinical signs included apathy, prone/lateral recumbency, spontaneous vocalization, respiratory disturbance, eyelid closure, and seizures were observed in females. Tremors and disturbances in gait were observed in 2 females only. Tremors were not reported in males.
- Skin reddening and scab formation on the tail of test and control animals.

Body Weights: There were no treatment-related observations in body weight up to the mid dose of 6 mmol Gd/kg and in males up to 20 mmol Gd/kg. In females receiving the high dose (20 mmol Gd/kg), a slight but significant increase in body weight was observed on days 3 and 20.

Feed and Water Consumption: No treatment-related finding was observed food and water consumption up to the high dose (20 mmol Gd/kg).

Ophthalmoscopy: No treatment-related findings were observed up to the high dose.

ECG: Not conducted

Hematology: There were significant increases in neutrophils ($p < 0.01$) and monocytes but not lymphocytes

Coagulation: An increase in fibrinogen was observed in one high dose male on day 2

Clinical Chemistry: No treatment-related effects..

Urinalysis: No treatment-related effects.

Gross Pathology:

At the high dose (20 mmolGd/kg), two females and one male were necropsied. Bilateral pale discolored kidneys, considered treatment-related, was observed in one female.

No macroscopic findings were noted in the necropsied male. Male number 2, a satellite animal designated for toxicokinetics was not necropsied.

Pale kidney discoloration was seen in 1/10 females (2.0 mmol/kg), 1/10 males (6.0 mmol/kg) and 1/10 males (20 mmol/kg) ..A histopathological correlate was found only in 1 male each treated with 6 or 20 mmol Gd/kg.

Organ Weights: No Biologically significant changes.

Histopathology:

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

Day 3 final sacrifice: Histopathological findings included the following:

- Dose-related vacuolation of the renal proximal tubular epithelium in males and females of all treatment groups
- Hepatocellular vacuolation of the centrilobular zone in high dose (20 mmolGd/kg) males and females. The vacuolation was more pronounced in females.
- Injection site vasculitis/perivasculitis was observed in animal of both sexes in all treatment groups. Perivascular hemorrhage was observed in all study groups including controls Table 36: Predominant histopathological findings on sacrifice day 3.

	K0							
	Group 1 control		Group 2		Group 3		Group 4	
	0 mmol Gd/kg		2 mmol Gd/kg		6 mmol Gd/kg		20 mmol Gd/kg	
	M	F	M	F	M	F	M	F
Organ	10	10	10	10	10	10	10	8
Kidney - vacuolation, corticotubular epithelium	0	0	9 (1.0)	8 (1.0)	10 (1.6)	10 (1.2)	10 (2.3)	8 (2.1)
Liver - vacuolation, hepatocellular, predominantly centrilobular	0	0	0	0	0	0	2 (1.0)	6 (1.8)
Application site - vasculitis/perivasculitis	0	0	0	2 (1.5)	1 (2.0)	1 (1.0)	3 (1.0)	2 (1.0)
- hemorrhage, perivascular	4 (1.5)	6 (1.2)	5 (1.0)	6 (1.3)	7 (1.0)	9 (1.6)	10 (1.4)	5 (1.6)

K0 = animals sacrificed at terminal sacrifice

M = male

F = female

Grading:

minimal (1), slight (2), moderate (3), marked (4), massive (5), present (ungraded) (p)

Day 22 (recovery) sacrifice:

- Dose-related corticotubular vacuolation in both sexes at the mid (6 mmol/kg) and high (20 mmol Gd/kg) dose groups.
- Liver vacuolation was resolved in animals in the high dose treatment group.
- Treatment-related changes at the injection site were resolved by recovery sacrifice.

Table 37: Predominant histopathological findings on sacrifice day 22

	R1							
	Group 1		Group 2		Group 3		Group 4	
	control		ZK 135079 – treated animals					
	0 mmol Gd/kg		2 mmol Gd/kg		6 mmol Gd/kg		20 mmol Gd/kg	
	M	F	M	F	M	F	M	F
Organ	6	6	6	6	6	6	5	6
Kidney - vacuolation, corticotubular epithelium	0	0	0	0	3 (1.0)	2 (1.0)	5 (1.8)	6 (1.3)
Liver - vacuolation, hepatocellular, predominantly centrilobular	0	0	-	-	-	-	0	0
Application site - vasculitis/perivasculitis	1 (1.0)	0	-	-	-	-	0	1 (1.0)
- hemorrhage, perivascular	0	0	-	-	-	-	0	0

K0 = animals sacrificed at terminal sacrifice

M = male

F = female

Grading:

minimal (1), slight (2), moderate (3), marked (4), massive (5), present (ungraded) (p)

- = not evaluated in this group

Special Evaluation: None**Toxicokinetics:**

- Pooled pharmacokinetic (PK) parameters were evaluated for males and females since no gender differences in PK were observed.
- Maximal serum Gd concentrations were observed at the 5-min sampling point.
- Although C_{max} increased by a factor of 2.92 in relation to the dose increase from 2 to 6 mmol Gd/kg, the increase was less than dose proportional
- Average systemic exposure (AUC(0-6h)) increased linearly with increasing dose

Table 38: Pharmacokinetic parameters determined in study A28309

Parameter	Unit	Dose (mmol/kg)					
		2		6		20	
		Male	Female	Male	Female	Male	Female
C_{max}	[mmol/L]	7.06	7.85	21.3	22.2	49.6	49.6
AUC(0-6h)	[mmol/L x h]	3.28	3.50	10.0	9.56	33.8	34.7
AUC	[mmol/L x h]	3.28	3.5	10.0	9.56	33.8	34.7
$t_{1/2}$	[h]	0.699	0.766	0.675	0.686	0.572	0.609
$C_{max}/Dose$	[mmol/L]/ [mmol/kg]	3.53	3.93	3.54	3.71	2.48	2.48
AUC(0-6h)/Dose	[mmol/L x h]/ [mmol/kg]	1.64	1.75	1.67	1.59	1.69	1.73
Parameter	Unit	Dose (mmol/kg)					
		2		6		20	
		Male + Female		Male + Female		Male + Female	
C_{max}	[mmol/L]	7.46		21.7		49.6	
AUC(0-6h)	[mmol/L x h]	3.39		9.79		34.2	
AUC	[mmol/L x h]	3.39		9.80		34.3	
$t_{1/2}$	[h]	0.736		0.679		0.592	
$C_{max}/Dose$	[mmol/L]/ [mmol/kg]	3.73		3.62		2.48	
AUC(0-6h)/Dose	[mmol/L x h]/ [mmol/kg]	1.69		1.63		1.71	

C_{max} Maximum Gd concentration in plasma after drug administration
 $t_{1/2}$ Apparent terminal half-life
 AUC(0-6h) Area under the concentration versus time curve from dosing time to 6 h post dose
 AUC Area under the concentration versus time curve from dosing time to infinity
 $C_{max}/Dose$ Dose normalized C_{max} -value
 AUC(0-6h)/Dose Dose normalized AUC(0-6h)-value
 N/A Not applicable

Stability and Homogeneity: Acceptable. Based on the Certificate of Analysis.

Conclusions:

- Mortality occurred at the high dose (20 mmol Gd/kg) in 4/42 study animals but no specific organ damage was identified at necropsy.
- Vacuolation of the renal proximal epithelial cells accompanied by increased kidney weight was observed at the low dose and higher.
- The sponsor determined a NOAEL of 6 mmolGd/kg. Since renal tabulation occurred beginning at the low dose (2 mmolGd/kg), A NOAEL was not established
- Local reactions at the drug application site were reversible.
- A linear, dose-dependent increase in the mean systemic exposure (AUC 0-6h) was observed following the single intravenous administration of gadobutrol.

- There was no sex difference in the pharmacokinetic data.

Reviewer's comments: Individual animal data (Appendix 1) was not provided. Besides mortalities observed at the high dose of gadobutrol tested in this study, the only other finding that appeared significant was vacuolation of the renal proximal tubule epithelium previously described with Gadolinium-based contrast agents. The vacuolation was however not accompanied by concomitant effect(s) on kidney function. The observed local injection site reactions (vasculitis/perivasculitis) are noted as important findings in this class of compounds. This finding is of importance in the label. Overall, I agree with the findings of this single-dose toxicity study of gadobutrol administered intravenously in rats.

6.1.1.2 Report No. PH-36304 (AT06032) (Submitted in amendment 0010 of 10/12/2010):

PH-36304

Study title: Extended Single Dose Toxicity Study in Neonatal Rats after Intravenous Administration on PND 4 with a Following Recovery Period up to Day 28 (First Revised Final Report of PH-36204)	
Study no.:	T4079918; Pathology No. 7607
Study report location:	NDA 201-277 4.2.3.1.1, 566 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, GDD-GED Toxicology, 42096 Wuppertal, Germany
Date of study initiation:	July 20, 2009
GLP compliance:	Yes (OECD GLP; Revised German Principles of GLP), Signed May 07, 2010; page 3
QA statement:	Yes; Signed August 30, 2010; page 4
Drug, lot #, and % purity:	Gadobutrol (SH L 562 BB; lot #: N/A, Purity: 100%; page 16

Key Study Findings:

- All pups survived to scheduled sacrifices except two high dose, recovery group females.
- No clinical findings were observed in main or recovery group animals
- There were no remarkable adverse effect on body weight gain at doses up to 6 mmolGd/kg
- A decrease in alanine aminotransaminase (ALAT) and glutamate dehydrogenase (GLDH) enzymes were observed at the high dose. No changes in plasma creatinine, urea and albumin in males and females at up to the high dose.
- No treatment-related changes were observed in urinalysis
- Renal cortical tubular vacuolation was observed at all doses of Gadobutrol tested

- Only gadolinium was evaluated in the TK analysis. Gd was determined in plasma, skin, liver, kidneys, heart and bone (sternum) after i.v administration on PND 4.
- The exposure of animals was evaluated with regard to sex, dose, concentration versus time course within the dosing interval
- Evaluation of skin, liver, kidney, heart, aorta and sternum did not reveal the presence of Gadolinium in these tissues.
- Systemic exposure and exposure to organs and tissues was dose proportional, while amounts of Gd remaining in the tissues 24h after administration was between 0.01 and 0.9% of initial dose.

Methods	
Doses:	0 (vehicle control), 0.6, 2.0, 6.0 mmolGd/kg Gadobutrol (SH L562 BB). The 0.6 and 2.0 mmol Gd/kg doses were diluted with 0.9% NaCl solution). The 6.0 mmol Gd/kg was used as undiluted formulation.
Frequency of dosing:	Once; Single dose on PND 4
Route of administration:	Intravenous
Dose volume:	6 mL/kg
Formulation/Vehicle:	Gadobutrol (SH L562 BB) solution / 0.9% NaCl
Species/Strain:	Rat / Wistar (Hsd Cpb:WU); (b) (4)
Number/Sex/Group:	Main: 10/sex/group Recovery: 10/sex/group
Age:	4 days
Weight:	Males (11.3 g); Females (11.0 g)
Satellite groups:	12/sex/group
Unique study design:	None
Deviation from study protocol:	According to the sponsor, no deviations were identified which affected the quality and integrity of the study or the results
PND = postnatal day; b.w = body weight	

Objectives/Design/Dose selection: This study was conducted to evaluate the potential toxic effects and toxicokinetics of a single intravenous dose of gadobutrol f(SH L562 BB) administered intravenously to male and female neonatal rats on PND 4. Additional objectives included a determination of the reversibility, continuation or a delayed occurrence of toxicity including the dose-response relationship following a single exposure. The study was also designed to establish a NOAEL.

Design: The study design is described in the following Table:

Table 39: Study Design (Report PH-36304)

Groups	Animals/sex	Treatment	Dose	Dose	Dose vol.
--------	-------------	-----------	------	------	-----------

					(mmolGd/kg)	multiple (by BSA)	(mL/kg)
	M	R	S				
1	10	10	12	0.9% NaCl	0	0	6
2	10	10	12	SH L562BB	0.6	0.97x (1.0x)	6
3	10	10	12	SH L562BB	2.0	3.2x (3.0x)	6
4	10	10	12	SH L562BB	6.0	9.7x (10.0x)	6
Reviewer's Table; M, R or S = Main, Recovery or Satellite groups; BSA = Body surface area; x = human dose multiples							

- Dosages were based on the results of a feasibility study (T3079917) in which SH L 562BB was administered as a single intravenous dose to pups in doses of 0, 0.6, 2.0 or 6.0 mmolGd/kg body weight on post-natal day (PND) 4.
- The 6 mmolGd/kg was the highest maximal feasible dose in 4-day old pups.
- Following Gadobutrol/saline administration, animals in the main group were sacrificed on PND 5 and fixed *in toto* for histopathological evaluation.
- Blood sample was collected for clinical pathology.
- Recovery group animals were administered gadobutrol/saline as animals in the main group and kept for a 28-day treatment-free period to determine reversibility, continuation or delayed occurrence of toxicity effects.
- Satellite group animals were administered test or control articles and sacrificed within 24h post administration and were used to determine Gd concentration in plasma, skin, liver, kidneys, heart and sternum.

Observations and Results:

Observations:

- Animals were examined for morbidity and/or mortality twice a day.
- Body weight was determined on day 1 in the main groups and weekly in recovery groups on days 7, 14, 21 and 28. Body weights were also recorded immediately prior to necropsies.
- Clinical chemistry was performed on day 2 in the main groups and on day 25 in the recovery groups
- Urinalysis was conducted on day 23 only in recovery group animals.
- Necropsy was performed on days 2 and 29 in main and recovery groups, respectively.
- Intravenous blood sampling for plasma toxicokinetics (TK) was done at 5 min, 3, 7 and 24h) post-administration in satellite pups (3 per sex) on PND 4.
- Tissue samples obtained from skin, liver, kidney, heart and sternum at the same time points for plasma TK sampling, were preserved for a determination of Gd concentrations.

Results:

Mortality: All pups survived to scheduled sacrifices except two high dose (6.0 mmol Gd/kg), recovery group females sacrificed on day 15 due to decreased motility, diminished muscular tone uncoordinated gait and tilted head.

Clinical Signs: No clinical findings were observed in main or recovery group pups except the clinical signs described in two females that were sacrificed on day 15 post-treatment.

Body Weights: No remarkable adverse effect on body weight gain was observed in recovery group males (up to 6.0 mmol Gd/kg) and females (0.6 mmol Gd/kg). At 6 mmolGd/kg, a significant body weight reduction was noted in recovery group females on day 4. This effect was reversed at recovery.

Clinical Pathology: According to the sponsor, no reference values of control animals of same strain and comparable age range were available from the performing laboratory for comparison

Clinical Chemistry: There was a decrease in alanine aminotransaminase (ALAT) enzyme activity in males administered 6 mmolGd/kg (high dose) 1 day after dosing. A similar finding was not seen at lower doses.

A decrease in glutamate dehydrogenase (GLDH) was reported in high dose females 24h post-dose.

There were no changes in plasma creatinine, urea and albumin in males and females at up to the high dose.

Urinalysis: No treatment-related changes were observed

Pathology:

a) Necropsy:

Two females were sacrificed before schedule. No gross pathological findings were reported at terminal or recovery sacrifice.

b) Organ Weights:

At 6 mmolGd/kg there was a decrease in mean absolute liver weight in recovery male and female animals relative to pups given the lower doses

At the high dose, absolute kidney weights were 8% or 19% lower than control male or females, respectively.

At 6 mmol/kg, there was a slight non-significant 4% increase in brain weight in males compared to controls. At the same dose in females, there was an 8% increase in brain weight. Brain weights were calculated as a percentage of terminal body weight

c) Histopathology:

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

- Histopathology of two high dose females in unscheduled revealed sacrifice atrophy of splenic white pulp, a lowered hematopoietic bone marrow activity and flat follicular epithelium of the thyroid. These findings were not considered treatment-related.
- In animals in the main group, cortical tubular vacuolation of the kidney epithelium was observed at 2 mmolGd/kg and above. At 2 mmolGd/kg and up, enlarged and/or increased microglia cells was observed in the cerebrum. No adverse effects on the brain were revealed by immunohistochemistry.
- Signs of mechanical trauma were noted at the injection site at doses up to 6 mmolGd/kg.
- In the recovery group, cortical tubular vacuolation was observed in animals in the high dose group. No similar findings were observed at the low dose. Findings at the 2 mmolGd/kg dose were not reported.
- No treatment-related changes were observed at the local injection site in recovery group animals
- No evidence of gadolinium was reported in the heart, liver, kidneys, stomach, aorta, and skin stained with von Kossa stain.

Special Evaluation: None

Toxicokinetics: Gd concentration in plasma, skin, kidneys, heart and sternum in pups was determined by validated ICP-MS method. Plasma and tissue samples were collected at 0.083, 3, 7 and 24h post i.v. administration on PND 4.

- Maximal plasma Gd concentrations were obtained at the first post-administration sampling time of 0.083h (5 min). Gd concentration decreased to <0.3%(plasma), less than 10 % (skin), 24 to 60% (liver), 50 to 62 % (kidneys), and less than 3% (heart and sternum) of the C_{max} at 24h.
- At 24h, the fraction of the remaining dose ranged from 0.011 - 0.014% in the heart, 0.8-0.9% in the kidney and 0.033 to 0.036 % in the liver.
- There was no evidence of sex-related differences in plasma and tissue TK values.
- Plasma AUC(0-24) and C_{max} values increased approximately dose-proportionally when increasing the dose from 0.6 to 2 mmol/kg. A less than dose-proportional increase of C_{max} was observed when the dose increased from 2 to 6 mmolGd/kg
- In tissues and organs, there was a dose proportional increase in AUC in the liver, heart and sternum and a less than dose-proportional increase in the skin and kidney at 0.6 to 2 mmolGd/kg.

- The increase in Cmax was dose-proportional in the kidney and sternum but less than dose-proportional in the liver and skin.
- Overall, the systemic exposure and exposure to organs and tissues was dose proportional, while amounts of Gd remaining in the tissues 24h after administration was between 0.01 and 0.9% of initial dose.

Dosing Solution Analysis: The test article, prepared fresh each day was administered diluted in 0.9% NaCl. The formulation was stable in the clear formulations in the period of use. Correct test substance concentrations, as described in the study design were used for the dose formulation analysis. Formulations were found to be stable for 24h at room temperature. The concentrations of the formulations samples were within the acceptable range of 80-120% of the normal concentration. No drug substance was found in the placebo batches.

Sponsor's Stability statement (GLP): The test article is stable for at least 36 months at room temperature.

Data Basis: The shelf-life of commercial Gadovist solution is 3 years. During product development Schering AG has compiled formal stability data for Gadovist solution in 100 ml glass bottles which support a shelf-life of up to 60 months at room temperature (see Stability Report QE1 093.5/97, front page appended here). This shelf-life is not fully exploited in this statement since the shelf-life of commercial Gadovist solution in bottles has also been set to 36 months.

Conclusions:

- All pups survived to scheduled sacrifices except two high dose, recovery group females.
- No clinical findings were observed in main or recovery group animals
- There were no remarkable adverse effect on body weight gain at doses up to 6 mmolGd/kg
- A decrease in alanine aminotransaminase (ALAT) and glutamate dehydrogenase (GLDH) enzymes were observed at the high dose. No changes in plasma creatinine, urea and albumin in males and females at up to the high dose.
- No treatment-related changes were observed in urinalysis
- Renal cortical tubular vacuolation was observed at all doses of Gadobutrol tested
- There was no evidence of skin and tissue mineralization
- Systemic exposure and exposure to organs and tissues was dose proportional, while amounts of Gd remaining in the tissues 24h after administration was between 0.01 and 0.9% of initial dose.

Reviewer's Comments: I agree with the study findings and conclusions

6.1.2 Single-dose toxicity studies in dogs

Single dose intravenous toxicity of gadobutrol was evaluated in one pivotal extended study of gadobutrol in dogs (A41318) and two supportive studies (A41286 and 9883).

6.1.2.1 Report No. A41318

Study title: Systemic toxicity study in male and female dogs with single intravenous administration and observation periods of 3 and 22 days

Study no.:	TXST20070301
Study report location:	NDA201277-: 4.2.3.1.1
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	January 15, 2008
GLP compliance:	Yes (x), No ()
QA statement:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562BB) 1.0 mmol Gd/ml Batch I: 63711307 (99.99%) Batch II: 64712306 (98.8%) Batch III: 64712307 (94.4%)

Key Study Findings:

- No mortality was reported.
- No treatment-related findings in body weight, food consumption or ophthalmology, ECG, blood coagulation or urinalysis were observed.
- Renal tubular vacuolation was observed at day 2 sacrifice. However, the finding of vacuolation was persistent in the mid- and high dose treatment groups at the end of the recovery period
- Pharmacokinetic (PK) analysis revealed a less than dose-dependent increase in mean systemic
- No gender differences were reflected in the PK findings.
- The NOAEL could not be determined based on the dose-dependent renal tubular vacuolation beginning at the low dose that persisted into recovery phase..

Methods

Doses:	0, 0.3, 1.7, and 10.0 mmol Gd/kg (or 1.6x, 9.2x and 54.1x the maximum intended clinical dose based on BSA, respectively)
Frequency of dosing:	Single dose
Route of administration:	Intravenous; injection speed – 8 ml/min
Dose volume:	0, 0.3, 1.7, and 10.0 ml/kg, corresponding to 0, 0.3, 1.7, and 10.0 mmol Gd/kg, respectively
Formulation/Vehicle:	Gadobutrol - SH L562BB (aqueous solution) / 0.9% (w/v) saline
Species/Strain:	Dog/Beagle
Number/Sex/Group:	5/sex/group
Age (at initiation):	10 months
Weight:	Males (6.9 – 8.9 kg), Females (6.2 – 8.9 kg)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Room temperature upper limit exceeded on 2/23/2008. Deviation did not impact study results

BSA = Body surface area

Observations:

- Toxicity was evaluated by clinical examination, mortality, food consumption, body weight, blood pressure, ECG/heart rate, ophthalmoscopy, and clinical tests of nervous system function, hematology, urinalysis, biochemistry and coagulation and toxicokinetics.
- Blood samples for determining Gd concentration were taken immediately after administration and at 20 minutes, 1, 3, 7 and 24 hours.
- Necropsy and histopathological examination was performed on all animals either on day 3 or 22. Postmortem examination included organ/tissue sampling and organ weight determinations.

Table 40: Study groups (A41318)

Groups	Animals (No. and sex)	Test/control article	Daily Dose (mmol Gd/kg)	Dose multiples of HD	Vol. (ml/kg)
1	3M / 3F * 2M / 2F **	Saline (0.9% NaCl)	0 mmol/kg	0	10
2		Gadobutrol SH L562BB	0.3 mmol/kg	1.62x	0.3
3			1.7 mmol/kg	9.2x	1.7
4			10.0 mmol/kg	54.1x	10

Reviewer's Table adapted from sponsor's table; M=Male, F=Female; * = Day 3 sacrifice; ** = Day 22 sacrifice, HD = intended human dose

Observations and Results

Mortality: There was no treatment-related mortality and all animals survived to scheduled sacrifice

Body Weights: There were no treatment-related effects.

Food Consumption: There were no treatment-related effects.

Ophthalmoscopy: No treatment-related findings were observed.

ECG: An increase in heart rate associated with decreases in the RR-interval and QT-time was observed in high dose animals 5 min after administration. This effect was considered dose-related

Hematology: There were no treatment-related effects. The decrease ($p < 0.05$) in MCHC observed in group 2 males (low dose; 0.3 mmol Gd/kg or 1.62x HD) and an increase ($p < 0.05$) in lymphocyte count in group 4 males (high dose; 10 mmol Gd/kg or 54X HD) were not dose-dependent.

Coagulation: No treatment-related effect was observed.

Clinical Chemistry: No treatment-related effect was observed

Urinalysis: No treatment-related effects were observed.

Gross Pathology: There were no treatment-related macroscopic findings.

Organ Weights: There were no treatment-related organ weight changes

Histopathology:

Adequate Battery: yes (x), no ()

Peer Review: yes (), no ()

Microscopic findings:

A dose-dependent vacuolation of the renal proximal tubular epithelium was observed in animals sacrificed on day 2 beginning from the low dose (0.3 mmol Gd/kg) upwards.

The effect was not completely reversed in recovery group animals since vacuolation was observed in 1 of 2 males and 2 and 2 of 2 females in the mid-dose and all high dose group animals.

Special Evaluation: None

Toxicokinetics:

- Gd concentration was determined in plasma samples 0.333, 1, 3, 7 and 24 h post-dose.. All Gd levels in plasma were expressed as $\mu\text{mol Gd/L}$. Dose proportionality was assessed using dose normalized C_{max} and AUC(0-24h)-values.
- No sex differences in systemic exposure to gadobutrol were observed and pharmacokinetic parameters were evaluated for the combined mean parameters.
- Increase in C_{max} was less than dose-proportional by a factor of 4.42 when dose increased from 0.3 to 1.7 mmol Gd/kg and by a factor of 10.6 in the dose increase from 1.7 to 10.0 mmol Gd/kg.
- The increase in mean systemic exposure (AUC (0-24h)) was less than dose proportional and the 5.6-fold increase in dose from 0.3 to 1.7 mmol Gd/kg resulted in a 4.2 fold increase of systemic exposure, while the 33.3-fold dose increase from 0.3 to 10 mmolGd/kg resulted in an 18.5-fold increase in AUC.

Stability and Homogeneity: Acceptable. Based on the Certificate of Analysis.

According to the sponsor, homogeneity testing of the Gadobutrol solution was not performed and the drug product was administered undiluted to test animals. However, drug products, when formulated as solution, were generally recognized to have homogeneity within the batch.

Conclusions:

- A NOAEL was not established.
- There was no treatment-related mortality at any of the administered doses.
- No treatment-related findings were observed in body weight, food consumption or ophthalmology, ECG, blood coagulation or urinalysis.
- Renal proximal tubular vacuolation persisted into the recovery period in mid and high dose treatment groups.
- Hematological findings were variable and not dose-dependent.
- No treatment-related gross pathological changes were observed.
- Pharmacokinetic evaluation showed a less than dose-dependent increase in the mean systemic exposure (AUC (0-24h)).
- No sex differences were observed in the pharmacokinetic profile.

Reviewer's comments: I agree with the findings and conclusions from this study.

6.2 Repeat-Dose Toxicity

6.2.1 Repeat-dose Toxicity study in rats

Reports of five intravenous repeat-dose toxicity studies in rats were submitted namely: SG/069, A08936, 9926, 9658 and A03528. Key findings for all reports and a review of Reports 9586 and A08936 are included in this review. A summary of the submitted studies is provided in the following Table:

Table 41: Summary of repeat-dose intravenous studies in the rat

	Intravenous repeat-dose toxicity reports in rats				
	A08936	9926	SG/069	9658	A03528
Type of study	Systemic toxicity	Systemic toxicity	DRF	Systemic toxicity	Systemic toxicity
Gender (M or M/F)	M/F	M	M/F	M/F	M/F
Drug formulation	562BB	562A	562A	562A	562A
Doses (mmol/kg)	0, 0.6, 1.2, 3	0, 1	0, 0.5 (0.5M) 4.5 (1.0M)	0, 1, 2.5, 5	0, 0.1, 0.25, 0.75
Dosing period	4 weeks (7 days/wk)	2 days	8 days	4 weeks (5 days/wk)	4 weeks (7 days/wk)
Postdose period	10 weeks	none	none	8 weeks	2 weeks
GLP (Y/N)	Y	Y	N	Y	Y
Reviewed (Y/N)	Y	N	N	Y	N
NOAEL		ND	ND	ND	M: 0.75 mmol/kg F: 2.5 mmol/kg
Key findings	Y	Y	Y	Y	Y

Reviewer's table based on sponsor's data; M=males; M/F=males and females; 562A, 562BB = SH L562A, SH L 562B; Y/N = yes/no; NOAEL = no observed adverse effect level; DRF = Dose-range finding study; ND = not determined

6.2.1.1. Report 9658:

Study title: Systemic tolerance study in rats after daily intravenous administration over about 4 weeks (16-18 applications) including a reversibility study

Study no.: **TX 91.139**
 Study report location: **CTD Module 4.2.3.2.,**
 Conducting laboratory and location: **Bayer Schering Pharma AG, Berlin, Germany**
 Date of study initiation: **N/A; Study conducted August – October, 1991**
 GLP compliance: **Yes (x), No ()**
 QA statement: **Yes (x), No ()**
 Drug, lot #, and % purity: **Gadobutrol (SH L562A; Substance Batch #: 411710 Formulation Batch #: G/094/B; G/094/B**

Methods (Report 9658)

Doses: 0, 1.0, 2.5, 5.0 mmol/kg
 Frequency of dosing: Daily; 4 weeks (5 days/week)
 Route of administration: Intravenous
 Dose volume: 10 ml/kg (groups 1,4,5 and 6); 2 and 5 ml/kg (groups 2 and 3, respectively)
 Formulation/Vehicle: SH L562A (0.5 mmol/mL); 0.9% NaCl
 Species/Strain: Rat/Han:WIST
 Number/Sex/Group: 10/sex/group; 4 groups
 Age: 9-11 weeks
 Weight: Males (195 - 256g), Females (159 - 205g)
 Satellite groups: 20 males/group (2 groups, # 5-6)
 Unique study design: None
 Deviation from study protocol: No protocol deviations were reported

Key findings:

- Two of four deaths in females were treatment-related
- No clinical signs were observed at the low and mid doses
- A dose-dependent increase in absolute and relative kidney weight occurred in males and females as from 1mmol or 2.5 mmol/kg, respectively.
- A treatment-related renal proximal tubule vacuolation was observed in males and females beginning at the low dose
- Based on the findings, NOAEL was <1.0 mmolGd/kg

At 1.0 mmolGd/kg (1.6x MHD), a significant dose-dependent increase in absolute and relative kidney weight was observed in male rats. A dose-dependent vacuolization of renal proximal tubular epithelial cells was observed in males and females as from 1.0 mmol/kg. At 2.5 mmol/kg (4x MHD) and higher, there was a dose-dependent increase in kidney weight (absolute and relative).

4 deaths among females was reported. In animals sacrificed in week 12 corresponding to week 8 after the 4-week treatment period, an increase in absolute and relative kidney weight in addition to proximal tubular vacuolization were observed.

Dose multiples (Report 9658):

Dose (mmol/kg)	LD	MD	HD	NOAEL
	1	2.5	5	
Dose multiple (based on BSA)	1.62x	4.1x	8.1x	NOAEL was not established

LD, MD, HD = low, mid and high dose, respectively

Study Design (Report 9658):

Group	Test formulation	Dose (mmol ZK 135.079 per kg body weight)	Concentration (mmol ZK 135.079/ml)	Application volume (ml/kg)	Application rate (ml/min)	No. of animals and sex
1 (control)	0.9 % (w/v) NaCl-solution	0	0	10 (vehicle)	6	10M/10F
2	SH L 562 A	1	0.5	2	6	10M/10F
3	SH L 562 A	2.5	0.5	5	6	10M/10F
4	SH L 562 A	5	0.5	10	6	10M/10F
5 (control)	0.9 % (w/v) NaCl-solution	0	0	10	6	20M
6	SH L 562 A	5	0.5	10	6	20M

Mortality: Four deaths in females animals were reported as follows:

No. of death	Treatment group/dose (mmol/kg)	Time of death	Comment
1	III (2.5)	Week 2	Accident; no signs
1	IV (5)	Week 5	Accident; no signs
1	IV (5)	Day 4	Treatment-related
1	IV (5)	Day 11	Treatment-related

Reviewer's summary of mortality data.

Two deaths in the high dose group were preceded by spasmodic twitches, biting of objects and unconsciousness. Clinical signs did not accompany the other 2 deaths.

Clinical Signs: No clinical signs were observed at 1 and 2.5 mmolGd/kg or 1.6x and 4x multiples of the human dose based on body surface area. Based on clinical signs NOAEL was 2.5 mmol Gd/kg (or 4x the clinical dose).

Body weight: No treatment related effects

Feed Consumption: No treatment related effects

Water Consumption: No treatment related effects

Ophthalmology: No treatment related effects

Hematology: There were no treatment-related effects on hematology or blood coagulation. The following sponsor Table shows some statistically significant hematology findings:

Table 42: Some statistically significant hematology values (Report 9658)

Parameter	Increase ↑ Decrease ↓	Group	Sex	Day of examination	Statistical significance	Reason
Erythrocyte count	↓	4	M	23	p < 0.01	R, S
MCH	↑	4	M	23	p < 0.01	R, S
Leucocyte count	↑	6	M	23	p < 0.05	R, Ch
Monocyte count	↑	6	M	23	p < 0.05	R, S
Lymphocyte count	↑	6	M	23	p < 0.05	R, Ch
Hematocrit	(↓)	4	M	23	p < 0.05	S
Reticulocyte count absolute } relative }	↑	6	M	23	p < 0.05	Ch R, Ch
R = within the reference range S = very slight change (not biologically relevant) when compared to the controls Ch = chance finding, not observed in group 4 (same dose, same time point) () = very slight						

Clinical chemistry:

- Decreased values relative to controls were observed in the relative and absolute total alpha (α 1 and α 2) globulin level.
- There was an increase in the absolute and relative β -globulin level.
- There was no compound-related effect on testosterone, LH or FSH levels

Urinalysis: No treatment-related effects

Gross Pathology: No treatment-related effects in animals at scheduled sacrifice. Mortality in 2 group 4 (high dose) females was treatment related. The following sponsor Table is a summary of the macroscopic findings:

Table 43: Macroscopic findings in controls and gadobutrol (SH L562 A)-treated animals sacrificed after 4 weeks or at the end of the recovery period

Organ/Finding	Group 1 Control 10M/10F	Group 2 1 mmol/kg 10M/10F	Group 3 2.5 mmol/kg 10M/9F	Group 4 5 mmol/kg 10M/7F	Group 5 Control 20M	Group 6 5 mmol/kg 20M
Kidney: pelvic dilatation	2M			1M	2M	
Thymus: punctiform/focal reddening	4M/1F	3M	3M/3F	4M/2F	2M	2M
Testes: diminished in size both sides left side	1M		1M			
Epididymides: abscess-like lesion (right tail) nodule (right head/left tail)	1M		1M	1M		1M
Uterus: hydrometra (both horns)	4F	4F	2F	1F		
Ovaries: cystic dilatation of ovarian sac (both sides)		2F				
Submandibular gland: reddening edematous	1M 3M/2F	4M/1F	4M/2F	2M 1M/1F		
Mandibular lymph node: reddened (both sides)					2M	
Skin: blood-soaked area (in subcutis of cervical region) focal alopecia/thinning of fur	3M/1F	1M/4F	4M/4F	3M/2F 1M	1M	
Iliac lymph node: reddening/focal reddening enlarged/swollen diminished in size	1M 1F	1F	1F			
Pituitary gland: anterior lobe: nodule	1F					

Organ Weights:

- A dose-dependent increase in absolute and relative kidney weight was observed as from the low dose (1 mmolGd/kg) in males and from the mid dose (2.5 mmolGd/kg) in females.
- An increased absolute and relative kidney weight was also observed in recovery group animals (5 mmolGd/kg) at the post-recovery period sacrifice.

Histopathology:

Adequate Battery: Yes (x), No ()

Peer Review: Yes (), No (x)

Histopathological Findings:

Treatment-related, dose-dependent microscopic findings namely, vacuolation of proximal tubule epithelium were reported in both sexes as from the low dose (1 mmolGd/kg or 1.6x the intended clinical dose). Similar findings were observed in recovery animals

Special Evaluation: None

Toxicokinetics: TK was not performed

Dosing Solution Analysis: No data available

Conclusion: The major significant findings in this study were as follows:

Four mortalities among females were reported. Two animals from groups 3 and 4 died on weeks 2 and 4 respectively. There were no clinical findings and cause of death was reported as accidental.

The other 2 deaths occurred in the high dose group died on days 4 and 11, 10 min after administration. Signs included twitchings, biting of objects and unconsciousness. The deaths were treatment-related

The renal changes include an increase in absolute and relative kidney weights and renal tubular vacuolization beginning at the low dose that persisted to the end of the 8-week post administration period. No concomitant change occurred in kidney function.

Reviewer's comments: According to the sponsor, a NOAEL of 2.5 mmol/kg was established in this study based on the deaths occurring at the high dose of 5.0 mmol/kg and mild decreases in hematological parameters in males high dose group. I do not agree with this NOAEL since renal tubular vacuolization beginning from the low dose (1 mmol Gd/kg) was reported. The NOAEL was less than 1.0 mmol/kg.

6.2.1.2 Report No. A08936:

Study title: Gadobutrol (SH L562BB) - Systemic toxicity study with daily intravenous administration to rats (M+F) followed by a subsequent observation period of approximately 10 weeks

Study no.: **TXST20020145 / KIST20020214**
 Study report location: **CTD 4.2.3.2.1, pages 1-933**
 Conducting laboratory and location: **Bayer Schering Pharma AG, Berlin, Germany**
 Date of study initiation: **June 10, 2002**
 GLP compliance: **Yes (x), No (); signed**
 QA statement: **Yes (x), No (); signed**
 Drug, lot #, and % purity: **Gadobutrol, lot #: N/A, purity – 100%**

Key Study Findings:

- Repeated administration of gadobutrol for four week at the doses tested was well-tolerated.
- The main finding in this study was renal tubular vacuolation. There were no concomitant degenerative or inflammatory changes identified histologically
- A single male death at the high dose was reported
- Based on the finding of renal tubular vacuolation occurring as from the low dose and higher in both male and female animals, the NOAEL was less than the low dose and could not be determined.

Methods

Doses: 0, 0.6, 1.2, and 3.0 mmol/kg
 Frequency of dosing: daily
 Route of administration: Intravenous; 6 mL/min
 Dose volume: 0.6 – 3.0 mL
 Formulation/Vehicle: Gadobutrol batch no. 69713703; formulation SH L562BB batch no. N21007) / Sodium chloride 0.9%, Batch Nos. I: 1203A92; II: 2222A91
 Species/Strain: Rat, Wistar (Shoe:WIST); (b) (4)
 Number/Sex/Group: 4 groups; 10/sex/group
 Age: Males (6-9 weeks); females (6-11 weeks)
 Weight (at initiation): male: 208-320 g; female: 153-201 g
 Satellite groups: 1 group; 9/sex
 Unique study design: none
 Deviation from study protocol: none

Treatment schedule (Report No. A08936)

Group	Number of animals/sex	Compound	Dose per day [mmol Gd/kg]	Application volume
1 (control)	10M +10F 9M + 9F*	vehicle	-	3.0
2	10M +10F 9M + 9F*	SH L562BB	0.6	0.6
3	10M +10F 9M + 9F*	SH L562BB	1.2	1.2
4	10M +10F 9M + 9F*	SH L562BB	3.0	3.0

* = satellite animals for plasma level determination and recovery period.

Concentrations of gadolinium were determined in serum samples taken 5 min, 20 min, 1 h, 3 h, 7 h and 24 h after daily administration of 0.6, 1.2 and 3.0 mmol/kg/day SH L 562BB on the first and last days of treatment (day 1 and 28). Blood was taken from 3 animals/sex per sampling time and dose group.

Dose multiples of Gadobutrol in Report No. A08936

Dose (mmol Gd/kg)	0.6	1.2	3.0
Dose multiple (Based on BSA)	0.97x	1.94x	4.86x

BSA = Body surface area; constructed by reviewer from sponsor's data

Observations and Results

Mortality: One group 4 male died on day 1 showing no clinical signs. Histopathology revealed severe myodegeneration and skeletal muscle inflammation.

Clinical Signs: No treatment-related effects.

Body Weights: No treatment-related effects

Food and water Consumption: No treatment-related effects.

Ophthalmoscopy: No treatment-related effects

ECG: Not conducted

Hematology: There were no treatment-related effects..

Coagulation: There were no treatment-related effects.

Clinical Chemistry: There was a significant treatment-related increase in beta globulins in females as from the low dose and a significant decrease in alpha globulins in high dose females.

Urinalysis: There were no treatment-related effects.

Gross Pathology:

Treatment-related macroscopic findings were restricted to the kidneys. Discoloration and enlargement was observed in both kidneys in 3/9 males at the high dose. Discoloration was observed in one high dose female. Similar findings were not observed in male or female recovery animals.

After sacrifice, pelvic dilatation was observed in 2/10 low dose females and 1/9 males in the high dose.

Reddening of the thymus was observed in all treated animals of both sexes at the post-treatment sacrifice and in recovery animals except the controls.

Organ Weights: A significant increase in absolute and/or relative kidney weight occurred in males and females. The increased kidney weight persisted in males from the mid dose (1.2 mmol/kg) upwards

Histopathology: Adequate Battery: Yes

TT 3: Organs selected for weighing and histological examination

Organ	W	Histol. examination					Organ	W	Histol. examination				
		Group							Group				
		K0				R1			K0				R1
		1	2	3	4	1 - 4			1	2	3	4	1 - 4
Liver	yes	P	P	P	P	P	Ovaries	yes	P	Fo	Fo	P	Fo
Kidneys	yes	P	P	P	P	P	Oviducts		P	Fo	Fo	P	Fo
Urinary bladder		P	P	P	P	P	Uterus						
Ureter		P	P	P	P	P	- horns	yes	P	Fo	Fo	P	Fo
Heart	yes	P	Fo	Fo	P	Fo	- corpus		P	Fo	Fo	P	Fo
Atrium		P	Fo	Fo	P	Fo	- cervix		P	Fo	Fo	P	Fo
Lung *	yes	P	Fo	Fo	P	Fo	Vagina		P	Fo	Fo	P	Fo
Aorta (thoracic)		P	Fo	Fo	P	Fo	Mammary glands		P	Fo	Fo	P	Fo
Vein (Vena cava caudalis)		Fo	Fo	Fo	Fo	Fo	Skin (Back-lumb. region)		P	Fo	Fo	P	Fo
Trachea		P	Fo	Fo	P	Fo	Thymus	yes	P	Fo	Fo	P	Fo
Esophagus		P	Fo	Fo	P	Fo	Spleen	yes	P	Fo	Fo	P	Fo
Larynx		P	Fo	Fo	P	Fo	Mandibular lymph node		P	Fo	Fo	P	Fo
Tongue		P	Fo	Fo	P	Fo	Iliac lymph node	yes	Fo	Fo	Fo	Fo	Fo
Salivary glands	yes ¹⁾ yes ¹⁾						Mesenteric lymph node		P	Fo	Fo	P	Fo
- mandibular gland		P	Fo	Fo	P	Fo	Sternum (bone marrow)		P	Fo	Fo	P	Fo
- sublingual gland		P	Fo	Fo	P	Fo	Femur (incl. bone marrow)		P	Fo	Fo	P	Fo
- parotid gland		P	Fo	Fo	P	Fo	Brain	yes					
Stomach		P	Fo	Fo	P	Fo	- cerebrum		P	Fo	Fo	P	Fo
Duodenum		P	Fo	Fo	P	Fo	- cerebellum		P	Fo	Fo	P	Fo
Jejunum		P	Fo	Fo	P	Fo	- medulla oblongata		P	Fo	Fo	P	Fo
Ileum		P	Fo	Fo	P	Fo	Spinal cord						
Caecum		P	Fo	Fo	P	Fo	- cervical		P	Fo	Fo	P	Fo
Colon		P	Fo	Fo	P	Fo	- thoracal		P	Fo	Fo	P	Fo
Rectum		P	Fo	Fo	P	Fo	- lumbal		P	Fo	Fo	P	Fo
Pancreas	yes	P	Fo	Fo	P	Fo	Harderian gland		P	Fo	Fo	P	Fo
Pituitary gland	yes	P	Fo	Fo	P	Fo	Eyes with optical nerve		P	Fo	Fo	P	Fo
Thyroid glands with parathyroid glands	yes	P	Fo	Fo	P	Fo	Periph. nerve (N. Saph.)		P	Fo	Fo	P	Fo
Adrenal glands	yes	P	Fo	Fo	P	Fo	Skeletal muscle		P	Fo	Fo	P	Fo
Testes	yes	P	Fo	Fo	P	Fo	Application site		P	Fo	Fo	P	Fo
Epididymides		P	Fo	Fo	P	Fo	Macroscopic findings		P	P	P	P	P
Prostate	yes	P	Fo	Fo	P	Fo	(if necessary for evaluation a diagnosis)						
Seminal vesicles	yes	P	Fo	Fo	P	Fo							

Organs/Tissues in bold print = paired examination W = weight determination
 * = fixation by instillation P = processed and examined histologically
 Fo = fixation of organ/tissue samples but not processed for histological examination
¹⁾ = weighed together
 K0 = scheduled sacrificed animals at the end of the treatment period
 R1 = animals sacrificed after the subsequent 10-week treatment free recovery period

Peer Review: Yes

Histological Findings:

- Vacuolation of renal tubular epithelium was observed as from the low dose and higher. Some vacuolation was also observed in the urothelium of the ureters and urinary bladder in male and female
- Lesions reversible at the end of the recovery period in all treatment groups
- Single cell necrosis was observed in 3/10 high dose males in addition to vacuolation. The finding was not observed in recovery animals
- Hepatocellular vacuolation occurred in both control and high dose groups, There was no similar finding in females in at the same dose.
- Histopathological examination of the skin revealed no treatment-related findings with no NSF-like skin lesions, namely, dermal thickening, proliferation of dermal fibroblasts, and presence of collagen bundles.
- Electron microscopy of the kidneys in treated-treated animals did not reveal any degenerative or cytotoxic effects on cellular ultrastructure.
- Vacuolation was reversible and evident only in the low dose recovery animals.

Bone marrow: An examination of bone marrow myelograms was not performed.

Special Evaluation: None

Toxicokinetics: PK data is described below and summarized in the following sponsor Tables:

- Maximum serum Gd concentrations occurred on both days (days 1 and 28) at the first sampling time (0.0833h or 5 min)
- There was no gender difference in the serum Gd
- The terminal $t_{1/2}$ of 0.3 h (or 20 min) was independent of dose or day of treatment
- Elimination was rapid resulting in serum levels below the LLOQ (10 $\mu\text{mol/L}$) occurring within 7h of administration in all animals and on both sampling days at all doses except 0.6 mmol/kg
- The systemic exposure (C_{max} and $\text{AUC}_{0.1-3\text{h}}$) increased linearly and proportionally in relation to the dose
- No accumulation of Gd was observed
- Systemic exposure (C_{max} and $\text{AUC}_{0.1-3\text{h}}$) increased linearly and dose proportionally
- Total clearance was 6-7 mL/min/kg and the apparent volume of distribution (V_z) was approximately 0.2 mL/kg for all dose group.

Table 44: Mean Gd levels in serum [$\mu\text{mol/L}$] rats after daily intravenous administration of gadobutrol over 4 weeks

Time[h]	Day 1			Day 28		
	Dose [mmol Gd/kg]					
	0.6	1.2	3.0	0.6	1.2	3.0
0.0833	2820	5749	14550 ¹⁾	2731	6408	14785
0.333	1099	2302	5605	1322	2673	6994
1	208	396	1266	234	454	1577
3	2.28	7.52	15.2 ¹⁾	0	5.77	32.7
7	0	0	0	0	0	0
24	0	0	0	0	0	0

Table 45: Mean pharmacokinetic parameters of Gd in rats after daily intravenous administration of gadobutrol over 4 weeks

Parameters	[unit]	Dose (mmol Gd/kg)					
		0.6		1.2		3.0	
		day 1	day 28	day 1	day 28	day 1	day 28
C_{max}	$[\mu\text{mol/L}]$	2820	2731	5749	6408	14550 ¹⁾	14785
$\text{AUC}_{0.1-3\text{h}}$	$[\mu\text{mol}\cdot\text{h/L}]$	1136	n.e.	2310	2638	6092	7191
$C_{\text{av}, 0.1-3\text{h}}$	$[\mu\text{mol/L}]$	389	n.e.	792	904	2088	2465
$\text{AUC}_{0-\infty}$	$[\mu\text{mol}\cdot\text{h/L}]$	1398	n.e.	2849	3233	7453	8590
Cl	$[\text{mL}/\text{min}/\text{kg}]$	7.15	n.e.	7.02	6.19	6.71	5.82
V_z	$[\text{L}/\text{kg}]$	0.19	n.e.	0.20	0.16	0.18	0.18
R		n.e.		1.14		1.18	
DF (C_{max})		1	1	1.02	1.17	1.03	1.08
DF (AUC)		1	n.e.	1.02	1 ²⁾	1.07	1.09

C_{max} maximum measured concentration of drug in serum after drug administration. C_{max} was observed at the first measurement at 5 minutes post administration.

$\text{AUC}_{0.1-3\text{h}}$ area under the concentration versus time curve from the first sampling time to 3 h

$C_{\text{av}, 0.1-3\text{h}}$ average serum concentration from the first sampling time to 3 h

$\text{AUC}_{0-\infty}$ area under the concentration versus time curve from the dosing time to infinity

Cl clearance rate

V_z volume of distribution during the apparent terminal elimination phase

R accumulation factor; $\text{AUC}_{0.1-3\text{h}, \text{day 28}}/\text{AUC}_{0.1-3\text{h}, \text{day 1}}$

DF Dose proportionality factor; the increase of the systemic exposure (C_{max} , AUC) in relation to the dose increase

n.e. not evaluable

1) the data for animal 145 (female, samples at 5 min and 3 h) were excluded from the mean on day 1, because the values were implausible and could not be reanalyzed due to lack of sample material

2) the dose factor for day 28 was calculated in relation to the 1.2 mmol Gd/kg group, because the $\text{AUC}_{0.1-3\text{h}}$ for the 0.6 mmol Gd/kg group was not available.

Stability and Homogeneity: Acceptable. Based on the Certificate of Analysis.

According to the sponsor, homogeneity testing of the Gadobutrol solution was not performed and the drug product was administered undiluted to test animals. However, drug products, when formulated as solution, were generally recognized to have homogeneity within the batch.

Conclusions: The following conclusions were made based on the findings:

- Repeated administration of gadobutrol for four week at the doses tested was well-tolerated.
- The main finding in this study was renal tubular vacuolation. There were no concomitant degenerative or inflammatory changes identified histologically
- A single male death at the high dose was reported
- Based on the finding of renal tubular vacuolation occurring as from the low dose and higher in both male and female animals, the NOAEL was less than the low dose and could not be determined.

Reviewer's comments: I agree with the findings of this study. However, the significance of the treatment-related increase in beta globulins in females as from the low dose and a significant decrease in alpha globulins in high dose females is not clear.

6.2.2 Repeat-dose Toxicity study in dogs

Three intravenous repeat-dose toxicity studies were conducted in dogs using formulations SH L562 A (Report A031), SH L 562AA (Report SG135) and SH L 562 BB (Report A10548; pivotal study).

6.2.2.1 Report No. A10548:

Study title: Gadobutrol (SH L562BB) - Systemic toxicity study with daily intravenous administration to male and female dogs followed by a subsequent observation period of 8 weeks

Study no.:	TXST20020146
Study report location:	CTD 4.2.3.2.1, pages 1-874
Conducting laboratory and location:	Bayer Pharma Schering, Germany
Date of study initiation:	September 23, 2002
GLP compliance:	Yes (x), no ()
QA statement:	Yes (x), no ()
Drug, lot #, and % purity:	Gadobutrol, batch no. 69713703, %purity: 100%

Key Study Findings:

- Daily intravenous administration of gadobutrol for 4 weeks to dogs appeared well tolerated.
- No adverse effects were observed at the low dose (0.3 mmol Gd/kg). At the mid dose (1.0 mmol Gd/kg) and higher, there was nausea, apathy and increase of heart rate, reddening of visible mucosa and/or inner surface of the ear.
- Effects observed at the mid dose level were reversible in 8 weeks.
- Pharmacokinetics revealed no gender-specific differences.
- Systemic exposure increased linearly over the test dose range.

- There was no Gd accumulation.

Due to the short half-life, exposure to Gd was limited to 3 - 7 hours post-dose.

Methods

Doses: 0, 0.3, 1.0 and 3.0 mmol Gd/kg
 Frequency of dosing: Once/day for 4 weeks (28-31 times)
 Route of administration: Intravenous; 9mL/min
 Dose volume: 1-3 mL
 Formulation/Vehicle: Gadobutrol (SH L562BB)/saline
 Species/Strain: Dog/Beagle
 Number/Sex/Group: 3 or 5/sex/group
 Age: male: 11 months; female: 10 months
 Weight: male: 9.2 – 11.7 kg; female: 7.2 – 9.9 kg
 Satellite groups: none
 Unique study design: none
 Deviation from study protocol: No significant deviations were recorded

Treatment schedule (Report No. A10548)

Table 46: Treatment schedule (A10548)

Group	No./sex	Test/ control article	Dose/day (mmol Gd/kg)	Dose multiples (x-MHD)	Dose vol. (mL)	Conc. mmolGd/L
1 (control)	3M + 3F 2M* + 2F*	0.9% NaCl	0	0	3.0	0
2	3M + 3F	SH L 562BB	0.3	1.62x	0.3	1.0
3	3M + 3F 2M* + 2F*		1.0	5.4x	1.0	1.0
4	3M + 3F		3.0	16.2x	3.0	1.0

. M = male; F = female; * = recovery M/F

Observations and Results

Mortality: No death was reported.

Clinical Signs:

Sporadic nausea and vomiting, limited to the mid dose (1.0 mmol Gd/kg or 5.4x the human dose) were observed in both sexes during treatment period. Similar signs did not occur in control, low and high dose groups.

Reddening of the mucosa, one of the signs of hypersensitivity reactions to drugs in dogs, was observed 1 hr post dose as from day 15 in high dose (3.0 mmol Gd/kg or 16.2-fold the human dose) male and female animals. A similar finding was not reported at lower doses and recovery animals.

Body Weights: No treatment-related effects.

Feed Consumption: No treatment-related effects.

Water Consumption: No treatment-related effects.

Ophthalmoscopy: No treatment-related effects.

ECG and Heart rate: At the high, but not the low or mid doses, a transient increase in heart rate was observed in both sexes after 1 hr of treatment. The finding was no longer present at 24 h. No changes were reported in the ECG.

Nervous system function: Visual neurological examinations revealed changes in single reflexes in individual animals compared to pre-values. Similar findings were observed in control animals.

Blood Pressure: No treatment-related effects.

Hematology: No treatment-related effects were observed.

Coagulation: No treatment-related effects were observed.

Clinical Chemistry: No treatment-related effects were observed.

Urinalysis: A significant increase in specific gravity was observed in mid-dose male and female animals on day 21. A reversible increase in urinary NAG and GGT was observed in mid-dose females on days 3 and 21. The changes were not dose-dependent

Gross Pathology: Discoloration and paleness of the kidneys was observed in all treatment and recovery groups except controls. The effect was not gender-specific. Gross findings were consistent with a histopathological finding of renal tubular vacuolation.

Organ Weights: No treatment-related change in organ weight was observed.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

- Vacuolation of the proximal tubular epithelium in the kidneys, observed the low dose upwards, was not fully reversible in all treatment groups
- In the revised version of the pathology report, no NSF-like skin lesions (thickening of the dermis, proliferation of dermal fibroblasts, presence of collagen) were reported.

Bone marrow: Bone marrow examination was not performed.

Statistical analysis: Statistical analysis was performed as described in the methods .

Special Evaluation: None

Toxicokinetics:

Serum Gd concentrations: Gd serum was determined on days 1 and 28.

In the control group, serum Gd was below the LLOQ of 10 µmol/L. Serum Gd levels in low, mid and high dose groups are shown in the Table below:

Table 47: Mean serum Gd concentrations (+/-SD) after i.v administration of gadobutrol at the low, mid and high dose levels

Timepoint	Group 2 0.3 mmol Gd/kg		Group 3 1.0 mmol Gd/kg		Group 4 1.0 mmol Gd/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
5 min	1731 ± 91.8	1694 ± 183	5215 ± 414	5630 ± 400	16585 ± 673	16612 ± 2166
20 min	1016 ± 62.4	1023 ± 131	3241 ± 122	3240 ± 207	10881 ± 1092	11062 ± 1631
1 h	519 ± 60.7	510 ± 107	1598 ± 153	1573 ± 203	5618 ± 1128	5743 ± 1362
3 h	90.7 ± 23.3	87.3 ± 18.6	232 ± 38.9	230 ± 53.9	978 ± 415	1041 ± 515
7 h	0	0	0	0	20.6 ± 19.9	23.5 ± 21.3
24 h	0	0	0	0	0	0

0 : <LLOQ of 10 µmol/L

Pharmacokinetic parameters: A summary of PK parameters and a Table of PK vales are shown below:

- There was no gender difference in the PK values reported and tabulated results do not reflect any differences between the sexes

- Mean $AUC_{(0.1-3h)}$ indicated that systemic exposure increased dose-linearly. Dose proportionality factors of 0.92 and 1.06 were established for the mid and high doses respectively.
- There was no change in dose proportionality of 0.94 and 1.09 for the mid and high dose groups on day 28

Parameter	0.3 mmol Gd/kg				1.0 mmol Gd/kg				3.0 mmol Gd/kg			
	Day 1		Day 28		Day 1		Day 28		Day 1		Day 28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max} [$\mu\text{mol/L}$]	1731	91.8	1694	183	5215	414	5630	400	16585	673	16612	2166
$DF(C_{max})$	N/A		N/A		0.90		1.00		0.96		0.98	
$C_{av, 0.1-3h}$ [$\mu\text{mol/L}$]	502	43.9	496	78.7	1543	76.5	1548	147	5325	826	5433	1072
$AUC_{0.1-3h}$ [$\mu\text{mol} \times \text{h/L}$]	1465	128	1448	230	4500	223	4517	428	15532	2411	15848	3127
$DF(AUC_{0.1-3h})$	N/A		N/A		0.92		0.94		1.06		1.09	
$R(AUC_{0.1-3h})$			0.98	0.08			1.01	0.11			1.02	0.11

C_{max} Gd-serum concentration measured in the first sample taken after dosing (5 min postdose)
 C_{av} average serum concentration from the first sampling time to 3 h postdose
 $(= AUC(0.1-3h)/\text{time interval})$
 $AUC_{(0.1-3h)}$ area under the concentration-time curve from the first sampling time to 3 h postdose
 $R(AUC_{(0.1-3h)})$ accumulation factor $(= AUC(0.1-3h, \text{day 28})/AUC(0.1-3h, \text{day 1}))$
 $DF(C_{max})$ Dose proportionality factor for C_{max}
 $(= [C_{max, \text{Dose X}}/C_{max, 0.3 \text{ mmol/kg}}] / [D(\text{Dose X})/D(0.3 \text{ mmol/kg})])$
 $DF(AUC_{0.1-3h})$ Dose proportionality factor for $AUC_{0.1-3h}$
 $(= [AUC_{0.1-3h}(\text{Dose X})/AUC_{0.1-3h}(0.3 \text{ mmol Gd/kg})] / [D(\text{Dose X})/D(0.3 \text{ mmol Gd/kg})])$
 N number of animals

Stability and Homogeneity: Acceptable. Based on the Certificate of Analysis.

According to the sponsor, homogeneity testing of the Gadobutrol solution was not performed and the drug product was administered undiluted to test animals. However, drug products, when formulated as solution, were generally recognized to have homogeneity within the batch.

Conclusions:

- Daily intravenous administration of gadobutrol for 4 weeks to dogs appeared well tolerated.
- No adverse effects were observed at the low dose (0.3 mmol Gd/kg). At the mid dose (1.0 mmol Gd/kg) and higher, there was nausea, apathy and increase of heart rate, reddening of visible mucosa and/or inner surface of the ear.
- Effects observed at the mid dose level were reversible in 8 weeks.
- Pharmacokinetics revealed no gender-specific differences.
- Systemic exposure increased linearly over the test dose range.
- There was no accumulation.
- Due to the short half-life, exposure to Gd was limited to 3 - 7 hours post-dose.

Reviewer's comments: I agree with the findings and conclusions

7 Genetic Toxicology

Introduction: Genotoxicity assessment of gadobutrol was performed using *in vitro* bacterial reverse mutation test, chromosomal aberration test in human peripheral blood lymphocytes, the hypoxanthine phosphoribosylguanine transferase (HGPRT) locus in cultured Chinese hamster V79 cells, and in the *in vivo* bone marrow mouse micronucleus test. The SH L562A (0.5 mmol/mL) formulation was used in all studies.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Three reverse mutation studies (9503, 9501 and 9502) of gadobutrol using the direct plate incorporation and the pre-incubation methods were conducted.

7.1.1 Report No. 9503:

Study title: Evaluation of SH L562A (ZK 135079) in the Ames Salmonella/Microsome mutagenicity test

Study no.:	TX91.134
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	June-July, 1991
GLP compliance:	Yes (x), No ()
QA statement:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol batch no. 411710, (SH L 562 A,; batch no. G/094-1B, 0.5 mmol/mL), lot #: N/A; Purity: N/A

Key Study Findings: In the *in vitro* bacterial reverse mutation assay (Ames test) with the direct plate incorporation method, Gadobutrol was not mutagenic in the absence or presence of extrinsic metabolic activation. None of the five tester strains showed an increase in revertant mutant colonies. Growth inhibition of the background lawn was not observed and there were no precipitates in the agar.

Methods

Strains:	<i>Salmonella typhimurium</i> tester strains, TA1535, TA100, TA 1537, TA 1538, TA 98
Concentrations in definitive study:	Direct plate incorporation method with and without metabolic activation: 0.1875, 0.375, 0.75, 1.5, 3.0, 6.0 mg/plate of gadobutrol
Basis of concentration selection:	Concentrations selected were sufficiently high (6000 µg maximum concentration was used), hence no preliminary dose range finding study was conducted.
Negative control:	DMSO (for Test article); Phosphate buffer, DMSO (for positive controls)
Positive control:	Without S9-activation: 9-Acridinamide HCl (100 ug), 2-Nitro-9H-fluorene (10 ug), Sodium Azide (5 ug) With S9-activation: Anthracene-2-amine (2 or 5 ug), Benzo(a)pyrene(10ug), Cyclophosphamide (400 ug)
Formulation/Vehicle:	Gadobutrol; DMSO/Phosphate buffer
Incubation & sampling time:	- Direct plate incorporation test / ca. 72 hrs - 3 replicate plates ± S9-activation - 2 independent assays - Colonies were counted after incubation with test, negative and positive control articles

Study Validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). Positive controls produced expected responses and induced marked increases in revertant colony numbers with all strains. Dose selection for the plate incorporation method was adequate. The S9 concentration was within acceptable limits.

Results:

- At the concentrations tested, there was no increase in revertants in all the five tester strains of *S. typhimurium* tested for mutagenic activity using the direct plate incorporation method. Three replicate plates were evaluated.
- Positive controls with known mutagens induced expected increases in revertant colony numbers with all strains.
- No growth inhibition in the background lawn was observed and there were no precipitates in the presence of the S9 mix.
- There were no cytotoxic or genotoxic effects.

Conclusion: The findings did not indicate that gadobutrol formulation SH L 562A tested up to a concentration of 6000 ug/plate was mutagenic in the bacterial reverse mutation test.

Reviewer's comments: I agree with the sponsor's conclusions.

7.1.2 Report No. 9501:

Study title: Evaluation of SH L562A (ZK 135079) in the Ames Salmonella/Microsome mutagenicity test with preincubation

Study no.: **TX91.135**

Study report location: **Module 4.2.3.3.1; 20 pages**

Conducting laboratory and location: **Bayer Schering Pharma AG, Berlin, Germany**

Date of study initiation: **July, 1991**

GLP compliance: **Yes**

QA statement: **Yes**

Drug, lot #, and % purity: **Gadobutrol batch no. 411710, (SH L 562 A,; batch no. G/094-1B, 0.5 mmol/mL), lot #: N/A; Purity: N/A**

Key Study Findings: Using the preincubation method, Gadobutrol formulation SH L 562A was not mutagenic in the Salmonella/Microsome mutagenicity (Ames) test. Similar to the findings reported in Report 9503, none of the five tester strains (TA1535, TA100, TA 1537, TA 1538, and TA 98) showed a significant increase in revertants under the test conditions for this study. There was no dose-dependent increase in the number of revertant colonies. Positive control chemicals induced marked increases in revertants colonies with all strains tested.

Methods

Strains: *Salmonella typhimurium* tester strains, TA1535, TA100, TA 1537, TA 1538, TA 98

Concentrations in definitive study: 0.1875, 0.375, 0.75, 1.5, 3.0 and 6.0 mg/plate

Basis of concentration selection: Concentrations selected were sufficiently high (6000 µg maximum concentration was used), hence sponsor did not conduct a preliminary dose range finding study.

Negative control: Phosphate buffer (for test article)
Phosphate buffer and DMSO (for positive controls)

Positive controls: 9-Acridinamide HCl; batch no. 191078
Anthracene-2-amine; batch no. 271174
2-Nitrofluorene; batch no. 270175
Benzo(a)pyrene; batch no. 4031497
Sodium azide; batch no. 2238
Cyclophosphamide; batch no. 098492
N-nitrosodimethylamine; batch no. 0427094

Formulation/Vehicle: SH L 562A / Phosphate buffer
Phosphate buffer/DMSO (positive controls)

Incubation & sampling time: - Pre-incubation test /1 hour and ca. 72 hours
- 3 replicate plates ± S-9 activation
- 1 independent assay
- Colonies counted after incubation for test, negative and positive control articles

Report No. 9501: Positive controls

Tester strain	S-9 mix	Positive control	Concentration/plate (µg)
TA1535	-	Sodium azide	5
TA1535	+	Anthracene-2-amine	5
TA1535	+		
TA100	-	Sodium azide	5
TA100	+	N-nitrosodimethylamine	5 ul
TA100	+	Anthracene-2-amine	5
TA100	+	Benzo(a)pyrene	5 or 10
TA 1537	-	9-Acridinamide	60
TA 1537	-	9-Acridinamide	80
TA 1537	+	Anthracene-2-amine	5
TA 1537	+	Benzo(a)pyrene	5 or 10
TA 1538	-	2-Nitro-9H-fluorene	10
TA 1538	+	Anthracene-2-amine	5
TA 1538	+	Benzo(a)pyrene	5 or 10
TA 98	-	2-Nitro-9H-fluorene	10
TA 98	+	Anthracene-2-amine	5
TA 98	+	Benzo(a)pyrene	5 or 10

Study Validity: Selection of bacterial tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). Positive controls produced the expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose (i.e., 5000 µg/plate).

Results: The negative and positive controls with known mutagens produced the expected numbers of revertant colonies. None of the five tester strains used showed increased reversion to prototrophy with gadobutrol (SH L 562A) at the concentrations tested either in the absence or presence of metabolic activation. Growth inhibition of the background lawn was not observed and there were no precipitates in the agar.

Conclusion: The results of the Ames Salmonella/Microsome mutagenicity test using the preincubation method did not indicate that gadobutrol is a mutagen. The findings are in agreement with the results of a similar study using the direct plate method.

Reviewer's comments: The findings of this Ames reverse mutation assay (preincubation method) study evaluating the mutagenic potential of gadobutrol (SH L 562A) agrees with the results of Report 9503 in which the same Salmonella typhimurium strains were used in the direct plate incorporation method. Unlike the direct plate incorporation method, the bacterial suspension, test material and buffer or S9-mix were mixed together and incubated before plating on agar. In the former, the bacterial suspension, test article, buffer or the S9 mix are plated directly on the agar plate prior to incubation.

I agree with the conclusions of Reports 9503 and 9501. Appropriate negative and positive controls were used.

Report No. 9502:

Study title: Evaluation of SH L562A (ZK 135079) in a bacterial mutagenicity test with Escherichia coli, strain WP2uvrA

Study no.:	TX91.103
Study report location:	Module 4.2.3.3.1; 15 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	July 1991
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Gadobutrol (SH L 562 A, 0.5 mmol/mL), lot #: N/A (Purity: N/A)

Key Study Findings: Under the conditions of this study, gadobutrol did not show a positive mutagenic potential in the Ames test using both the direct plate incorporation and the preincubation methods in the presence and absence of metabolic activation.

Methods

Strains: *Escherichia coli*, strain WP2uvrA

Concentrations in definitive study: **Assay #1 (direct plate incorporation test):** 0.303, 0.61, 1.21, 2.4, 4.8, 10.0 mg/plate
Assay #2 (pre-incubation test): 0.1894, 0.3788, 0.7575, 1.515, 3.03, 6.06 mg/plate

Basis of concentration selection: Concentrations selected were sufficiently high (at least 6000 µg maximum concentration was used), hence sponsor did not conduct a preliminary dose range finding study.

Negative control: Phosphate buffer (for test article)
 Phosphate buffer and DMSO (for positive Controls)

Positive controls: Anthracene-2-amine; batch no. 271174; Ethyl methanesulfonate; batch no. 81003; N-methyl-N1-nitro-nitrosoguanidine, batch no. 026F36701

Formulation/Vehicle: SH L 562A/ Phosphate buffer
 Phosphate buffer/DMSO (positive controls)

Incubation & sampling time: - Direct plate incorporation test: 48-72 hrs
 - Pre-incubation test: 1hr and 48-72 hrs at 37°C
 - Pre-incubation test 1 hr and ca. 72 hours
 - 3 replicate plates ± S-9 activation
 - 1 independent assay
 - Colonies counted after incubation for test, negative and positive control articles

Report No. 9502: Positive controls for Ames test using E.coli strain WP2uvrA

S-9 mix	Positive control	Conc./plate (µg or ul)	
		Assay #1	Assay #2
-	Ethyl methanesulfonate	5 ul	2 ul
+	Anthracene-2-amine	10 ug	20 ug
+	N-methyl-N1-nitro-nitrosoguanidine	5 ug	ND
ND = no data			

Study Validity: Based on Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996), selection of bacterial tester strains was adequate based upon. Positive controls produced the expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose (i.e., 5000 µg/plate).

Results: Escherichia coli strain WP2uvrA did not show increased reversion to prototrophy in assays with gadobutrol at the concentrations tested in the presence of extrinsic metabolic activation (S9 mix) using the direct plate incorporation and the pre-incubation methods. Growth was not observed in the background lawn and no precipitates were observed in the agar. Negative controls and positive controls produced the expected increase in revertant colonies.

Reviewer's comment: I agree with the findings of this assay.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Summary: Two independent *in-vitro* chromosomal aberration studies (Reports 9616 and 9972) were submitted. The effect of gadobutrol (SH L 562 A) on chromosome number and structure were determined in human lymphocytes in the presence or in the absence of a metabolic activation system. Gadobutrol was tested at the highest concentration of 6000 ug/mL. The duration of treatment was 20 and 27 hours without the metabolic activation system and 3, 17 and 24 h in the presence of metabolic activation. Results showed no increase in frequency of emergence of chromosomal aberrations in either study. The positive controls produced expected results in both studies.

7.2.1 Report No. 9972:

Study title: Evaluation of the clastogenic potential of SH L562A in human peripheral blood lymphocytes

Study no.:	TX91.213
Study report location:	Module 4.2.3.3.1; 23 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, Germany
Date of study initiation:	Studies were conducted in September, September, 1991
GLP compliance:	Yes (x), no ()
QA statement:	Yes (x), no ()
Drug, lot #, and % purity:	Gadobutrol/ZK135079 (SH L562A), lot # and % purity: N/A

Key Study Findings: The findings of this study did not indicate any clastogenic potential of SH L562A in human peripheral blood lymphocytes *in vitro* without or with an extrinsic metabolizing system. SH L562A was tested up to the highest recommended concentration level of 2% (v/v) or approximately 6.05 mg ZK135079/ml.

Methods: In vitro Clastogenicity testing

Cell line: Human peripheral blood lymphocytes (HPBL)

Concentrations in definitive study: **Without S9:**
20 h (first harvest): 0.61, 3.03 and 6.05 mg/mL
27 h (second harvest): 3.03 and 6.05 mg/mL
With S9:
3h + 17h recovery: 0.61, 3.03 and 6.05 mg/mL
3h + 24h recovery: 4.54 and 6.05 mg/mL.

Basis of concentration selection: Solubility in McCoy's 5a culture medium and found to be soluble at all concentrations tested (maximum concentration was approximately 6000 ug/mL)

Negative control: Water

Positive control: Triaziquone (ZK 68301; batch No. PT 170809)
Cyclophosphamide (ZK 56889, batch No. 050504). The controls are consistent with FDA/CFSAN Redbook recommendations)

Formulation/Vehicle: Gadobutrol (SH L 562 A), Mc Coy's culture medium

Incubation & sampling time: **Without activation:**
20 and 27 h
With activation:
3h followed by 17h recovery
3h followed by 24h recovery

Methods:

- Blood (lymphocyte) cultures were prepared in duplicate and incubated at 37°C, 5% CO₂.
- Assays were performed without and with S-9 mix at concentrations up to 6 mg/mL.
- For the evaluation of chromosomal aberrations, 100 cells per replicate were scored i.e., 200 cells per concentration level except for the positive control in which only 100 cells were scored to prove the positive response.
- For cultured treated without S9, there was continuous treatment for 20 and 27 h. In the presence of S9, cultures were treated for 4h with either 17h or 24h recovery.
- All metaphase spreads were examined for both chromatid and chromosome aberrations (achromatic lesions, breaks, acentric fragments, deletions, and exchange figures).
- The clastogenic potential of gadobutrol was evaluated by calculating the breakage rate and the percentage of aberrant cells. A break incidence of up to 3% was classified as a negative response especially if a dose-dependent response did not exist.

Study Validity: The study was considered valid for the following reasons:

- Appropriate positive controls were employed according to FDA/CFSAN Redbook guidelines and produced expected results
- Cells were evaluated in replicates.
- Concentrations tested were in accordance with current practice
- The counting method was in compliance with the currently accepted procedure and therefore considered valid.
- A test article induced a positive response when the percentage of cells with aberrations was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group.
- The conditions of the assays were appropriate given the use of the limit dose of 4 hr incubations and toxicity measured in the 20 hr incubation (FDA/CFSAN Redbook guidelines).
- The dose selection based upon mitotic index was acceptable.

Results: The results of the study are summarized in the following Tables:

Table 48: Report 9972 - Chromosome aberration test performed without metabolic activation

Metabolic activation	Treatment Period (h)	Test article	Concentration (mg/mL)	Polyploidy cells (%)	Aberrant Cells (%)
No Activation	20	Medium control	0	1	0
		Gadobutrol	0.61	0	1
			3.03	0	0.5
			6.05	2	0
	27	Triaziquone	1×10^{-7} M	ND	20*
		Medium control	0	1	0
		Gadobutrol	3.03	0	1
			6.05	1	0.5

Reviewer's Table based on sponsor's summary Table; * = $p < 0.05$ compared to concurrent solvent control; ND = not evaluated; Gadobutrol was tested at the highest recommended concentration of 1×10^{-2} M (ca. 6.05 mg/mL)

Table 49: Report 9972 - Chromosome aberration test performed with metabolic activation

Metabolic activation	Treatment Period (h)	Test article	Concentration (mg/mL)	Polyploidy cells (%)	Aberrant Cells (%)
With Activation	3h + 17 h recovery	Medium control	0	0	0
		Gadobutrol	0.61	1	1.0
			3.03	1	0
			6.05	2	1.5
	3h + 24h recovery	Cyclophosphamide	2×10^{-5} M	ND	21*
		Medium control	0	3	0.5
		Gadobutrol	4.54	1	1.0
			6.05	2	0

Reviewer's Table based on sponsor's summary Table; * = $p < 0.05$ compared to concurrent solvent control; ND = not evaluated; Gadobutrol was tested at the highest recommended concentration of 1×10^{-2} M (ca. 6.05 mg/mL)

Conclusions:

- In the absence of metabolic activation, the solvent and positive control values were within the expected range.
- There was no increase in the percentage of aberrant cells in the blood cultures treated with the concentrations of gadobutrol tested at the 20 and 27 hour harvest times.
- No statistical analysis was performed since the result was negative. Triaziquone was clastogenic.
- In the presence of metabolic activation, the solvent and positive control values were within the expected range.
- There was no increase in the percentage of aberrant cells in the cultures treated with gadobutrol at the concentrations tested as compared to the solvent control.
- At both harvesting times, the mitotic indices did not show a reduction when compared to the concurrent vehicle control.
- No analysis was performed on the negative results. The positive control Cyclophosphamide was clastogenic.
- There was no increase in the frequency of chromosomal aberration in this study and in the parallel study report 9616 (not reviewed).

Reviewer's comment: I agree with conclusions

7.2.2 Report No. 9592:**Study title: SH L 562 A (ZK 135079) - Evaluation of gene mutations in mammalian cells in culture: HGPRT-test with V79 cells**

Study no.: TX91.136, TX 91.137
Study report location: Module 4.2.3.3.1; 25 pages
Conducting laboratory and location: Bayer Schering Pharma AG, Berlin, FR Germany
Date of study initiation: July, 1991
GLP compliance: Yes (x), no ()
QA statement: Yes (x), no ()
Drug, lot #, and % purity: Gadobutrol (batch No. 411710)/ZK135079 (batch No. G/094-1B) (SH L562A), , lot # and % purity: N/A

Key Study Findings:

- Cells used in each of two assays were exposed for 4 h to gadobutrol (SH L 562 A) at concentrations ranging from 0.3 – 6 mg/mL.
- Negative and positive controls with known mutagens were tested in the absence and in the presence of S9 mix and produced expected range of mutant colonies.
- Compared to negative controls, the assays did not show an increase in mutant colonies in the cultures treated with gadobutrol at the highest recommended dose of 10^{-2} M.
- The HGPRT assay did not indicate any mutagenic potential in the in vitro HGPRT gene mutation test using Chinese hamster V79 cell line with or without extrinsic metabolic activation.

Methods:

Cell line: Cultured Chinese hamster V79
Concentrations in definitive study: Assays 1 and 2: 0.3, 0.6, 1.51, 3.02, 6.05 mg/mL
Basis of concentration selection: Standard criteria for dose selection as described in methods below
Negative control: 1% DMSO
Positive control: Ethyl methanesulfonate (ZK 77.522, batch no. 81003); 7,12-Dimethylbenz(a)anthracene (ZK 30.923, batch no. 1685EL); (The controls are consistent with FDA/CFSAN Redbook recommendations); Vehicles: Minimum Essential Medium (MEM), Dimethyl Sulfoxide (DMSO)
Formulation/Vehicle: Gadobutrol (SH L 562 A), MEM
Incubation & sampling time: Pulse treatment for 4h without or with S9 mix

Methods: V79 cell line cultures were propagated at 37°C and 5%CO₂. Doses were selected based on the criteria that the highest dose evaluated should be toxic, causing a reduction in plating efficiency (cell survival) or should correspond to the substance's

solubility limit (i.e. produce a precipitation in the culture). Five doses up to 10^{-2} M were tested for each assay namely, 0.3, 0.6, 1.51, 3.02, 6.05 mg/mL. The negative controls were untreated cultures and cell DMSO-treated cultures.

Study Validity: For negative controls, the spontaneous mutation rate should range below 40 mutants per 10^6 cells. Positive controls should cause an approximately 10-fold or greater increase in mutation frequency. The test substance was classified as mutagenic if it induced reproducibly at one of the test concentrations a mutation frequency 3-fold higher than the spontaneous mutant frequency in the experiment.

Results and Conclusion: No cytotoxicity was observed with gadobutrol up to the highest concentration tested (10^{-2} M) with or without metabolic activation (S9 mix). A high level of cytotoxicity was observed with the positive controls, Ethyl methane sulfonate (EMS) and N-Methyl-N-nitro-N-nitrosoguanidine (MNNG).

Gadobutrol did not show a mutagenic potential in the HGPRT/V79 mammalian cell gene mutation test whether in the absence or in the presence of rat liver S9 mix in two independent assays up to the highest recommended dose of 10^{-2} M. The results are shown in the following Tables:

Table 50: Mammalian cell gene mutation test (HGPRT/V79 cell line) - Assay 1

Assay 1	Test article	Concentration (mg/mL)	Mutant colonies (per 10^6 cells)	
			Without Metabolic Activation	With Metabolic Activation
	Medium control	0	5	1
	Dimethyl sulfoxide	0 (1%)	contaminated	2
	Vehicle control	0 (2%)	10	n.d.
	Gadobutrol	0.3	6	1
		0.6	10	3
		1.51	13	1
		3.02	13	2
		6.05	10	5
	N-Methyl-N-nitro-N-nitrosoguanidine (MNNG)	0.2 ug/mL	1260	n.d.
	7,12-Dimethylbenz[a]anthracene	15 ug/mL	n.d.	183

Reviewer's Table adapted from sponsor's summary Table; Values are means of 2 replicate cultures; n.d = not evaluated; Gadobutrol was tested up to the highest recommended concentration of 1×10^{-2} M (or 6.05 mg/mL)

Table 51: Mammalian cell gene mutation test (HGPRT/V79 cell line)- Assay 2

Assay 2	Test article	Concentration (mg/mL)	Mutant colonies (per 10 ⁶ cells)	
			Without Metabolic Activation	With Metabolic Activation
	Medium control	0	5	3
	Dimethyl sulfoxide	0 (1%)	3	4
	Vehicle control	0 (2%)	n.d	2
	Gadobutrol	0.3	1	4
		0.6	2	4
		1.51	2	2
		3.02	1	3
		6.05	2	1
	Ethyl methane sulfonate (EMS)	0.2 ug/mL	239	n.d
	7,12-Dimethylbenz[a]anthracene	15 ug/mL	n.d	145

Reviewer's Table adapted from sponsor's summary Table; Values are means of 2 replicate cultures; n.d = not evaluated; Gadobutrol was tested up to the highest recommended concentration of 1×10^{-2} M (or 6.05 mg/mL)

Reviewer's comment: I agree with the findings and conclusions.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

7.3.1 Report No. 9823

Study title: Studies on the mutagenic potential of SH L562A in the mouse
Microsome test

Study no.:	TX 91.173
Study report location:	Module 4.2.3.3.2, 16 pages
Conducting laboratory and location:	Bayer Schering Pharma AG, Berlin, FR Germany
Date of study initiation:	August, 1991 (Date of dosing)
GLP compliance:	Yes (x), no ()
QA statement:	Yes (x), no ()
Drug, lot #, and % purity:	Gadobutrol (SH L562 A; batch No. 411710)/ZK135079 (batch No. G/094-1B) (SH L562A), , lot # : N/A, % purity: N/A

Key Study Findings:

- Gadobutrol was negative in the *in vivo* mouse bone marrow micronucleus test.
- The positive control, Triaziquone, gave the expected increase in the micronucleated polychromatic or normochromatic erythrocytes in male and female mice.

Methods

Doses in definitive study: Gadobutrol: 2, 4 or 8 mmol/kg
Saline: 16 mL/kg
Frequency of dosing: Once
Route of administration: Intravenous
Dose volume: 0.2 mL/min
Formulation/Vehicle: Gadobutrol (SH L 562 A)/0.9% (w/v) NaCl solution
Species/Strain: Mice/NMRI (9-10 weeks)
Number/Sex/Group: 5/sex/dose group per time point
Satellite groups: None
Basis of dose selection: Range finding study using 2 acute toxicity studies in male and female mice at 50 mL/kg
Negative control: Physiological saline
Positive control: Triaziquone (ZK 68.301), batch no. PT 170809; 0.15 mg/mL (10 mL/kg, single i.p administration)

Study Validity: Range finding study using 2 acute toxicity studies (Reports 9345 and 9346) in male and female mice at 50 mL/kg showed weak signs of toxicity. The highest dose of gadobutrol tested (8 mmol/kg), was in accordance with CFSAN Redbook 2000, OECD recommendations.

The study was deemed valid for the following reasons:

- 1) previous pharmacokinetic assessments demonstrated systemic exposure,
- 2) dosing appeared to be adequate based upon the results of the dose-ranging study,
- 3) preparation and administration of the test substance was acceptable,
- 4) the species and number of animals/sex/group were acceptable,
- 5) tissue sampling and analysis was acceptable,
- 6) positive controls exhibited appropriate responses

Exposure conditions: Five mice per sex from each of the negative control and the gadobutrol groups were sacrificed at 24, 48 and 72h after treatment. Positive control animals were sacrificed 24h after treatment.

Analysis:

- Number of replicates: Not applicable
- Sampling time: Bone marrow sampling took place at 24, 48 and 72 h after dosing
- Cells evaluated: Polychromatic (PCE) and Normochromatic (NCE) erythrocytes in bone marrow
- Counting method: The bone marrow from the femur was flushed and smears were taken on slides. 2000 polychromatic erythrocytes (PCE) per animal were then evaluated for incidence of micronuclei and 1000 red blood cells per animal were counted for determination of the ratio of polychromatic to all erythrocytes.

- Criteria for positive results: The following criteria were established for a positive response: a statistically significant result ($p < 0.05$) for which the median number of micronucleated polychromatic erythrocytes per 2000 cells evaluated should not be less than 5 or 3 for 24 hour and 48 hour sampling, respectively.
- Statistical evaluation: Statistical evaluation was conducted for proportion of micronucleated PCE or NCE and for PCE/NCE ratio..

Results

- Male and female animals responded similarly to the administration of the test substance
- No sign of toxicity was observed at the high dose (8 mmol/kg) of gadobutrol. However, one animal at the lowest dose (2 mmolGd/kg) of gadobutrol died 2 days post administration
- The Table below summarizes the result of the in vivo mouse micronucleus assay
- No positive responses were observed following the administration of gadobutrol at any of the three doses tested at 24, 48 or 72h
- PCE and NCE counts and PCE/NCE ratio were not statistically significant
- The positive control compound, Triaziquone, produced the expected increase in the micronucleated polychromatic cell counts

Table 52: Results - In vivo mouse bone marrow micronucleus assay

Report No.:	9823	Study No.:	TX 91.173	Location	Module:	4.2.3.3.2	
Test Article:	Gadobutrol						
Report Title:	Studies on the mutagenic potential of SH L562A in the mouse micronucleus test						
Test Article	Dose [mmol/kg]	Route of administration	Sampling time [hours]	No. of Animals	MPCE(%) [Mean \pm S.D.]	MNCE(%) [Mean \pm S.D.]	PCE/NCE Ratio [Mean \pm S.D.]
Vehicle control	0 (16 mL/kg)	intravenous	24	5M/5F	0.95 \pm 0.28	0.80 \pm 0.42	1.10 \pm 0.08
Gadobutrol	2	intravenous	24	5M/5F	1.15 \pm 0.67	0.60 \pm 0.70	1.10 \pm 0.07
	4	intravenous	24	5M/5F	0.95 \pm 0.44	1.10 \pm 0.57	1.12 \pm 0.05
	8	intravenous	24	5M/5F	0.95 \pm 0.44	0.90 \pm 0.74	1.13 \pm 0.06
Vehicle control	0 (16 mL/kg)	intravenous	48	5M/5F	0.75 \pm 0.42	0.60 \pm 0.97	1.10 \pm 0.03
Gadobutrol	2	intravenous	48	5M/5F	1.11 \pm 0.49	0.67 \pm 0.50	1.10 \pm 0.05
	4	intravenous	48	5M/5F	1.00 \pm 0.33	0.60 \pm 0.52	1.09 \pm 0.06
	8	intravenous	48	5M/5F	0.90 \pm 0.21	0.80 \pm 0.63	1.10 \pm 0.05
Vehicle control	0 (16 mL/kg)	intravenous	72	5M/5F	0.95 \pm 0.37	1.00 \pm 0.67	1.08 \pm 0.06
Gadobutrol	2	intravenous	72	5M/5F	1.10 \pm 0.46	0.50 \pm 0.71	1.08 \pm 0.05
	4	intravenous	72	5M/5F	0.85 \pm 0.34	0.70 \pm 0.48	1.08 \pm 0.04
	8	intravenous	72	5M/5F	0.80 \pm 0.26	0.70 \pm 0.67	1.12 \pm 0.06
Triaziquone (positive control)	0.15 mg/kg	intraperitoneal	24	5M/5F	12.15* \pm 1.47	0.60 \pm 0.52	0.83* \pm 0.03

MPCE: micronucleated polychromatic erythrocytes

MNCE: micronucleated normochromatic erythrocytes

* $p < 0.05$, ANOVA for each variable independently, values of MPCE and MNCE were arcsin-transformed
SH L562A (0.5 mol/L Gadobutrol)

Conclusions:

- There were no signs of toxicity or bone marrow depression in the animals studied in this assay
- Increased numbers of polychromatic red blood cells with micronuclei were observed in
- mice dosed with Triaziquone.
- Mice administered gadobutrol did not show induction of micronuclei irrespective of the time of collection, dose, or sex.

Reviewer's comments: One female animal in the low dose group died. The time and circumstance of death was not described. Although this death was reported, 5F animals were still reported in the low dose group in the Table of results. The significance of this death is not clear to the reviewer.

Overall, I agree with the findings and conclusions.

7.4 Other Genetic Toxicity Studies

None

8 Reproductive and Developmental Toxicology**9.1 Fertility and Early Embryonic Development****Report No. A39049**

Study title: Gadobutrol (SH L562BB) - Study of fertility and early embryonic development to implantation in the rat after daily intravenous (i.v.) administration

Study no.: TXST20070103

Study report location: Module 4.2.3.5.1

Conducting laboratory and location: Bayer Schering Pharma, 13353 Berlin, Germany

Date of study initiation: July 05, 2007

GLP compliance: Yes (OECD GLP; Chemical Laws, Germany); signed 01/27/2009

QA statement: Yes; signed 01/28/2009

Drug, lot #, and % purity: Gadobutrol (formulation: SH 562BB), lot #: not provided, %purity: not provided

Key Study Findings: Gadobutrol (SH L562BB; 1.0 mmol/L) was administered i.v to male and female rats in daily doses of 0.6, 2.2 and 7.5 mmolGd/kg. Over a period of 4

weeks prior to mating, during mating and in the postmating period, males received at least 43 consecutive doses. Females received at least 23 consecutive doses in the 2-week premating period, during the mating period lasting about 2 weeks and through day 7 of presumed gestation.

In the toxicokinetic study, there was a dose-dependent increase in AUC (0-24h) and C_{max} on days 1, 22 and 43. On day 1, a less than dose-proportional increase in AUC(0-24h) and C_{max} was observed in the increase to 2.2 mmolGd/kg but more than dose-proportionally when increasing from 2.2 to 7.5 mmolGd/kg.

The main findings from this study are summarized by the following NOAELs:

Parameters	NOAEL (mmolGd/kg/day)
Systemic Toxicity	2.2 (3.6x-MHD)
Reproductive Performance	7.5 (12.2x-MHD)

Methods

Doses: 0 (vehicle), 0.6, 2.2 and 7.5 mmolGd/kg Gadobutrol
 Frequency of dosing: Once/day for 4 weeks
 Dose volume: 0.6 - 7.5 mL/kg
 Route of administration: Intravenous
 Formulation/Vehicle: Gadobutrol (SH L562BB, 1 mmol Gd/mL) / 0.9 % NaCl
 Species/Strain: Rat/CrlGlxBrl Han: WI SPF; (b) (4)
 Number/Sex/Group: Groups 1, 2 and 3 (20/sex); Group 4 (20M+22F)
 Satellite groups: 6M per group
 Age: Males: sexually mature males were at least 8 weeks old at the beginning of treatment. Exact age was not provided.
 Females: virgin, sexually mature females were at least 10 weeks old at the beginning of treatment. Exact age was not provided.
 Body weight: males: 217-257 g; females: 148-176 g at receipt
 Study design: The study design is described in Table 1 below
 Deviation from study protocol: Deviations were described in Section 5.2.5 of the study report
 Notes: M = males; F = females

Dose selection: The doses used in this study were based on the findings of three studies in rats:

- 1). Fertility study (SG124) using gadobutrol formulation SH 562A (0.5 mmol/mL)
- 2). Repeat-dose toxicity study A08936 with SH L562BB (1.0 mmol/mL) and
- 3). Orienting study (PH 35165) of the effect of SH L562BB on pre- and postnatal development including maternal function.

The doses and NOAELs in these studies are summarized in the Table below:

Table 53: Doses in range-finding studies

Study	Type of Study	Doses			NOAEL (mmolGd/kg)
		Low	Mid	High	
SG124	8-day repeat-dose Fertility study	0.25	0.75	2.5	2.5
A08936	4-week repeat-dose Toxicity study	0.6	1.2	3.0	M (1.2); F (3.0)
PH 35165	Pre/postnatal development study	5.0	7.5	10.0	5.0

Study Design: In a study designed to evaluate the toxicity of Gadobutrol (formulation SH L562BB, 1.0 mmol/mL) on fertility and early embryonic development to implantation in the rat after repeated daily intravenous administration, animals were randomized to one of four main study groups and received gadobutrol (SH L562BB; 0.6, 2.2, or 7.5 mmolGd/kg) or physiological saline, 0.9 % NaCl (7.5 mL/kg; control group) via the tail vein. Males were treated for 4 weeks before the start of cohabitation (day 1-29), through the cohabitation period until the day prior to necropsy, receiving at least 43 consecutive daily doses. Females were treated for 14 days before the start of cohabitation, through the subsequent mating period lasting up to 15 days and until day 7 of presumed gestation (G7). Females received at least 23 consecutive daily doses, depending on successful mating.

Table 54: Study Design (main and satellite groups) – Report A39049

Group	Number of animals/sex	Test/control article	Dose/day mmol Gd/kg	Dose multiple (by BSA)	Dose volume (mL/kg)
1 (control)	20M + 20F 6M *	Control (0.9% NaCl)	0	0	7.5
2	20M + 20F 6M*	SH L 562BB	0.6	1.0x	0.6
3	20M + 20F 6M *	SH L 562BB	2.2	3.6x	2.2
4	20M + 22F [#] 6M *	SH L 562BB	7.5	12.2x	7.5

M = male; F = female; * = Satellite animals for determination of active substance levels; [#] 2 female animals were added to this group due to mortality in two female rats on the first day of treatment.

Observations and Results: Survival, clinical signs, body weight and food consumption were recorded in all animals in the premating, mating and postmating and gestation period. Details are provided in the respective sections.

Mortality: All animals survived to scheduled sacrifice except two females in the high dose group that died on treatment day 1. The deaths, preceded by clonic-tonic seizures and respiratory distress, were treatment-related. NOAEL for mortality was 2.2 mmolGd/kg (or 3.6x-MHD).

Clinical Signs: Daily clinical observations were performed.

Results: In males, no clinical signs were observed up to the high dose of 7.5 mmolGd/kg (12.2-fold the intended human dose based on BSA). Local intolerance at the injection site occurred in animals in the control and all treatment groups and manifested as scab formation, skin reddening or brown discoloration of tail skin. Local intolerance, also noted in the controls, and observed as injuries and tail wounds at the mid dose (2.2 mmolGd/kg). Reddened skin was observed in females at the high dose (7.5 mmolGd/kg).

Body Weight: In males, body weight during the pre mating, mating or post mating periods was calculated as group means. In females, body weight was recorded during pre mating on days 1, 4, 8, 11 and 15; on all days of the mating period; on gestation days 0-7, 9, 12 and 15 and the day of sacrifice. Gain in body weight was calculated for the different periods based on the individual absolute body weight.

Results: There was no drug-related effect on body weight or body weight gain in both sexes up to 7.5 mmolGd/kg.

Feed Consumption: In females, food consumption was recorded for the periods between days 1-8 and 8-15 of the pre mating period and for the periods between days 0-3, 3-6, 6-9, and 9-12 of the gestation period.

Results: There were no remarkable effects on food consumption up to the high dose in males and females.

Estrus, Mating and Mating Performance

Estrus: In all females, the estrus cycle was monitored 14 days before and 15 days after commencing treatment.

Mating: In the afternoon of day 15 of treatment, females were caged overnight with male partners. They were examined for vaginal plugs or sperm in the vaginal smears in the morning. This day was designated day 0 of gestation (day 0 p.c.) if there was evidence of successful mating. In the event of successful mating, the mated females were removed and housed individually. This procedure was repeated each day for a maximum of 15 days until there was evidence of a successful mating in individual females. For females without evidence of mating during the whole mating period, the last day of the mating period was designated as day 0 p.c. in order to determine the day of sacrifice.

Results: No effect of treatment on mating performance was observed in animals of both sexes in any study group. Mating occurred within the first four days and up to day 15 of mating period.

Toxicokinetics: A validated ICP-MS method was used in determining plasma concentrations of Gadolinium in satellite rats. Blood samples were collected days 1, 22 and 43 at 24h on day 1. On days 22 and 43, samples were collected at predose and at 0.083, 3 and 7 h post treatment. Exposure (AUC) was evaluated with regard to dependence on dose, concentration vs. time course within the dosing interval and repeated administration.

Results: There was a dose-dependent increase in AUC (0-24h) and Cmax on days 1, 22 and 43. On day 1, a less than dose-proportional increase in AUC (0-24h) and Cmax was observed in the increase to 2.2 mmolGd/kg but more than dose-proportionally when increasing from 2.2 to 7.5 mmolGd/kg. On Day 22 and 43 following repeated dosing, the increase in AUC(0-24h) and Cmax was comparable to the values of these parameters on Day 1 in the low and high doses. For 2.2 mmolGd/kg dose group, a higher exposure was observed compared to Day 1. Plasma concentration occurred at 0.083 h (5 min) after administration followed by a rapid decrease. On all sampling days, Gd concentration was between 0.007 and 0.05 % of Cmax.

Table 55: Summary of AUC(0-24) and Cmax values (Report A39049)

TK Parameter	Sampling time (days)	Doses (mmolGd/kg)		
		LD	MD	HD
		0.6	2.2	7.5
AUC (0-24) $\mu\text{mol} \times \text{h/L}$	1	1126	3131	15218
	22	1414	5455	16688
	43	1404	6562	19013
Cmax ($\mu\text{mol/L}$)	1	2070	6079	25506
	22	2503	10216	24556
	43	2613	8706	28715

Reviewer's Table adapted from sponsor's Table

Dosing Solution Analysis: Test solutions were used as provided to the conducting laboratory by the sponsor.

Necropsy: Group incidences/percentages of parental necropsy observations were reported.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Animals were sacrificed by excess CO₂ inhalation. Dams were sacrificed between days 15 and 18 post-coitum. Males of all groups were sacrificed on days 44-46. All animals were examined macroscopically for pathological changes. Parental necropsy observations were described as group incidences and group percentages. Kidney weights and reproduction parameters were evaluated in animals in the main groups. The number of corpora lutea, the number and uterine position of implantation sites, viable and dead fetuses and early and late resorptions were recorded. The placentae

were examined macroscopically. Absolute and relative kidney weights were determined for all animals in the main study groups.

Mating data:

Percentage of females per group with sperm in the vaginal smear on days 1-4, 5-8 and 9-15 of the mating period

Insemination index (number of inseminated animals as percentage of the number of mated females per group)

Pregnancy index (number of pregnant animals as percentage of the number of mated females per group)

Fertility index (number of pregnant animals as percentage of the number of inseminated females).

Reproduction data: Group means of corpora lutea, implantations, fetuses (total, viable and dead), resorptions and postimplantation loss per dam. Percentages of preimplantational loss (corpora lutea minus implantations), of resorptions (early/late) and dead fetuses (based on the number of implantations) postimplantational loss (resorptions and dead fetuses) per group. Group medians and quartiles of percentages of preimplantational loss, dead fetuses, resorptions and postimplantational loss per dam. Percentages of viable/dead fetuses and early/late resorptions per group

Results: In males, there were no macroscopic findings at the low dose. A dose-dependent and treatment-related enlargement of the kidney was observed at ≥ 2.2 mmolGd/kg. Injection site necrosis was observed in control and high dose animals. A liver enlargement and red nodule in the lung were observed at the low dose. The findings were not dose-dependent. There were no treatment-related gross changes noted up to the mid dose in female animals. The enlargement of the kidneys in two dams of the high dose group was treatment-related. A treatment-related necrosis at the injection site occurred in one low-dose female was not dose-dependent.

Table 56: Mating Data (Report A39049)

PARAMETER	TREATMENT GROUPS (Treated prior to mating through day 7 of presumed gestation)			
	Doses (mmol Gd/kg)			
	1 0.9 % NaCl	2 0.6	2 2.2	4 7.5
Premating Period				
(M/F Assigned) - No. of females at start	20/20	20/20	20/20	20/22
No. of females dead/sacrificed in premating period	0	0	0	2
No. of days prior to mating	2	2	3	2
Mating Period				
No. of females at start of mating period	20	20	20	20

No. of females Sperm positive	20	19	20	18
Gestation Period				
No. of females with defined day 0 p.c during gestation	20	19	20	18
No. aborted/premature delivery	0	1	0	0
No. of nonpregnant females	0	0	1	0
No. of pregnant females	20	18	19	18

Table 57: Reproduction Parameters (Study No. A39049)

PARAMETER	TREATMENT GROUPS (Treated prior to mating through day 7 of presumed gestation)			
	(1)	(2)	(3)	(4)
	0.9 % NaCl	0.6 mmol Gd/kg	2.2 mmolGd/kg	7.5 mmolGd/kg
M/F assigned	20/20	20/20	20/20	20/22
Dam Deaths	0	0	0	2
No. Pregnant	20	18	19	18
Total No. Fetuses	209	201	218	193
Total Live Fetuses	208	201	218	193
% Live Fetuses	99.52	100.0	100.0	100.0
% Dead Fetuses	0.48	0.0	0.0	0.0
Live Litters				
Mean Litter Corpora Lutea	12.5	13.5	13.1	12.6
Mean Litter Implants	10.9	11.8	12.1	11.4
Mean Litter Resorption				
%Total Resorption	3.69	5.19	5.22	5.85
%Early Resorption	100.0	90.91	100.0	100.0
%Late Resorption	0.00	9.09	0.00	0.00
Mean Fetal Death				
Mean Litter Live Pups				
Pre-implantation Loss (% CL per group)	12.9	12.8	7.3	9.7
Post-implantation Loss (%)	9.0	11.0	12.0	12.0
CL=corpora lutea				

Conclusions

Based on findings of this study, the NOAEL for systemic toxicity was 2.2 (or 3.6x-MHD mmolGd/kg/day. NOAEL for reproductive performance was 7.5 mmolGd/kg/day (or 12.2x-MHD).

Reviewer's comment: Agree.

9.2 Embryonic Fetal Development

Report A34150

Study title: Gadobutrol (SH L562BB) - Study for effects on embryo-fetal development in rats after daily intravenous administration from days 6 to 17 of gestation

Study no.: **TXST20060144**
Study report location: **Module 4.2.3.5.2**
Conducting laboratory and location: **Bayer Schering Pharma, 13353 Berlin, Germany**
Date of study initiation: **July 20, 2006**
GLP compliance: **Yes (OECD GLP; Chemicals Law, Germany); signed 3/19/2009**
QA statement: **Yes; signed 03/19/2009**
Drug, lot #, and % purity: **Gadobutrol, lot #: not provided, %purity: not provided**

Key Study Findings: Gadobutrol was administered intravenously to rats in doses of 5.0, 7.5 and 10.0 mmolGd/kg/day during days 6 through 17 of the gestation period. Local intolerance at the injection site was observed at ≥ 7.5 mmolGd/kg and mortality occurred at the high dose (10 mmolGd/kg). There was embryotoxicity at the 7.5 mmolGd/kg dose or higher with incidences of skeletal variations occurring at these doses. There was a reduction in fetal body weight in the high dose group. There were however no structural findings in fetuses up to the 10 mmolGd/kg high dose. PK indicated a linear and dose-dependent increase in the mean systemic exposure to Gd after a single i.v administration.

The main clinical findings from this study are summarized by the following NOAELs:

Parameters	NOAEL (mmolGd/kg/day)
Systemic Toxicity	5.0 (8.1x-MHD)
Embryofetal Toxicity	5.0 (8.1x-MHD)

Doses:	0 (control), 5.0, 7.5 and 10.0 mmolGd/kg
Frequency of dosing:	Once/Day
Dose volume:	5.0-10.0 mL/kg
Route of administration:	Intravenous
Formulation/Vehicle:	Gadobutrol (SH 562BB, 1.0 mmol/L) / 0.9 % NaCl
Species/Strain:	Rat/Wistar (female), Crl: WI (HAN) (b) (4)
Number/Sex/Group:	Main groups: 20/group Satellite groups: 8/group
Age:	At least 10 weeks old on Day 0 of gestation
Body Weight:	172-204g on Day 0 of gestation
Satellite groups:	8 females/group
Study design:	The study design is described in the Study Design Table below
Deviation from study protocol:	One fetus designated for skeletal examination was missing. Some blood samples for TK parameters were not collected at the designated time

- The findings from two intravenous dose-range finding studies (A30997 and A576) were the basis for dose selection in study A34150.
- In the dose-range finding embryo-fetal toxicity Report A30997, gadobutrol formulation (SH 562BB) was administered to 32 inseminated females as 5.0, 7.5 and 10.0 mmolGd/kg (corresponding to 8.1, 12.2 and 16.2 X MHD) on gestation days 6 to 17. The mid-dose of 7.5 mmolGd/kg/day was well tolerated.
- In Report 576 (Study TX 91.310), Gadobutrol formulation SH L562 A (0.5 mmolGd/L; 0.5, 1.5 and 5.0 mmolGd/kg or physiological saline control) was tested for embryo-fetal developmental toxicity using successfully copulated rats. There was no treatment-related effect at the mid dose (1.5 mmolGd/kg). An increase in post-implantation loss, a decrease in number of viable fetuses per litter, delayed ossification occurred at 5.0 mmolGd/kg.
- Based on the findings of these dose-range finding studies, gadobutrol dose levels of 5, 7.5 and 10 mmolGd/kg were selected for the definitive developmental toxicity study in Wistar rats.

Study Design: In a study designed to evaluate the potential toxicity of Gadobutrol administered by daily intravenous bolus injection on embryo-fetal development in pregnant rats. eighty inseminated female Wistar rats were randomized to one of four groups of 20 animals per group. An additional eight rats per group (satellite groups) designated for toxicokinetic analyses were dosed in the same manner as rats in the main study groups.

Table 58: Study Design (main and satellite groups) - Report A34150

Group	Number of Females (Main/Satellite)	Test/control article	Dose/day mmol Gd/kg)	Dose multiple (by BSA)	Dose volume (mL/kg)
1 (control)	20 / 8*	0.9% NaCl	0	0	10.0
2 (LD)	20 / 8*	SH L 562BB	5.0	8.1	5.0
3 (MD)	20 / 8*	SH L 562BB	7.5	12.2	7.5
4 (HD)	20 / 8*	SH L 562BB	10.0	16.2	10.0

Sponsor's Table reconstructed by Reviewer; LD, MD, HD = Low, Mid or High dose groups; * = Satellite animals for determination of active substance levels

Animals were individually housed except when cohabited for mating. They were at least 10 weeks old on day 0 of gestation at which time, they weighed 172-204 g. Gadobutrol was administered i.v (tail vein) from gestation day 6 through 17. Each dose of the drug or 0.9% NaCl (control group) was administered in 12 consecutive injections. Dosage levels were 0 (normal saline), 5.0, 7.5 and 10.0 mmolGd/kg/day. These doses corresponded to 8.1, 12.2 and 16.2 X MHD for low, mid and high dose groups, respectively. Dose volumes, adjusted daily to actual body weights, were administered at the injection speed of 1.0 mL/minute.

Observations and Results

The effect of gadobutrol was evaluated by clinical signs, body weight, food consumption, and reproduction indices. Survival, clinical signs and body weight were recorded daily from day 0 to day 21 post-coitum (p.c). Body weight was recorded at least for days 0, 6, 9, 12, 15, 17, and 21.

Body weight gain was calculated for different gestational periods based on absolute body weight. A *corrected body weight* on day 21 was derived by deducting the intact uterine weight from the respective individual animal body weight. The *corrected body weight change* was calculated by subtracting the body weight at the start of treatment on day 6 p.c from the corrected body weight. Food consumption was measured for gestation periods between days 0-6, 6-9, 9-12, 12-15, 15-17 and 17-21. Blood samples were collected from four satellite animals per group on days 6 and 17 for a determination of toxicokinetic parameters.

Table 59: Toxicokinetic parameters

Parameter	Symbol	Way of calculation
Maximum Gd concentration	C_{max}	Taken as directly determined from the plasma concentration-time profile
Sampling time of C_{max}	t_{max}	Taken as directly determined from the plasma concentration-time profile
Area under the concentration-time curve from zero to 24 h	AUC(0-24h)	Non-compartmental analysis applying the mixed linear/logarithmic trapezoidal rule; extrapolation of the area from the first data point to the time of administration by linear connection of the first data point with the origin (t_0 ; $C_0 = C_1$)
Dose normalized AUC(0-24h)	AUC(0-24h)/dose	AUC(0- t_{last}) divided by the dose
Accumulation ratio	R	$R = \text{AUC}(0-24h), \text{ day 17 p.c.} / \text{AUC}(0-24h), \text{ day 6 p.c.}$

All dams in the main group surviving to day 21 p.c were sacrificed by excess CO₂-inhalation. Necropsy was performed and ovaries and uteri removed. The pregnancy status of surviving satellite animals was determined at sacrifice on day 21 p.c. All dams were examined macroscopically for number of corpora lutea per ovary, number and nature of implantation sites, number of viable and dead fetuses and early and late resorptions.

At necropsy, fetuses were individually weighed and examined for abnormalities (malformations and variations). All fetuses were examined for gross external abnormalities and their sex determined. Fetal livers with macroscopic findings were also examined microscopically. The remaining fetuses were eviscerated and prepared for fetal skeletal examination.

Mortality: Four deaths were recorded. Three dams died (one control animal on day 8 p.c and one each in main and satellite high dose (16.2X MHD) group on p.c days 8 and 9, respectively). No clinical signs were observed prior to death. Since macroscopic examination did not reveal any findings, the cause of death was determined as unclear. The fourth death was a high dose satellite dam sacrificed moribund on day 6 p.c following prone lateral or supine position seizures and respiratory distress. Death possibly resulted from vascular puncture and was not considered treatment-related.

Clinical Signs: One control group female had a premature delivery on gestation day 21. Low dose (5.0 mmolGd/kg): There were no treatment-related findings besides an occurrence of skin discoloration and scab formation noted also in controls. At 7.5 mmolGd/kg and up, observed treatment-related signs included apathy, vocalization, clonic-tonic seizures, tachypnea and prone-lateral or supine position. Similar treatment-related findings were noted in the acute toxicity study A28236. Dose-dependent signs of local drug intolerance at the injection site were noted in the mid and high dose groups. These signs included discoloration of the skin, scab formation, injuries and wounds, skin weeping, necrosis and ulcers. Auto-mutilation occurred at the high dose.

Body Weight: No effect on body weight or body weight change was observed up to the mid dose of 7.5 mmolGd/kg. Body weight was however slightly decreased at the high dose of 10 mmolGd/kg when calculated a corrected body weight change.

Feed Consumption: There was no effect on food consumption at the low dose of 5.0 mmolGd/kg (8.1X MHD). However, a decrease in food consumption was observed at the mid dose (7.5 mmolGd/kg; 12.2 X MHD) and higher.

Toxicokinetics: There was a linear increase in mean systemic exposure ((AUC 0-24h) on day 6 and on the last day of administration (G17). Accumulation ratios (R) were between 1.09 and 1.43 indicating a minimal tendency for gadobutrol to accumulate. TK findings are summarized in the sponsor's Table provided below:

Table 60: Mean PK parameters in female Wistar rats after daily intravenous treatment with 5, 7.5 and 10 mmolGd/kg gadobutrol on days 6 -17 of gestation

Parameter	Unit	Day p.c.	Dose (mmol/kg)		
			5.0	7.5	10
C_{max}	[mmol/L]	6	18.8	26.5	33.2
		17	18.6	29.5	40.2
$C_{max}/Dose$	[mmol/L]/ [mmol/kg]	6	3.76	3.53	3.32
		17	3.72	3.93	4.02
AUC(0-24h)	[mmol/L x h]	6	9.02	12.5	17.4
		17	9.82	17.3	24.9
AUC(0-24h)/ Dose	[mmol/L x h]/ [mmol/kg]	6	1.80	1.67	1.74
		17	1.96	2.31	2.49
R		17	1.09	1.38	1.43

Dosing Solution Analysis: Test solutions were used as provided to the conducting laboratory by the sponsor.

Necropsy: Scab formation was observed at the injection site starting from the low dose. Since the presence of scab was comparable to that in control animals, it was not considered treatment-related. There was however, a dose-dependent increase in scab formation as from the mid dose that was determined to be dose-related. A blood soaked cutis was observed in one high dose satellite animal sacrificed at blood sampling. The death was not considered treatment-related. All other macroscopic findings including thinning of fur and a discoloration of skin were considered incidental finding and not treatment-related.

Reproduction data: Reproduction data, based on the findings from 18, 19, 20 and 19 dams (control, low, mid and high dose groups), were unimpaired up to the maximal dose (10 mmolGd/kg). The number of surviving dams was calculated as follows:

Table 61: Summary of Maternal Survival

	TREATMENT GROUPS (Treated from Gestation Day 6 through 17)			
	1 0.9 % NaCl	2 5.0 mmolGd/kg	3 7.5 mmolGd/kg	4 10.0 mmolGd/kg
No. Assigned	20	20	20	20
Dam death	1	0	0	1
No. Eliminated during Gestation	1	0	0	0
Premature delivery	1	0	0	0
Defined day 0 p.c	18	20	20	19
- Not pregnant	0	1 (5%)	0	0
- Pregnant	18 (100%)	19 (95%)	20 (100%)	19 (100%)
Pregnant with viable fetuses	18	19	20	19
Pregnant with total Resorption	0	0	0	0

Two dams prematurely delivered on gestation day 21 in the high dose satellite group. The occurrence of premature delivery was not considered treatment related as a similar occurrence was reported in controls of the main group.

Table 62: Fertility indices (Report A34150)

	TREATMENT GROUPS (Treated from Gestation Day 6 through 17)			
	1 0.9 % NaCl	2 5.0 mmolGd/kg	3 7.5 mmolGd/kg	4 10.0 mmolGd/kg
Defined day 0	20	20	20	20
No. inseminated/%	20 (100%)	20 (100%)	20 (100%)	20 (100%)
No. pregnant /%	19 (95%)	19 (95%)	20 (100%)	19 (95%)
Fertility index	19 (95%)	19 (95%)	20 (100%)	19 (95%)

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Table 63: Implantation sites and losses, and fetal data

	TREATMENT GROUPS (Treated from Gestation Day 6 through 17)			
	1 0.9 % NaCl	2 5.0 mmolGd/kg	3 7.5 mmolGd/kg	4 10.0 mmolGd/kg
Calculated animals	18	19	20	19
Mean corpora lutea per animal	12.4	12.3	13.2	12.1
Mean no. of Implantation sites per animal	11.7	11.1	12.0	10.8
% Pre-implantation loss	5.4	9.8	9.5	10.1
% Post-implantation loss	6.6	6.2	6.3	6.8
Total live fetuses (/ % per group)	197 (10.9)	198 (10.4)	224 (11.2)	192 (10.1)
Total resorptions / group	14	13	15	14
Early resorptions (% / group)	14 (100)	11 (84.6)	14 (93.3)	12 (85.7)
Late resorptions (% / group)	0 (0.0)	2 (15.4)	1 (6.7)	2 (14.3)
% viable male fetuses	50.3	52.5	50.4	46.8
% viable females fetuses	49.8	47.5	49.5	53.1
Live litters	18	19	20	19
Litter body weight (g)	56.6	54.2	55.9	50.2
Male fetal weight (g)	5.3	5.3	5.1	5.1
Fetal weight (g)	5.1	5.1	4.9	4.8

The average number of corpora Lutea and rate of implantation loss were comparable across the study groups. The rate of post implantation loss was not affected up to the high dose of 10 mmolGd/kg. No fetal death occurred up to the high dose (10.0 mmolGd/kg). There was no impairment to the sex ratio. The fetal body weight was not affected up to the mid dose. However, there was a 10% reduction in fetal weight at the high dose.

Offspring (Malformations, Variations, etc.): There were no test article related external changes up to the high dose. There were also no treatment-related visceral malformations or variations. Where variations occurred they were either comparable to controls or were not dependent on dose.

Conclusions: The main conclusions from this study are listed as follows:

Gadobutrol was administered intravenously to rats in doses of 5.0, 7.5 and 10.0 mmolGd/kg/day during day 6 through 17 of the gestation period. Local intolerance at the injection site was observed at ≥ 7.5 mmolGd/kg and mortality occurred at the high dose (10 mmolGd/kg). There was embryotoxicity at the 7.5 mmolGd/kg dose or higher with incidences of increased skeletal variations occurring at these doses. There was a reduction in fetal body weight in the high dose group. There were however no malformations in fetuses up to the 10 mmolGd/kg high dose. PK indicated a linear and dose-dependent increase in the mean systemic exposure to Gd after a single ivy administration.

Reviewer's comment: Agree. However, one of the reported deaths in the study occurring at the high dose and in which a finding of seizure was described is very likely a treatment-related mortality.

Report A36661

Study Title: Gadobutrol (SH L562BB) - Study for effects on embryo-fetal development in rabbits after daily intravenous administration from day 6 to day 18 of gestation	
Study no.:	TXST20060242
Report Date:	February 10, 2009
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	Bayer Schering Pharma, Berlin, Germany
Date of study initiation:	January 22, 2007
In-Life Dates:	January 2007 – May 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Gadobutrol, Lot #: N/A, % Purity: N/A

Key Study Findings: This study was initiated with 20 presumed pregnant dams per group. Based on a finding of pale kidneys that occurred dose-dependently at all doses except controls, a NOAEL for maternal toxicity in pregnant dams was not established. A minimal incidence of embryotoxicity manifested as skeletal variations occurred beginning from 2.5 mmol Gd/kg/day. There was a linear and dose-proportional increase in mean systemic exposure (AUC(0-24)) following both single and repeated administration of gadobutrol. There appeared to be a tendency towards accumulation of the test compound at the high dose. The increase in Cmax was dose proportional.

Methods: Report A36661	
Doses:	0 (vehicle), 2.5, 5 and 10 mmol Gd/kg/day
Frequency of dosing:	Once daily (Gestation day 6 through 18)
Dose volume:	10, 2.5, 5 and 10 mL/kg (control, low, mid and high doses, respectively. Dose volumes were adjusted daily based on individual body weight; Injection speed: 5 mL/min)
Route of administration:	Intravenous
Formulation/Vehicle:	Gadobutrol (ZK135079), formulation SH L562BB 1.0 mmol Gd/mL/Vehicle - 0.9% (w/v) NaCl
Species/Strain:	Rabbit/New Zealand White SPF (b) (4)
Number/Sex/Group:	20/females/group (4 groups)
Satellite groups:	4/females/group
Study design:	See below

Study Design: This study was designed to assess the maternal health and fetal effects of gadobutrol (SH L562BB) when administered to pregnant rabbits. Eighty New Zealand White, SPF, female rabbits weighing 2.83 - 4.11 kg (on day 0 of gestation) were naturally impregnated with males and randomized to four treatment groups. Rabbits were supplied by (b) (4). The age of the animals was not recorded. No information was provided on the number, ages and weight of the breeder used for inseminating the study animals. Females were administered daily intravenous doses of 0.9% NaCl (control) or gadobutrol on day 6-18 of the presumed gestation. Dosages of 0, 2.5, 5 and 10 mmolGd/kg/day of SH L562BB were used. Each rabbit received 13 doses of SH L562BB or saline (control group).

Table 64: Study Design (A36661)

Group (animals)	Treatment	Dose (mmol Gd/kg/day)	Dose multiple (by BSA)	Dose Vol. (mL/kg/day)
1 (20)	0.9% NaCl	0	0	10
2 (20)	SH L562BB	2.5	8.1x MHD	2.5
3 (20)	SH L562BB	5	16.2x MHD	5
4 (20)	SH L562BB	10	32.4x MHD	10

Reviewer's Table; BSA = Body surface area

Dose selection: Dosages were selected based on the results of a dose-finding, non-pivotal, embryo-fetal developmental study in rabbits in which 10 mmol Gd/kg was found to be the maximal feasible dose.

Table 65: Dose selection (Report A36661)

Study (Report / Number)	A32272 / TXST20066068
Test article	Gadobutrol formulation SH L562BB (1 mmol Gd/mL)
Doses	5, 7.5 and 10 mmol Gd/kg/day on gestation days 6-18
Maternal toxicity	≥5 mmol Gd/kg; NOAEL – not established
Fetotoxicity	≥7.5 mmol Gd/kg; NOAEL – not established

Reviewer's Table

Observations and Results; All rabbits were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 6, 9, 12, 15, 18, 21, 24 and 28 of presumed gestation. Feed consumption values were determined for the period between gestation days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24 and 24-28. Blood samples were collected from the four satellite animals per dose group on days 6 and 18 for a determination of toxicokinetic parameters. Samples were collected at 0.083 (5 min), 1, 3, 7 and 24 h post-dose. Satellite animals were sacrificed after the final blood sampling on day 18. Dams were sacrificed by lethal injection (T61 euthanasia solution) on day 28 of presumed gestation. Ovaries and uteri were removed and kidneys were weighed. The number of corpora lutea per ovary, implantations, live and dead fetuses, and early and late resorptions were recorded. Indices for pre-implantation and post-implantation loss were calculated. Live fetuses were weighed, examined for external, visceral and skeletal alterations and sex determined.

Mortality: The study was initiated with 20 presumed pregnant females. A total of nine dams died or were sacrificed moribund during the gestation period. One death occurred at the mid-dose (5 mmol Gd/kg) group. All other mortalities or sacrifice occurred at the high (10 mmol Gd/kg) group.

Table 66: Maternal Survival (A36661)

	Groups			
	1	2	3	4
	0 mmol Gd/kg	2.5 mmol Gd/kg	5 mmol Gd/kg	10 mmol Gd/kg
No. at study initiation	20	20	20	20
No. dead or sac During gestation	0	0	1	4
No. with abortion	0	0	0	2
No. delivered prematurely	0	0	0	2
Total dead/sac	0	0	1	8
Mortality (%)	0	0	5	40
No. survived	20	20	19	12

Reviewer's Table

Clinical Signs: No treatment-related effects were observed at 2.5 mmol Gd/kg. Scab formation at the injection site was observed at 5 mmol Gd/kg. At the high dose, a reduction or absence of defecation occurred at the high dose. Prone, lateral or supine posture; seizures and increased respiration occurred in high dose group animals that died or were sacrificed.

Signs of intolerance at injection site were observed in the skin of the ear in two high dose dams.

Body Weight: There were no treatment-related effects on body weight up to 5 mmol Gd/kg.

Feed Consumption: Food consumption was considered not affected up to 5 mmol Gd/kg.

Toxicokinetics: Pharmacokinetic results are shown in the following sponsor Table:

Table 67: Mean pharmacokinetic parameters in female rabbits after daily intravenous

Parameter	Unit	Day p.c.	Dose (mmol/kg)		
			2.5	5.0	10.0
C _{max}	[mmol/L]	6	17.2 ± 3.41	27.4 ± 1.16	45.8 ± 0.933
		18	14.4 ± 1.36	26.1 ± 1.47	49.9 ± 4.33
AUC(0-24h)	[mmol/L x h]	6	15.3 ± 5.01	26.0 ± 2.69	53.3 ± 7.61
		18	12.9 ± 1.49	27.7 ± 2.76	86.5 ± 19.2
AUC(0-24h)/D	[mmol/L x h]/ [mmol/kg]	6	6.10 ± 2.00	5.21 ± 0.537	5.33 ± 0.761
		18	5.18 ± 0.597	5.55 ± 0.551	8.65 ± 1.92

C_{max} Maximum observed concentration of drug in plasma after drug administration
 AUC(0-24h) Area under the concentration versus time curve from dosing time to 24 h post-dose
 AUC(0-24h)/D AUC(0-24h) divided by the dose

Plasma C_{max} was observed in all study groups at the 5 min (0.083 h) time point. Similar to C_{max}, systemic exposure (AUC(0-24h)) increased dose-dependently with the increase being equal to the increase in dose. However, on gestation Day 18 a 3-fold increase in AUC was observed for the 2-fold dose increase from 5 to 10 mmol Gd/kg. Mean AUC (0-24 h) values were constant over the entire treatment period with no evidence of a tendency to accumulate. However, a mean accumulation ratio of 1.68 in the high dose group indicated a potential for an accumulation of gadobutrol at the high dose.

Dosing Solution Analysis: Test solutions were used as provided to the conducting laboratory by the sponsor.

Necropsy: Pale, discolored kidneys were observed in 1/20 (mid-dose) and 3/12 (high dose) dams that died or were sacrificed prematurely. An accentuated liver lobular pattern was observed in 1/20 mid-dose dams. The liver accentuation was not dose-dependent.

Organ weight: Gadobutrol had no effect on absolute kidney weight at the low dose (2.5 mmol Gd/kg). An increase in kidney weight was noted at the mid-dose of 5 mmol Gd/kg and higher.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Table 68: Implantation sites and losses, and fetal data (Report A36661)

PARAMETER	Treatment Groups (Treated on Gestation Days 6 -18)			
	1	2	3	4
	0.9 % NaCl	2.5 mmol Gd/kg	5 mmol Gd/kg	10 mmol Gd/kg
Dams assigned	20	20	20	20
Pregnancies (%)	20 (100)	20 (100)	19 (95)	12 (60)
Live Litters	20	19	19	11
Corpora Lutea (Mean Litter)	10.8	11.1	12.1	9.6
Implants (Mean Litter)	9.9	10.0	10.7	8.0
Total Fetuses	189	188	196	87
Total Live Fetuses	188	187	193	87
Live Fetuses (Mean Litter)	9.5	9.4	10.3	7.3
Total Dead Fetuses	1	1	3	0
Total Resorbed Fetuses	8	12	8	9
Early Resorptions / %	6 / 75.0	8 / 66.7	6 / 75.0	6 / 66.7
Late Resorptions / %	2 / 25.0	4 / 33.3	2 / 25.0	3 / 33.3
Pre-Implant Loss (%) ^A	8.8	9.5	11.3	16.5
Post-Implant Loss (%) ^B	4.6	6.5	5.4	9.4
Gravid Uterine Weights (g)	505	486	531	399
Fetal weights, g (M/F)	36.7/35.3	34.4/34.9	35.4/34.4	36.1/35.1
Sex Ratio (M/F)	1.04	0.99	1.03	1.03
^A Pre-implantation Loss: No. of Corpora Lutea – No. of Implantations/No. of Corpora Lutea x100				
^B Post-implantation loss: No. of Live fetuses/No. of Implants x100				

Offspring (Malformations, Variations, etc.): Occurrence of fetal deaths were reported in 1, 1, and 3 fetuses in the control, low (2.5), and mid (5.0) mmol Gd/kg/day groups, respectively. The three deaths in the mid dose occurred in different litters and were considered incidental. Fetal weight was not affected up to the high dose of 10 mmol Gd/kg/day. There were no treatment-related external structural findings. Although there was an increased incidence of visceral variations observed at the mid dose (5 mmol Gd/kg/day), the findings were single occurrences that were not dose-dependent. No treatment-related skeletal findings classifiable as malformations were observed. Non-treatment-related skeletal malformations were observed in control, low, mid and high groups.

Conclusions: No animals died or were sacrificed in the control and low dose groups, however, in the mid and high dose groups 1/20 and 4/20 animals died during gestation, respectively. At the high dose, 2/20 high dose animals aborted their fetuses. The same number in the same group delivered their fetuses prematurely. 10 mmol Gd/kg was therefore considered a lethal dose. High dose group animals that died naturally or were sacrificed moribund had clinical signs of apathy, prone-lateral or supine position, tremors and seizures prior to death. This dose was therefore considered greater than the maximal tolerated dose (MTD) of 5 mmolGd/kg.

The finding of pale kidneys occurred dose-dependently and was observed at all doses except controls. Therefore, a NOAEL for maternal toxicity in pregnant dams based on the macroscopic finding in the kidneys in dams of all groups receiving Gadobutrol was not established.

A low incidence of embryotoxicity, manifested as skeletal variations, occurred starting from 2.5 mmol Gd/kg/day.

There was a linear and dose-proportional increase in mean systemic exposure (AUC(0-24)) following both single and repeated administration of gadobutrol. Based on the R factor of 1.68, there was a tendency towards accumulation of the test compound at the high dose. Similarly, the increase in Cmax was dose proportional and slightly less than the dose increase.

Reviewer's comment: Agree

Report A894:

Study title: ZK 135.079 - Embryotoxicity including teratogenicity study in Cynomolgus monkeys after daily intravenous administration from day 20 to day 50 of gestation	
Study no.:	TX 92.075 / HD-1054-014-068
Report Date:	January 20, 1994
Study report location:	Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 07, 1992
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Gadobutrol (ZK 135079/SH L562A), lot #: N/A, %Purity: N/A

Key Study Findings: The major finding in this study was the occurrence of abortions in the high dose group (8.1x MHD)

Methods: Report No. A894	
Doses:	0 (vehicle control), 0.75 and 2.5 mmolGd/kg/day; 31 consecutive doses were administered.
Frequency of dosing:	Once daily (Gestation day 20 through 50)
Dose volume:	5, 1.5 and 5 mL/kg (control. Low and high doses, respectively. Dose volumes were injected at a dose rate of 10 mL/min
Route of administration:	Intravenous
Formulation/Vehicle:	Gadobutrol (ZK 135079/SH L562A, 0.5 Gd mmol/mL)/Vehicle – 0.9% NaCl
Species/Strain:	Monkey/Cynomolgus (Macaca fascicularis) (b) (4)
Number/Sex/Group:	12 Females/group ((3 groups – control, low and high doses)
Age:	Sexually mature; At least 4 years old
Body weight:	2.1 - 4.1 kg; 2.5 – 4.0 kg on day 20 post coitum
Satellite groups:	None
Study design:	See study design below
Deviation from study protocol:	No deviations were reported

Study Design (Report No. A894):

This study was conducted to assess the maternal health and fetal effects of gadobutrol (SH L 562A) in Cynomolgus monkey as an additional species to the rat and rabbit. According to the sponsor, embryotoxicity studies performed in non-human primates as a third species served to improve risk assessment with regard to potential teratogenic

effect. In addition, the monkey was similar to the human in terms of reproductive physiology, embryology and placentation. Please note that the Division did not request for this study.

Thirty six (36) Cynomolgus monkeys weighing 2.5 – 4.0 kg on day 20 post-coitum (p.c.) were supplied by (b) (4). The animals were at least four years old. Prior to the study, female animals were mated with untreated fertile male animals within two days of the theoretical middle of the menstrual cycle or based on the consistency of the cervical mucus. Mating period lasted for 18 hours. Following mating, the day on which the vaginal smear showed the presence of sperm was taken as day 0 of pregnancy. Pregnancy was confirmed by ultrasonography on days 18, 19 or 20.

Pregnant females were randomized to study groups of 12 animals per group on day 20. The animals were administered intravenous doses of 0.9% NaCl (control) or gadobutrol on day 20 through 50 of the presumed gestation. Dosages of 0, 0.75 and 2.5 mmol Gd/kg/day of SH L562A were used. Each monkey received thirty-one consecutive doses of SH L562A or saline (control group).

Table 69: Treatment scheme (Report No. A894)

Group (animals)	Treatment	Dose (mmol Gd/kg/day)	Dose multiple (by BSA)	Dose Vol. (mL/kg/day)
1 (12)	0.9% NaCl	0	0	5.0
2 (12)	SH L562A	0.75	2.4x MHD	1.5
3 (12)	SH L562A	2.5	8.1x MHD	5.0

Dosages were selected based on the results of the following studies:

Table 70: Dose selection (Report No. A894)

Study No.	Type of study	Finding(s)
TX 91.139 (Schering)	Repeat-dose Toxicity in rats	Mortality at 5 mmol Gd/kg
TX 91.230 (Schering)	Repeat-dose Toxicity in dogs	No toxicity at 2.5 mmol Gd/kg
TX 91.281 (Schering)	Dose-range finding study in rabbits	No toxicity at 5 mmol Gd/kg

Observations and Results: All animals were examined daily for survival and clinical signs from gestation day 20 until cesarean section was performed. Body weights were recorded on Days 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 97, and on the day of the cesarean section on day 100±1 of gestation. Blood samples were collected on the same days body weights were recorded. Samples were preserved for possible future use. Monitoring of pregnancy was performed by ultrasonography on days 30, 44, 58, 72 and 86 of gestation. An additional ultrasonography was performed when signs indicating possible abortion were observed. On day 100±1 of gestation cesarean section was

performed to terminate the pregnancies and the fetuses sacrificed. The fetuses weighed, sexed, examined for external abnormalities and necropsied.

Mortality: Abortions occurred in 1/12 and 5/12 control and high dose animals, respectively. No abortions occurred in animals in the low dose group. Although abortions in the high dose group were within the upper limit of the historical control, the incidence was considered treatment-related embryonic death.

Clinical Signs: No treatment-related clinical signs were observed. However, hematoma occurred at the site of injection in most animals.

Body Weight: No treatment-related effects on body weight were observed and mean body weight gain was comparable between treatment and control groups.

Feed Consumption: No information was provided on feed consumption.

Toxicokinetics: Not performed.

Dosing Solution Analysis: Test solution were used as provided to the conducting laboratory by the sponsor

Fetal Data: There were no treatment-related changes observed in fetal body or organ weights, fetal measurements or placenta weight.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Offspring (Malformations, Variations, etc.)

There were no external, visceral or skeletal abnormalities observed in the groups receiving the test article. Minor skeletal abnormalities were observed in all fetuses of the control group and the test compound. These defects were not considered treatment-related as there were similar occurrences in the controls. There were no malformations observed in the fetuses.

Conclusions: The major finding of note in this study was the occurrence of abortions in the high dose group (8.1x MHD).

Reviewer's comment: Agree

9.3 Prenatal and Postnatal Development

Report No. PH-35738

Study title: SH L 562 BB - Study for the effects on Pre- and Postnatal development in rats including maternal function and toxicokinetic investigation after intravenous administration

Study no.:	T7076752;
	Sponsor's No.TXEX200770003
Report Date:	February 17, 2009
Study report location:	Module 4.2.3.5.3
Conducting laboratory and location:	Bayer HealthCare (Bayer Schering Pharma AG), 42096 Wuppertal, Germany
Date of study initiation:	August 03, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Gadobutrol (ZK 135079/SH L562BB), lot #: N/A, %Purity: N/A

Key Study Findings: The main clinical findings from this study are summarized by the following NOAELs:

Parameters	NOAEL
Maternal effect (F ₀)	0.6 mmol Gd/kg/day
Pre- and Postnatal development of F ₁ generation	2.2 mmol Gd/kg/day
Late effect, including fertility testing, of F ₁ generation	7.5 mmol Gd/kg/day

Methods: Report No. PH-35738

Doses: 0 (vehicle), 0.6, 2.2 and 7.5 mmol Gd/kg/day
 Frequency of dosing: Once daily (Day 6 post-coitus through Day 21 post-partum)
 Dose volume: 7.5, 0.6, 2.2, and 7.5 mL/kg/day for control, low, mid and high doses, respectively. Injection speed: 1 ml/min
 Route of administration: Intravenous
 Formulation/Vehicle: Gadobutrol (ZK135079), formulation SH L562BB 1.0 mmol Gd/mL/Vehicle - 0.9% (w/v) NaCl
 Species/Strain: Rat/Wistar Hsd Cpb:WU SPF (b) (4)
 Age: At time of mating: Males and females were 12-14 weeks old
 Weight: At the time of mating, males were 391-473 g and females were 192-251 g
 Number/Sex/Group: 22 females/group (4 groups)
 Satellite groups: 5 females/group
 Study design: See Study groups below
 Deviation from study protocol:

Study Design (Report No. PH-35738):

Table 71: Study groups (Report No. PH-35738)

Group (animals)		Treatment	Dose (mmol Gd/kg/day)	Dose multiple (by BSA)	Dose Vol. (mL/kg/day)
Main	Satellite				
1 (22)	5	0.9% NaCl	0	0	10
2 (22)	5	SH L562BB	0.6	0.97x MHD	2.5
3 (22)	5	SH L562BB	2.2	3.6x MHD	5
4 (22)	5	SH L562BB	7.5	12.2x MHD	10

Reviewer's Table; BSA = Body surface area

Dosages were selected based on the results of studies SG 127 and PH-35165

Table 72: Dose selection (Report PH-35738)

Study Number	Doses (mmol Gd/kg/day)	Type of study	Finding(s)
SG 127	0.5, 1.5 and 4.5	Segment III Reproduction study	Up to 4.5 mmolGd/kg/day from day 15 p.c through day 21 p.p had no maternal toxicity effect NOAEL of 4.5 mmol Gd/kg/day for systemic toxicity and fertility in dams 1.5 mmol Gd/kg/day in the offspring
PH-35165	5, 7.5 and 10	Segment III dose range finding study	2/4 animals died at 10 mmol Gd/kg Local intolerance at 7.5 mmol Gd High dose for definitive study established as 7.5 mmol Gd/kg

Observations and Results (Optional Table)**Maternal findings (F₀ dams)****Survival –****Table 73: Survival data - F0 dams (Report PH-35738)**

Group	Mortality	Comments
Group 1 (Control)	2/22	1 died during lactation; 1 sacrificed at early lactation due to total litter loss
Group 2 (low dose)	--	-
Group 3 (mid dose)	-	-
Group 4 (high dose)	2/22	sacrificed at early lactation due to total litter loss
Totals	4	All other animals survived to scheduled sacrifice

Clinical signs, Body weight, Feed consumption, Uterine content, Necropsy and Toxicokinetics –

F₀ Dams

Survival:	Except for 4 dams (see Table above), all other dams survived to scheduled sacrifice
Clinical signs:	0.6 mmolGd/kg/day: no signs reported 2.2 mmolGd/kg/day: single, transient case of tremor 7.5 mmolGd/kg/day: sporadic signs (convulsions, hypoactivity, gasping, bleeding and discoloration at injection site) were considered treatment-related
Body weight:	During lactation, a statistically significant increase in body weight gain was observed at 7.5 mmolGd/kg/day
Feed consumption:	No effect on feed intake or water consumption was evident during gestation or subsequent lactation period
Uterine content:	The average number of implantation sites in F ₀ dams was comparable across the dose groups up to the high dose
Necropsy observation:	Enlarged kidneys; There was increased absolute/relative kidney weight at ≥ 2.2 mmolGd/kg/day. Similar findings were observed in the repeat dose toxicity study (A08936) in rats
Toxicokinetics:	On day 21p.p, AUC (0-24) and C _{max} increased dose-proportionally when dose was increased from 0.6 to 7.5 mmolGd/kg/day Following repeated dosing on day 21 p.p, there was no change in AUC (0-24) and C _{max} compared to day 6 p.c.
Dosing Solution Analysis	Test solutions were used as provided to the conducting laboratory by the sponsor
Other:	See Reproduction data

Reproduction Data (F₀ Dams):

The reproductive performance of F₀ Dams was assessed by the fertility, gestation and rearing indices, defined as:

Fertility Index (%) = No. of females with implantation sites / No. of Inseminated females x100

Gestation Index (%) = No. of females delivering pups / No. of females with implantation sites x 100

Rearing Index (%) = No. of females which reared pups / No. of females which littered.

Table 74: Reproduction data in F0 dams (Report PH-35738)

F₀ PARAMETERS	Treatment Groups (Treated on Gestation Days 6 p.c – 21 p.p)			
	1	2	3	4
	0.9 % NaCl	0.6 mmolGd/kg	2.2 mmolGd/kg	7.5 mmolGd/kg
No. of Dams assigned	22	22	22	22
Fertility Index (%) (Females with Implantations)	90.9	90.9	90.9	90.9
No. of females	20	20	20	20
Gestation Index (%) (Females which delivered)	100	100	100	95
No. of females	20	20	20	19
Rearing Index (%) (Females which reared pups)	94.7	100	100	89.5
No. of females	18	20	20	17
Duration of Gestation (days ± sd)	22.7 ± 0.6	22.8 ± 0.4	23.0 ± 0.2	22.8 ± 0.4
No. of F ₀ Implantation sites (Mean ± sd)	13.8 ± 3.1	14.4 ± 2.2	15.0 ± 1.7	14.6 ± 3.3
Prenatal loss (Mean ± sd)	0.5 ± 0.6	1.5 ± 2.5	1.2 ± 1.2	1.3 ± 1.2

Reviewer's Table constructed with sponsor's data; p.c = post-coitum; p.p = post-partum

There was no difference between control and treatment groups in the fertility index up to the high dose of 7.5 mmol Gd/kg/day. The gestation and rearing index were not affected at ≥ 2.2 mmol Gd/kg/day. However, the rearing index was considered affected at the high dose of 7.5 mmolGd/kg.

The duration of gestation was not affected by treatment up to the high dose of 7.5 mmolGd/kg/day.

Course of birth: Observation of the course of birth did not indicate any effect of gadobutrol up to the high dose of 7.5 mmol Gd/kg.

Lactation behavior: Increased number of pups showed a lack of milk spot at the high dose (7.5 mmolGd/kg) group. A similar observation was not made at doses ≤ 2.2 mmolGd/kg.

Prenatal loss: The prenatal loss was in the historical range and did not reveal any dose relationship. No treatment-related effect was present up to the high dose level.

Postnatal development (F₁ Pups):**F₁ Generation**

- Survival: Pup mortality: One F₁ male pup death occurred at 2.2 mmol Gd/kg.
There was complete litter loss in 2 F₀ dams at the high dose resulting in an increased number of dead pups (See pup survival indices below)
- Impaired pup viability: Occurred at the high dose (7.5 mmol Gd/kg/day; characterized by pups lacking milk spots, pale skin and early postnatal loss
- Clinical signs: No treatment-related effects
- Body weight: No treatment-related effects
- Feed consumption: No treatment-related effects
- Physical development: Not affected by treatment at ≥ 7.5 mmolGd/kg/day
No treatment-related malformations and gross pathology findings were observed
- Neurological assessment: Reflexes (surface righting, negative geotaxis, hearing, and pupillary reflex) and Behavior (motor activity test, water-maze test, memory and reversal learning performance) were not affected at ≥ 2.2 mmolGd/kg/day
Decreased motor activity at 7.5 mmolGd/kg/day was considered treatment-related
- Reproduction: At doses up to and including 7.5 mmolGd/kg, there were no treatment-related effects on:
Insemination, fertility and gestation rates, gestation time, number of implantation sites, prenatal loss, litter size, F₂ pup sex ratio, clinical observations, and body weights.
(See Table of Reproduction data)
- Gross Pathology: Findings include:
Microphthalmia in 1 Male at mid-dose
Bladder stones and reduced testis in 1 Male at high-dose
Cervical thickening/uterus with clear fluid: 1 Female at high-dose
Findings were not considered treatment-related

Table 75: Pup live birth and survival indices (PH-35738)

F₁ Parameters	Treatment Dose (mmolGd/kg/day)				Comment
	0	0.6	2.2	7.5	
Live birth index	98.6	99.3	97.5	95.7	Marginal decreases at high dose were considered treatment-related
Survival index (up to 4 p.p)	94.1	98.9	97.6	84.2	
Survival index (day 4 to 21 p.p)	99.3	98.8	99.4	100	

There were no gadobutrol-related effects on clinical signs, feed consumption, and body weight in the pre-mating, mating and gestation period or on gross pathology in male and female F₁ animals.

F₁ Reproductive performance

The insemination, fertility and gestation rate of F₁ females were in the historical control data range. There were no treatment-related effects up to the high dose of 7.5 mmolGd/kg. The number of implantation sites, duration of gestation, prenatal loss, litter size, number of stillborn pups, pup weight and sex ratio were not affected by treatment up to the high dose of 7.5 mmol Gd/kg.

Table 76: F₁ Reproductive performance

F₁ Parameters (Values are given as mean per female)	Treatment Groups			
	0.9 % NaCl	0.6 mmolGd/kg	2.2 mmolGd/kg	7.5 mmolGd/kg
No. of Implantations	14.6	14.1	14.2	14.3
Duration of Gestation (days)	22.4	22.6	22.6	22.5
No. of Live pups	13.5	13.0	12.6	13.1
Prenatal Loss	0.8	1.1	1.3	1.1
Live birth index (%)	98.4	100.0	97.6	99.0
Stillbirths (% per group)	1.7	0.0	2.2	1.1
No. of Dead pups	0	0	2	0
Weight of pups (g)	6.2	6.5	6.7	6.3
% sex ratio (on day 0 p.p)	47.9	51.9	48.2	46.9

Reviewer's Table constructed from sponsor's data; pups = F₂ generation

F₂ Generation

Survival:	Mortality in two F ₂ pups at 2.2 mmolGd/kg was not considered treatment-related as no dose dependence was observed
No. of living and dead F ₂ pups	Litter size and number of stillbirths was not affected by treatment up to the high dose
Birth weight:	Birth weight increased in the 2.2 mmolGd/kg group. Finding was not dose-related and was considered incidental
Body weight:	Not affected by treatment up to the high dose
External evaluation:	Microphthalmia and/or anophthalmia observed in low- and high-dose groups including controls. No dose-dependence was described
Male/Female ratio:	Not affected by treatment up to the high dose

Conclusions

Based on the findings from this study the sponsor determined a NOAEL of 0.6 mmolGd/kg/day (or 0.97x MHD) for maternal (F₀) effects. NOAELs of 2.2 and 7.5 mmolGd/kg/day or 3.6x and 12.2x MHD, respectively were determined for Pre- and postnatal development of the F₁ generation and for late effects (including fertility testing of the F₁ generation).

Reviewer's comments: Agree

9 Local tolerance

Local tolerance studies were conducted using the intravenous route of administration. Other routes including intraarterial, paravenous, intramuscular, and injection into the liver parenchyma were also tested.

9.1 Report No: 9622:

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

Volume #, and Page #:	Module 4.2.3.6.1; 9 pages
Conducting laboratory and location:	Schering AG, Berlin, Germany
Study #:	TX 91.130
Date of study initiation:	N/A; Studies conducted from September 17-24, 1991
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (ZK 135079; Formulation: SH L562 B, 1.0 mmolGd/mL); Saline vehicle ZK 135079 (411700); Formulation Batch SH L562 B (G/0094-2A)
Substance Batch number(s):	ZK 135079 (411700); Formulation Batch SH L562 B (G/0094-2A)
Animal species/strain/sex per dose:	Rabbit/New Zealand White/4 per sex; Breeder: (b) (4)
Age:	N/A
Weight:	Males (2.3-2.8 kg); Females (2.7-2.9 kg)
Doses/Vehicle:	0.5 mL/animal at an injection rate of 30 sec/animal
Duration/route:	Single, intravenous via congested and uncongested marginal ear vein

Key findings: Mild, transient reddening and swelling were observed at the administration site for 4 days after administration in the congestive or non-congestive state. Moderate reddening was also observed for 3 days after administration in the congestive condition. There were no abnormal histopathological findings.

Study Design: Two animals per sex each received 0.5 mL of Gadobutrol (SH L 562 B) into the uncongested marginal ear vein of the right ear in 30 sec. Saline was used as control and administered in the same manner as the test compound in the left ear. The remaining two animals per sex were similarly administered the test article in the same volume (0.5 mL) in the congested marginal ear vein of the right ear. Saline was injected into the marginal vein of the left ear as control. The treatment schedule is shown below.

Table 77: Treatment schedule (Report No. 9622)

Application site	Uncongested vein animal nos.	Congested vein animal nos.
right ear SH L 562 B	565 M	561 M
left ear 0.9 % (w/v) NaCl-solution (control)	567 M 574 F 577 F	564 M 571 F 579 F

The injection site was marked and clinical observations were made immediately after injection, 1 and 3h post application and at 24h intervals until day 8 after application. Animals were sacrificed on day 8 and samples taken from the area 1 cm proximal to the site of injection that was previously marked. The samples were examined histologically.

Results: The results are shown in the following sponsor Table:

Table 78: Local findings at injection site following administration of Gadobutrol SH L 562 B in the rabbit

Findings	Congested vein				Uncongested vein			
	left ear (control) 0.5 ml 0.9 % (w/v) NaCl-solution		right ear 0.5 ml SH L 562 B		left ear (control) 0.5 ml 0.9 % (w/v) NaCl-solution		right ear 0.5 ml SH L 562 B	
	M s (x-y)	F s (x-y)	M s (x-y)	F s (x-y)	M s (x-y)	F s (x-y)	M s (x-y)	F s (x-y)
reddening, slight [total ear, area around the vein, area of the puncture tract (0.3 x 0.3 cm to 4 x 3 cm)]			3/2 (1-3)	6/2 (1-4)			6/2 (1-3)	3/1 (1-2)
reddening, moderate [total ear, right or lateral half ear, area around the vein, area of the puncture tract (1 x 3 cm to 5 x 3 cm)]			5/2 (1-3)	6/2 (1-3)				
Swelling, slight [total ear, area around the vein, area of the puncture tract (0.3 x 0.3 cm to 2 x 5 cm)]			6/2 (1-3)	6/2 (1-2)			4/2 (1-1)	5/2 (1-2)
vein invisible				1/1 (3-3)				
bluish discolouration (vein, puncture tract, injection site)	4/2 (2-3)	8/2 (1-6)		4/2 (5-6)		1/1 (2-2)	1/1 (1-1)	
s = summation of findings/number of animals (x-y) = first day/last day of occurrence of a finding M = male F = female								

The main findings included slight reddening and swellings in the congested and uncongested veins. Bluish discolorations were observed at the injection site after the administration of gadobutrol as well as saline. No pathological findings were revealed from the histological examination of the control ears or in treated congested and uncongested ears.

Conclusions: No abnormal histopathological findings were observed in the ears of rabbits under congested and uncongested conditions. According to the sponsor, the results of the study of local tolerance reaction in a small vessel, as obtained in the rabbit could be relevant for administration into a small vessel as seen in infants and small children.

Reviewer's comments: I agree with the sponsors conclusion.

9.2 Report No: 9736:

Study title: SH L 562 B - Local tolerance test in the dog after a single intravenous injection into the vena cephalic antebrachii

Volume #, and Page #:	Module 4.2.3.6.1; 9 pages
Conducting laboratory and location:	Schering AG, Berlin, Germany
Study #:	TX 92.031
Date of study initiation:	N/A; Studies conducted in January, 1992
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (ZK 135079), lot# and %purity – N/A
Drug, lot #, and % purity:	Gadobutrol (ZK 135079; Formulation: SH L562 B, 1.0 mmolGd/mL); Saline vehicle ZK 135079 (411700); Formulation Batch SH L562 B (G/0094-2A)
Substance Batch number(s):	
number(s):	
Animal species/strain/sex per dose:	Dog/beagle; 2/sex; Breeder - (b) (4)
Age:	N/A
Weight:	Males (9.3-14.8 kg); Females (8.7-11.2 kg)
Doses/Vehicle:	1.5 mL/kg
Duration/route:	Single/intravenous; injection rate: 1mL/sec

Key findings: A single intravenous application of gadobutrol was tolerated at the local injection site in the forelimb cephalic vein of the dog. No local effects were observed at the site of injection.

Study Design: This study was designed to evaluate potential tolerance reaction following the application of gadobutrol (SH L 562 B) in the cephalic vein of the forelimb which corresponds in size to a similar vein in the adult human.

A dose of 1.5mL SH L 562 B/kg was used was administered in the right vein in four male and female beagle dog using an injection rate of 1mL/sec. 1.5 mL/kg saline was administered into the left vein as control. The administered dose was about 3-fold the maximal intended human dose. The site of injection was observed during and immediately after the injection and at 1 and 3 h and every 24h until the eight day after injection for signs of reaction. The dogs were not sacrificed at the end of study.

Results: There were no clearly defined treatment-related findings.

Conclusions: A single application of gadobutrol was tolerated at the local injection site in cephalic vein of the dog. The sponsor concluded that based on the findings, no local adverse effects may be anticipated at the local site of injection of gadobutrol in humans.

Reviewer's comments: I agree

9.3 Report No. 9569:

Study title: L 562 B – Local tolerance test in the rabbit (M+F) after a single injection into the central artery of the ear

Volume #, and Page #:	Module 4.2.3.6.1; 9 pages
Conducting laboratory and location:	Schering AG, Berlin, Germany
Study #:	TX 91.131
Date of study initiation:	N/A; Study conducted from September 18-25, 1991
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562 B)
Substance Batch number(s):	ZK 135079 (411700); Formulation Batch
number(s):	SH L562 B (G/0094-2A)
Animal species/strain/sex per dose:	Rabbit/New Zealand White; 2/sex; Breeder:
	(b) (4)
Age:	N/A
Weight:	Males (2.4-2.7 kg); Females (2.8-2.9 kg)
Doses/Vehicle:	0.5mL Gadobutrol/Saline vehicle
Duration/route:	intraarterial; ear artery

Key findings: A single intra-arterial application of gadobutrol was tolerated at the local injection site in the central ear artery of the Rabbit. No local effects were observed at the site of injection.

Study Design:

- 0.5 mL of SH L562B was injected into the central artery of the right ear of each male female rabbit
- 0.5 mL saline was injected into the same artery in the left ear
- After injection, the site was observed at 0.5, 2 and 4h and daily for 8d post-administration
- Rabbits were sacrificed on day 8 and tissue samples taken for histological examination

Results: Local findings at the injection site included paleness of skin, skin reddening and hematoma. The observed signs were seen in both test article-treated and control sites. 7 days after injection, histological examination did not reveal any pathological changes in either control or SH L562 B-treated ears.

Conclusions: The findings were not treatment-related and similar local hemodynamic changes might occur in humans under similar conditions of administration.

Reviewer's comment: I agree with the findings and conclusions.

9.4 Report No. 9566:

Study title: SH L562B – Local tolerance test in the rabbit (M+F) after a single paravenous injection

Volume #, and Page #:	Module 4.2.3.6.1; 10 pages
Conducting laboratory and location:	Schering AG, Berlin, Germany
Study #:	TX 91.133
Date of study initiation:	N/A; Study conducted from September 18-24, 1991
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562 B)
Substance Batch number(s):	ZK 135079 (411700); Formulation Batch
number(s):	SH L562 B (G/0094-2A)
Animal species/strain/sex per dose:	Rabbit/New Zealand White; 2/sex; Breeder:
	(b) (4)
Age:	N/A
Weight:	Males (2.9-3.2 kg); Females (2.6 kg)
Doses/Vehicle:	0.5mL Gadobutrol/Saline vehicle
Duration/route:	intra-arterial; ear artery

Key findings:

Study Design: 1.0 mL Gadobutrol (SH L562 B) was injected as a bolus paravenously at a point proximal to the right of the congested saphenous vein. Under the same condition, a paravenous injection of saline (1.0 mL) was administered proximal to the left congested saphenous vein

- The injection site was observed immediately, 2 and 4h after injection and at 24h intervals until 7 days after administration.
- Test animals were sacrificed on day 7 and the samples of the skin and perivascular tissue at injection site examined histologically.

Results:

- Local reaction observed at the site of test article injection included a slight to moderate reddening, swelling, bluish discoloration and hematoma.
- The injection site was blood stained.
- The effects were seen in both male and female animals.
- These signs were treatment-related and were not observed in the saline-treated injection site.
- Histological examination of tissue samples revealed an inflammatory reaction characterized by edema, proliferation of fibrocytes/fibroblasts and leucocyte infiltration at the application site. These findings were not present in the control site

Conclusions: The sponsor classified these local site reactions occurring after a single paravenous injection of gadobutrol as mild to moderate local irritations.

Reviewer's comment: The findings, though mild, indicate the potential of gadobutrol to provoke local reactions at the site of a paravenous drug administration.

9.5 Report No. 9599:

Study title: SH L 562B – Local irritation test in rabbits after a single intramuscular injection

Volume #, and Page #:	Module 4.2.3.6.1; 10 pages
Conducting laboratory and location:	Schering AG, Berlin, Germany
Study #:	TX 91.132
Date of study initiation:	N/A; Study conducted from September, 1991
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH L 562 B)
Substance Batch number(s):	ZK 135079 (411700); Formulation Batch
number(s):	SH L562 B (G/0094-2A)
Animal species/strain/sex per dose:	Rabbit/New Zealand White; 4/sex; Breeder:
	(b) (4)
Age:	N/A
Weight:	Males (2.3-2.7 kg); Females (2.5-2.7 kg)
Doses/Vehicle:	0.5mL Gadobutrol/Saline vehicle

Duration/route:

Intramuscular / sacrospinal muscles

Key findings: Intramuscular injection of gadobutrol did not provoke any local reactions of damage to muscular tissue

Study Design: 1.0 mL of SH L562 B was administered as a single dose into the sacrospinal muscle of four rabbits per sex. The injection sites were examined at 2, 4 and 24h and thereafter until day 3 or day 7 when 2 rabbits per sex were sacrificed for macroscopic and histological examination.

Results: No treatment-related irritation was observed after injection. Reddening and bleeding observed macroscopically with both test and control articles resolved by day 7. Histology revealed signs consistent with mechanical trauma at the injection site. No difference was observed between test article-treated and control animals

Conclusions: It can be concluded from the result that administration of gadobutrol into muscular tissue did not result in a local reaction at the site of injection.

Reviewer's comment: I agree with the findings of the study. It does not appear that an accidental administration of SH L562 B into muscular tissue in humans will result in muscular damage or local reaction to the test article.

10 Special Toxicology

10.1 Nephrogenic Systemic Fibrosis (NSF)

a). Introduction to NSF Studies

a). **General:** Nephrogenic systemic fibrosis (NSF) is a recently described, serious, fibrotic and highly debilitating disorder most frequently described in patients with end-stage renal disease or acute renal failure. While NSF primarily affects the skin, other organ compartments may also be involved. Although the etiology of NSF is not fully understood, exposure to gadolinium-containing contrast agents has been associated with the onset of symptoms. Considerable progress towards a better understanding of this disease has occurred since the first association between the clinical use of GBCAs and NSF was made (Grobner et al., 2006). Consequently, there are several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate <30 mL/min/1.73m²). The use of GBCAs is currently contraindicated in such individuals. The commonly reported hypothesis for the involvement of gadolinium in the pathomechanism of NSF is the in vivo dechelation of GBCAs. Although all GBCAs have the potential to release gadolinium ion (Gd³⁺), which in the context of renal failure, is an

important trigger for NSF, such a release is thought to more readily occur among the less stable gadolinium chelates. However, the genesis of NSF may be multifactorial (Sieber et al., 2009) and other triggers or co-triggers may play a role in its pathogenesis. A definitive diagnosis of NSF requires deep skin biopsy and histopathology. No effective treatment is currently available for NSF and prevention of disease is of key epidemiological importance.

b). Nonclinical Considerations in NSF: The sponsor submitted a series of non-GLP exploratory studies in intact and renally-impaired adult male rats. Potential skin lesions and possible changes in concentrations of gadolinium, zinc and copper were evaluated in the skin, liver, and femur in animals administered Gadobutrol or other GBCAs with different structural and ionic formations.

c). Gadolinium deposition in skin and other tissues: The study objective was to determine if repeated intravenous administrations of Gadobutrol and other marketed GBCAs, including Omniscan, Magnevist, Dotarem, OptiMARK and Multihance induced NSF-like lesions in rats. After 20 injections administered 5 times per week over four weeks, rats were sacrificed 5 days after the last dose. The skin (back and neck), liver and femur were removed to determine Gd, Zn and Cu concentration. No macroscopic or microscopic findings were observed in rats administered macrocyclic nonionic (Gadovist and Dotarem) or linear ionic (Magnevist, Multihance, Eovist and Ablavar) Gd chelates. In contrast, ulcer and crust formation, fibrosis, deposits of collagen and skin thickening were observed in rats administered the linear nonionic chelates, Omniscan and OptiMARK. The lowest Gd concentrations were observed in animals treated with the macrocyclic compounds (Gadovist and Dotarem) followed by the linear ionic compounds and highest amounts in Omniscan-treated rats. GBCA treatment had no effect on Zn or Cu levels in tissues or organs.

Progress has been made towards a better understanding of this disease since the first association between the clinical use of GBCAs and NSF was made in 2006. Consequently, there have been several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate <30 mL/min/1.73m²).

The sponsor submitted a series of non-GLP exploratory studies conducted in adult male rats in response to NSF. The studies reviewed involved GBCAs that are FDA-approved and those that have not yet attained approval in the U.S. For ease of reference, information on the nomenclature (Trade or Active Ingredient), U.S approval status, and NDA application numbers is provided in the following summary Table:

Table 79: Gadolinium-Based Contrast Agents (GBCAs)

Product		NDA or IND	Sponsor	Approval Yes (Year)
Trade Name	Established Name			
Magnevist	Gadopentetate dimeglumine	N 019596	Bayer	Yes (2000)

		N 021037		
ProHance	Gadoteridol	N 020131 N 021489	Bracco	Yes (1992)
Omniscan	Gadodiamide	N 020123 N 022066	GE Healthcare	Jan (1993)
OptiMARK	Gadoversetamide	N 020937 N 020975 N 020976	Covidien	Dec (1999)
MultiHance	Gadobenate dimeglumine	N 021357 N 021358	Bracco	Nov (2004)
Eovist/Primovist	Gadoxetate disodium	N 022090	Bayer	Jul (2008)
Ablavar/Vasovist	Gadofosveset trisodium	N 021711	Lantheus	Dec (2008)
Gadovist	Gadobutrol	N 201277 I 056410	Bayer	Pending as of 2010
Dotarem	Gadoterate	(b) (4)	Guerbet	In IND status

Table adapted from "Postmarketing Safety Evaluation of Gadolinium-Based Contrast Agents" for MRI by Dr. Lucie Yang

Table 80: Classification of GBCAs Based on Structure and Charge

Trade Name	Established Name	Structure	Charge
Omniscan	Gadodiamide	Linear	Nonionic
OptiMARK	Gadoversetamide	Linear	Nonionic
Magnevist	Gadopentetate dimeglumine	Linear	Ionic
MultiHance	Gadobenate dimeglumine	Linear	Ionic
Eovist	Gadoxetate disodium	Linear	Ionic
Ablavar/Vasovist	Gadofosveset trisodium	Linear	Ionic
ProHance	Gadoteridol	Macrocylic	Nonionic
Gadovist	Gadobutrol	Macrocylic	Nonionic
Dotarem	Gadoterate	Macrocylic	Ionic

Table adapted from "Postmarketing Safety Evaluation of Gadolinium-Based Contrast Agents" for MRI by Dr. Lucie Yang

Report A40180 examined the possibility of development of skin in rats following multiple administrations with GBCAs. In addition to evaluating Gd concentrations in various tissues after GBCA treatment, the study also investigated the role of excess ligand on the development of NSF-like lesions. Changes in the concentrations of Gadolinium, zinc and copper were evaluated in skin, liver and femur following 20 repeated daily intravenous administrations of Gadovist (formulation SH L562 BB; 1.0 mmol/mL) and other GBCAs including Gadodiamide - the drug substance of Omniscan® and Gadopentetate dimeglumine – the drug substance of Magnevist®. Other

compounds evaluated in this study were Gadovist[®], OptiMARK[®], MultiHance[®], Dotarem[®], Vasovist[®], Eovist[®], Gd-EDTA, Caldiamide (the calcium chelate in Omniscan) and saline.

Study A40160 evaluated the effect of endogenous Zinc (Zn) depletion in addition to repeated administration of different GBCAs (Gadovist[®], Omniscan[®], OptiMARK[®], Magnevist[®], Gadopentetate - the drug substance of Magnevist[®] without excess of free ligand), and Gd-EDTA) on the development of skin changes in rats. An additional objective was to evaluate the effect of endogenous zinc depletion in addition to the administration of GBCAs on the serum levels of zinc and copper.

Report A42495 evaluated the elimination time course of Gadolinium in skin tissue and the potential long-term Gd retention in skin tissue after intravenous administration of GBCAs (Gadovist, Omniscan, OptiMARK[®], Magnevist[®], MultiHance[®], Prohance[®] and Dotarem[®]).

In **Report A42496**, partially (5/6)-nephrectomized rats were administered Gadobutrol, Omniscan, Magnevist or OptiMARK, each at a dose of 2.5 mmol/kg once daily for 5 consecutive days to determine the effect of prolonged circulation time of GBCAs caused by reduced renal clearance on the Gd concentration and long-term retention of Gd in the skin of rats. The study also evaluated 5/6 nephrectomized rats as a model for prolonged circulation time of GBCAs seen in renal-impaired patients. Results showed a prolonged presence of Gd in serum in the 5/6-nephrectomized rats compared to non-nephrectomized control rats. The exposure and plasma half-life in 5/6 nephrectomized rats were prolonged compared to healthy rats.

Other studies reviewed included A39927, A47234, A47235, A47233 and A42715. Tabulated below are the study objectives for these studies:

Table 81: Objectives of Reports A39927, A47234, A47235, A47233 and A42715

Report	Objectives
A39927	Elucidation of the pathomechanisms of skin lesions in rats treated with i.v Gadodiamide (Omniscan) over a period of 1-8 days
A47234	Determination of the influence of residual Gd in the induction NSF-like lesions in rats treated i.v with Gadodiamide (Omniscan) using different time intervals
A47235	Determination of the origin and role of cytokines in the pathology of skin changes in Gadodiamide-treated rats
A47233	Elucidation of the potential of the lanthanoid series of elements to induce NSF-like lesions using Omniscan- and Optimark-treated rats
A47215	Evaluation of the stability of the Gd-complex and Gd-dissociation of GBCAs

10.1.2 Studies in Intact Rats

10.1.2.1 Report No. A40180:

Study title: Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs on occurrence of NSF-like skin lesions in rats

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 54
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM06350, KM07031
Date of study initiation:	October 11, 2006
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	See Doses/Vehicle (below)
Animal species/strain/sex per dose:	Rat/ Han Wistar (Crl: WI); (b) (4) 6 males/group
Age of animals:	8 weeks at initiation of study
Weight:	240-345g
Doses/Vehicle:	2.5 mmol/kg each of: Gadovist (Gadobutrol), Omniscan (Gadodiamide), Magnevist (Gadopentetate dimeglumine), OptiMARK (Gadoversetamide), MultiHance, Gadobenate dimeglumine, Dotarem (Gadoterate), Vasovist/Ablavar (Gadofosveset trisodium), Gd-EDTA, Eovist (Gadoxetate disodium), Caldiamide (the calcium chelate in Omniscan)/Saline vehicle.
Duration/route:	Daily injections for 4 weeks (5 days/week); Intravenous

Key findings:

- Significant macroscopic and microscopic lesions were observed in Omniscan-treated animals. However, no gross or histopathologic effects were observed in animals treated with Gadovist, Magnevist, MultiHance, Eovist, Dotarem and Vasovist.
- All animals treated with gadoversetamide (OptiMARK®) (containing 5% excess free ligand), developed microscopic skin changes. However, only 1 of 12 animals developed microscopic lesions when treated with standard OptiMARK formulation containing at least 10% excess free ligand.
- An increasing the level of excess free ligand in Gadodiamide or Gadoversetamide formulations up to 10% resulted in a decrease in skin Gd concentration in animals treated with these compounds compared to animals treated with either of these compounds with 0% excess ligand.
- No skin changes were observed in animals treated with Caldiamide (both 0.5 and 2.5 mmol/kg) and saline.

- Gd was detected in the skin, liver and femur in all animals five days after the last administration of Gd-containing substances. The amount of Gd differed among the contrast agents and across tissues with prominent differences being observed in the skin and femur.
- The non-ionic, linear compounds, Omniscan and OptiMARK, showed a higher accumulation in the skin and femur compared to other marketed products. Lower amounts were obtained with Gadovist, Magnevist and Multihance and the lowest Gd concentrations were detected in Eovist®-treated animals.

Objectives: This study was performed to determine a) if multiple intravenous administrations of GBCAs induced NSF-like lesions; b) amounts of Gd in tissues following such administration, and c) evaluate the role of excess ligand on the development of NSF-like skin lesions and Gd concentration in tissues.

Methods:

- 2.5 mmol/kg (25x clinical dose) of Gadovist, Dotarem, MultiHance, Magnevist, Eovist, Vasovist, OptiMARK and Omniscan were administered at the doses in the Table below.
- For comparability, 2.5 mmol/kg of Gadodiamide, Gadoversetamide and Caldiamide (calcium chelate in Gadodiamide) were studied.
- Lower doses (0.5 mmol Gd/kg) Gadodiamide and Caldiamide were evaluated to determine any potential dose relationship.
- Lower doses of Gd-EDTA were used (i.e. 0.1 and 0.05 mmol Gd/kg) due to low LD₅₀.
- Formulations of gadodiamide and gadoversetamide, without and with 5% (gadoversetamide) or 10% (gadodiamide) excess ligand, were tested at doses of 0.5 (gadodiamide only) and 2.5 mmol/kg, respectively.

<u>Test article</u>	<u>Test Dose</u>	<u>Dose (mmol Gd/kg)</u>	<u>Multiples of clinical dose</u>
Gadovist (M)	0.1	2.5	25x
Dotarem (M)	0.1	2.5	25x
MultiHance (L, I)	0.1	2.5	25x
Magnevist (L, I)	0.1	2.5	25x
Eovist (L, I)	0.025	1.0	40x
Vasovist (L, I)	0.03	1.0	33x
OptiMARK (L, NI)	0.1	2.5	25x
Omniscan (L, NI)	0.1	2.5	25x

_Reviewer's Table; (M) = Macrocyclic, (L, I) = Linear, Ionic, (L, NI) = Linear, Nonionic

- Test articles were administered intravenously daily five times per week for 4 weeks using 6 rats per group. Due to severe skin lesions, animals that received

2.5 mmolGd/kg Gadodiamide or Gadoversetamide were administered only 10 injections.

- Blood samples to determine serum levels of Zn and Cu were taken before treatment and 5 days after the last injection.
- Animals were sacrificed 5 days after the 20th injection.
- Tissue samples were taken from skin, femur, and liver to determine the Gd, Zn and Cu contents using Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES). Bound and free metal ions were not distinguishable by this method.
- Body weight and macroscopic changes of the skin were recorded at the time of each injection. Samples from femur and liver were collected and preserved.
- Organ weights were determined and histopathological evaluation of the skin was performed.

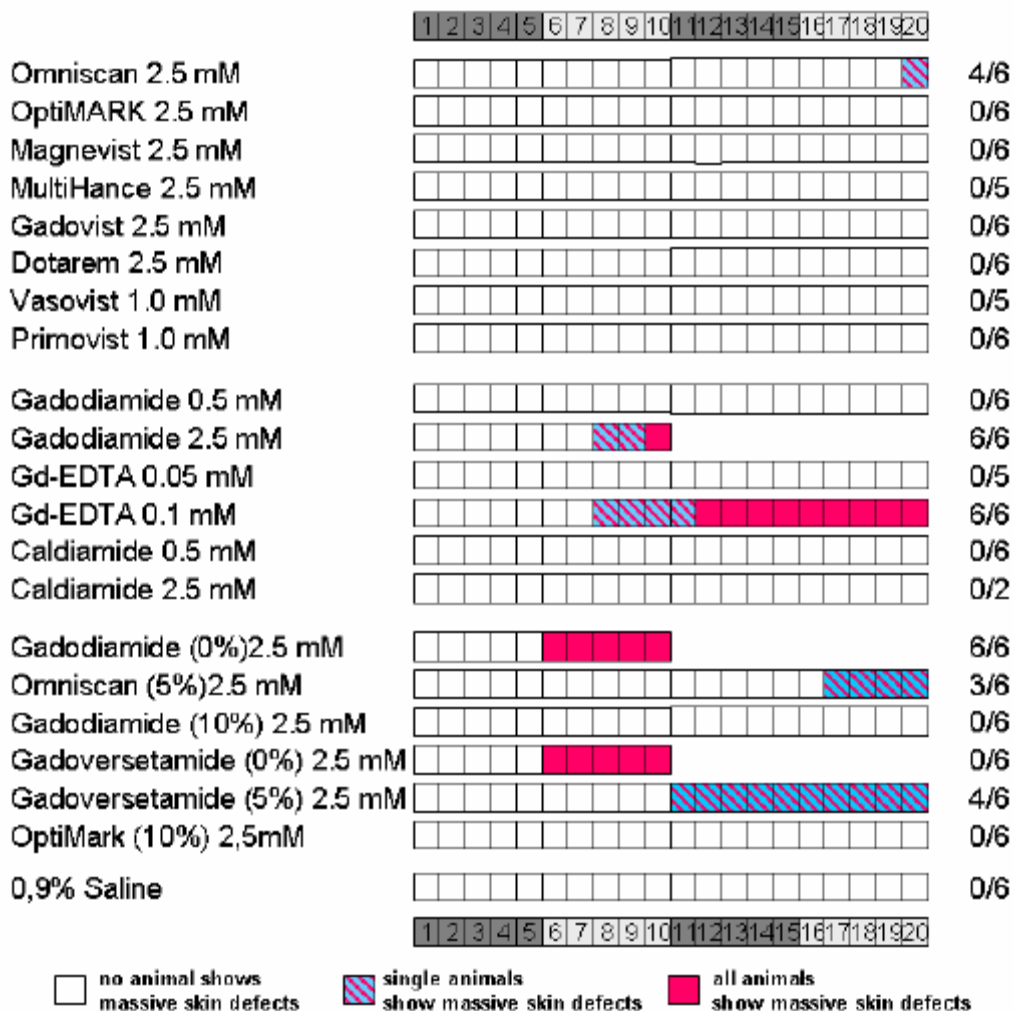
Results:

Body weight: GBCAs did not result in significant reduction in body weight gain.

Macroscopic skin changes:

From day 6 onwards, visible skin changes (erythema, multifocal ulcerations and multiple crusts) developed in all rats administered Gadodiamide (2.5mmol/kg; 0% excess free ligand), Gadoversetamide (2.5mmol/kg; 0% excess free ligand), and Gd-EDTA (0.1mmol/kg). Treatment was discontinued on the 10th day due to severe skin lesions.

The following Table summarizes the observed skin lesions:



Microscopic skin changes:

8 of 12 rats treated with Omniscan (Standard formulation with 5% excess free ligand) developed microscopic skin changes. Microscopic skin changes were not observed with Gadovist, Magnevist, Primovist, Vasovist, Multihance and Dotarem.

The microscopic findings are summarized in the Table below:

Table 82: Microscopic skin effects (Report A40180)

Test article	Dose (mmol/kg)	% Excess ligand	No. of animals with lesions	Presence of lesions
Omniscan	2.5			
OptiMARK	2.5			
Magnevist	2.5		0/6	
MultiHance	2.5		0/6	

Gadovist	2.5		0/6	No lesions
Dotarem	2.5		0/6	
Vasovist	1.0		0/6	
Eovist	1.0		0/6	
Gadodiamide	0.5		4/6	Lower severity skin changes
Gadodiamide	2.5			
Gd-EDTA	0.05			No lesions
Gd-EDTA	0.1			Epidermis: ulceration crusts, acanthosis Dermis: dermatitis, ↑ cellularity
Caldiamide	0.5			No lesions
Caldiamide	2.5			No lesions
Gadodiamide	2.5	0		Epidermis: ulceration crusts, acanthosis Dermis: dermatitis, ↑ cellularity
Omniscan	2.5	5	8/12	Lesions present
Gadodiamide	2.5	10	0/6	No lesions
Gadoversetamide	2.5	0		
Gadoversetamide	2.5	5	6/6	Epidermis: ulceration crusts, acanthosis Dermis: dermatitis, ↑ cellularity
OptiMARK	2.5	10	1/12	lesion
0.9% saline				

Reviewer's Table constructed with sponsor data; **Calcinosis cutis** (mineralization) was observed in animals from different treatment groups including those treated with caldiamide but not in animals treated with Gd-EDTA, Gadodiamide (0.5 and 2.5 mmol/kg, MultiHance or Dotarem, and saline controls

Tissue Gadolinium, Zinc and Copper:

a). Gadolinium concentrations in skin, femur and the liver (Data is summarized in Table below)

Skin: The highest concentration of Gadolinium in skin was observed in rats treated with formulations of Gadodiamide (2.5mmol/kg) or Gadoversetamide (2.5 mmol/kg) without excess ligand, Gd-EDTA (0.1 mmol/kg) and Omniscan®.

This was followed by animals treated with Gadodiamide (0.5mmol/kg), Gd-EDTA (0.05mmol/kg), Gadodiamide (2.5mmol/kg; 10% excess free ligand) and OptiMARK®.

The Gd concentrations in the Omniscan-treated rats was nearly 10-fold the amount detected following treatment with Magnevist, MultiHance or Vasovist and >30-fold the amount following Gadovist and Dotarem. The lowest amount of skin Gd was measured in animals treated with Eovist.

Femur: The highest concentration of Gd in bone occurred following the administration of Gd-EDTA (2.5 and 0.5 mmol/kg). This was followed by Gadodiamide, Omniscan, Gadoversetamide, and OptiMARK (regardless of level of excess free ligand in the formulation).

The lowest concentrations of Gd in bone occurred in animals treated with Gadovist, Dotarem and Eovist..

Liver: The highest levels of Gd were found after Gadodiamide and Gadoversetamide (each without excess free ligand), and after Gd-EDTA treatment.

Of the GBCAs tested, the highest Gd concentration in the liver was found after Omniscan treatment. Lower Gd concentrations were observed Magnevist, MultiHance, Gadovist, Dotarem or Vasovist. The lowest Gd concentration was obtained with Eovist.

Table 83: Gd concentrations in skin, femur and liver after GBCA injection in rats

Test article	Dose (mmol/kg)	Tissue Gd (nmol/g tissue)		
		Skin	Femur	Liver
Study KM06350				
Gadodiamide (Omniscan without excess ligand)	2.5	2202.7 ± 112.5	582.6 ± 72.8	2911.1 ± 244.7
OptiMARK	2.5	428.7 ± 85.9	360.4 ± 57.5	298.5 ± 50.0
Omniscan	2.5	1697.3 ± 244.1	581.8 ± 103.1	415.1 ± 59.5
Magnevist	2.5	184.0 ± 78.4	220.5 ± 60.5	216.8 ± 24.0
Gd-EDTA	0.05	730.8 ± 37.9	1169.0 ± 249.3	1321.7 ± 54.7
Eovist	1.0	10.0 ± 2.8	38.3 ± 9.8	25.9 ± 4.1
Dotarem	2.5	51.1 ± 11.5	46.0 ± 21.9	197.3 ± 30.1
MultiHance	2.5	82.5 ± 10.5	106.3 ± 3.9	214.4 ± 20.4
Gadovist	2.5	49.1 ± 8.8	69.3 ± 10.2	162.1 ± 27.2
Caldiamide	2.5	<10	<10	<10
Caldiamide	0.5	<10	<10	<10
Vasovist	1.0	109.8 ± 19.6	96.5 ± 11.3	154.2 ± 17.3
Gadodiamide (Omniscan without excess ligand)	0.5	608.4 ± 45.5	166.4 ± 34.7	779.1 ± 32.3
Gd-EDTA	0.1	1743.1 ± 107.9	2304.9 ± 306.7	2497.1 ± 149.1
Study KM07031				
Gadodiamide (Omniscan without excess ligand)	2.5	1536.4 ± 112.2	705.2 ± 33.6	2435.6 ± 148.7
Omniscan	2.5	1475.4 ± 524.6	707.1 ± 185.7	377.9 ± 71.3
Gadodiamide (Omniscan with 10% excess ligand)	2.5	590.1 ± 145.0	598.1 ± 148.3	375.6 ± 60.4
Gadoversetamide (OptiMARK without excess ligand) mmol/kg		1399.4 ± 301.3	510.2 ± 43.1	2798.8 ± 657.5
Gadoversetamide (OptiMARK with 5% excess ligand)	2.5	2040.4 ± 231.6	577.1 ± 102.7	579.0 ± 87.4
OptiMARK	2.5	429 ± 86	360 ± 58	299 ± 50
Saline 0.9%		<10	<10	<10

Reviewer's table constructed with sponsor's data

Zinc concentrations in skin, femur and the liver (Data not shown):

Treatment with Gadodiamide) and caldiamide, each at 2.5 mmol/kg, resulted in elevated Zn levels in the skin.

Comparable amounts of Zinc were observed with all other treatments. The levels of zinc measured in the femur or in the liver were comparable in all treatment groups.

Tissue copper (Cu) levels: There were no remarkable differences copper levels following treatment in the various groups.

Serum levels of Zinc and Copper: Compared to baseline, there was a decrease in Zn serum level after treatment with Gadoversetamide (2.5 mmol/kg; 5% excess ligand), and Optimark (2.5 mmol/kg). There was no change in Zn serum levels with other treatment groups. There was no change in serum Cu concentration after the treatment in all groups except in rats treated with 2.5mmol/kg Gadodiamide and 0.1mmol/kg Gd-EDTA.

Conclusions:

- Following the administration of GBCAs, significant macroscopic and microscopic skin lesions were observed in Omniscan-treated animals. However, there were no gross or histopathologic skin changes in animals treated with Gadovist, Magnevist, MultiHance, Eovist, Dotarem and Vasovist.
- Animals administered gadoversetamide (containing 5% excess free ligand) developed microscopic skin changes while similar changes developed in only 1/12 animals treated with the standard OptiMARK® formulation containing 10% excess free ligand.
- No skin changes were observed in animals treated with Caldiamide (0.5 or 2.5 mmol/kg) or animals administered saline.
- Increased free ligand in Gadodiamide and Gadoversetamide formulations up to 10% resulted in decreased skin Gd compared to animals treated with either compound lacking excess free ligand.
- Although most prominent in skin and bone, Gd was detected in varying amounts in the skin, liver and femur of all animals treated with Gd-containing compounds.
- The high Gd concentration in the skin was associated with the presence of skin lesions.
- The non-ionic, linear Gd-chelates, Omniscan® and OptiMARK®, showed higher amounts of Gd in skin and femur compared to other products. Lowest Gd in skin and femur occurred in Eovist®-treated animals.
- No remarkable differences were observed between treatment groups in serum and tissue concentrations of Zn and Cu.

Reviewer's comments: I agree with the conclusions of the sponsor that macroscopic and microscopic skin lesions were most extensively observed in animals treated with 2.5 mmol/kg of Gadodiamide or Gadoversetamide without excess free ligand, Omniscan and 0.1 mmol/kg of Gd-EDTA.

Since Gadodiamide and Gadoversetamide formulations containing a high level of excess free ligand resulted in little or no macroscopic or microscopic skin changes, the

sponsor postulated a protective effect of excess free ligand on skin changes. While plausible, it cannot be substantiated by the results of the study.

Since there were no skin changes in animals treated with caldiamide (calcium chelator in Omniscan), I agree with the sponsor that caldiamide might be excluded as a causative factor in the occurrence of Omniscan-induced skin lesions.

The results show a potential for Gd deposition in bone. In preclinical models very small amounts of Gd are retained in the bone and liver, and the amount retained correlates with the kinetic and thermodynamic stability of the GBCA with respect to Gd release in vitro (Aime and Caravan, 2009) showed an inverse relationship correlation between the amount of excess ligand present in Gd-containing contrast agents and the amount of Gd in the tissue, and further underline the importance of the inherent stability of these agents in the development of NSF.

10.1.2.2 Report No. A40160:

Study title: Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs and zinc depletion on occurrence of NSF-like skin lesions in rats.

Volume # and Page #:	Module 4.2.3.7.7, 47 pages
Conducting laboratory and location:	Bayer Schering Pharma/Bayer HealthCare, Germany
Study #:	KM06278
Date of study initiation:	June 2006
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Omniscan, Optimark, Magnevist, Gadovist, Gadopentetate, Gd-EDTA; lot #, N/A% purity: N/A
Animal species/strain/sex per dose:	Rat/ Male Han Wistar (CrI: WI), (b) (4)
Age:	8 weeks (at initiation of study)
Weight:	94-186 g (at study initiation)
Doses/Vehicle:	2.5 mmol/kg per test article
Duration/route:	5 consecutive injections/week for 5 weeks

Key findings:

- Results showed no remarkable effect of Zn on the NSF-like skin lesions.
- Gd-EDTA-treated animals developed macroscopic skin lesions irrespective of zinc content in diet. Rats administered Gadovist, OptiMARK, Magnevist, Omniscan or Gadopentetate showed no detectable skin lesions.

- Histopathologic lesions were present in animals treated with Omniscan/Gadodiamide or Gd-EDTA and fed either the zinc-depleted or control diets.
- No microscopic findings were observed in animals treated with OptiMARK, Magnevist, Gadovist or Omniscan regardless of diet type.
- No histopathologic findings were observed in untreated animals regardless of diet.
- There was no remarkable difference in skin Gd concentration between rats receiving the zinc-depleted diet and the control diet.

Methods:

- The study evaluated the effect of endogenous zinc depletion in addition to the administration of different GBCAs namely, Gadobutrol, Omniscan, OptiMARK, Magnevist, Gadopentetate (the drug substance of Magnevist with no excess free ligand) and Gd-EDTA, a Gd-based compound with low in-vivo stability.
- Groups of 6 male rats were each injected intravenously with Gadovist, Omniscan, Optimark, Magnevist, Gadopentetate, Gd-EDTA once daily for 5 weeks (i.e., 5 consecutive days per week or 25 days) at a high dose via the tail vein.
- Gadovist, Omniscan, Optimark, Magnevist and Gadopentetate were administered 2.5 mmol/kg, a dose 25-fold the standard clinical dose of 0.1 mmol Gd/kg.
- For comparability, similar doses were used for Gadodiamide and Gadopentate. Due to the high acute toxicity of Gd-EDTA, a significantly lower dose (0.1 mmol/kg) was used.
- Two groups of 6 male Wistar were treated with Gd-containing compounds and kept on a Zinc-depleted diet (Test group). The control group of rats were treated with GBCAs (including Gd-EDTA) and fed a control diet. Test and control groups were kept on respective diets 6 weeks prior to injection of contrast agents.

The study groups are described in the Table below:

Table 84: Study groups for Report A40160

Study group	Contrast agent	Dose (mmol/kg)	Diet
Test	OptiMARK	2.5	Zn-depleted Diet (8 mg Zn/kg)
	Magnevist		
	Omniscan (Gadodiamide without excess ligand)		
	Gadovist		
	Omniscan		
	Gadopentetate (Magnevist without excess ligand)		
	Gd-EDTA	0.1	
	Untreated	0	
Control	OptiMARK	2.5	Control Diet (65 mg Zn/kg)
	Magnevist		
	Omniscan (Gadodiamide without excess ligand)		
	Gadovist		
	Omniscan		
	Gadopentetate (Magnevist without excess ligand)		
	Gd-EDTA	0.1	
	Untreated	0	

Clinical examination: All animals in each group were examined at the time of each injection. Body weight was taken and possible pathological signs (as shown below) were scored as shown below:

0 reduction of weight to previous day	5 yellow urine
1 shaggy fur	6 by ears bald, bloody and scabby
2 Danders	8 bald areas
3 bloody areas	9 bloody rings under the eyes
4 aggressive/ pain during administration	

Body weight: Body weight was determined at time of each drug injection. Terminal organ weights were recorded at the end of the study.

Tissue sampling and histopathology:

Animals were sacrificed five days after the 25th injection of contrast agent or day 38 after the first injection). At necropsy, the skin was examined for gross lesions.

Samples of skin, femur and liver were taken from each animal for histopathologic examination. Microscopic findings were scored as described below:

Grade	Description of lesions
1	minimal, very few, very small
2	slight, few, small
3	moderate, moderate number, moderate size
4	marked, many large
5	massive, extensive number, extensive size

Gd, Zn and Cu concentrations in tissues and organs: Tissue probes of the skin, femur, liver, kidneys, spleen, lung, heart, and muscle were obtained to determine Gd, Zn and Cu concentrations using the Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) method.

Zinc and Copper concentration in the serum: Serum levels of Zn and Cu were determined in blood samples 6 weeks before the 1st, 5th, 10th, 15th, 20th, 25th and 5 days after the 25th injection

Results:

Body and Organ weight: An increase in body weight was observed in animals in all treatment groups with the exception of the Gd-EDTA and Omniscan/Gadodiamide groups. A decrease in weight due to Zn depletion was observed. Animals treated with Gd-EDTA lost weight in both diet groups (i.e., with and without Zn depletion). No remarkable differences were observed in kidney and liver weight between the treatment groups. Organ weight was recorded as percentage of body weight. No effect of Zn-depletion was detected in this study. There were no differences between the treatment groups in the weights of the heart, lungs and spleen.

Macroscopic and microscopic changes of the skin:

Macroscopic lesions: All animals administered Gd-EDTA developed macroscopic lesions (erythema, ulcerations and crusts) in the skin regardless of the dietary zinc.

The progress of lesion development was faster in animals fed the zinc-depleted diet. None of the rats that received OptiMARK, Magnevist, Gadovist, Omniscan or Gadopentetate developed any detectable skin lesions.

Microscopic lesions: No histopathologic findings were observed in animals treated with Gadovist, OptiMARK and Magnevist regardless of diet. The microscopic lesions in each diet group are summarized in the following sponsor tables:

	KM06278 / Zn deficient diet							
Group	1.1	1.2	1.3	1.4	1.5	1.6	1.7	K2
Tested article	OptiMARK*	Magnevist*	Omniscan/ Gado-diamide*	Gadovist	Omniscan	Gadopen-tetate*	Gd-EDTA	untreated
DOSE	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	0.1 mmol/kg	
n	5	4	5	6	6	5	6	3
Diagnosis	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity
no findings	5	4		6	5	4	1	3
Crust	-	-	5 2.8	-	1 1	1 2	2 1.5	-
Ulceration	-	-	5 2	-	-	1 1	1 2	-
Acanthosis	-	-	5 2	-	-	1 2	3 1.5	-
Acantholysis	-	-	1 2	-	-	-	-	-
Dermo-Epidermal Cleft(s)	-	-	2 1	-	-	1 1	2 1	-
Interface Dermatitis	-	-	5 2	-	-	1 3	4 2.2	-
Cellularity, Increased	-	-	5 1.8	-	-	1 2	4 1.2	-
Fibrosis/Sclerosis	-	-	5 1.6	-	-	1 1	4 1	-

* In some groups animals did not survive the treatment period due to anesthetic accident (at the study beginning n=6 animals per group)

	KM 06278 / Zn control diet							
Group	2.1	2.2	2.3	2.4	2.5	2.6	2.7	K1
Tested article	OptiMARK	Magnevist*	Omniscan/ Gado-diamide	Gadovist*	Omniscan	Gadopen-tetate*	Gd-EDTA	untreated
DOSE	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	2.5 mmol/kg	0.1 mmol/kg	
n	6	5	6	5	6	5	6	3
Diagnosis	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity	total affected mean severity
no findings	6	5		4	5	5		3
Crust	-	-	3 3	-	- -	-	2 1	-
Ulceration	-	-	2 2	-	- -	-	1 1	-
Acanthosis	-	-	5 1.4	-	1 1	-	5 1.4	-
Acantholysis	-	-	1 1	-	- -	-	-	-
Dermo-Epidermal Cleft(s)	-	-	2 1	-	- -	-	1 1	-
Interface Dermatitis	-	-	6 2	-	- -	-	6 1.3	-
Cellularity, Increased	-	-	5 1.4	-	- -	-	6 1.2	-
Fibrosis/Sclerosis	-	-	5 1	-	- -	-	6 1	-
Calcinosis cutis	-	-	-	1 2	-	-	-	-

* In some groups animals did not survive the treatment period due to anesthetic accident (at the study beginning n=6 animals per group)

- Animals treated with Omniscan and Gd-EDTA fed either Zn-depleted or control diets had extensive histopathologic lesions.

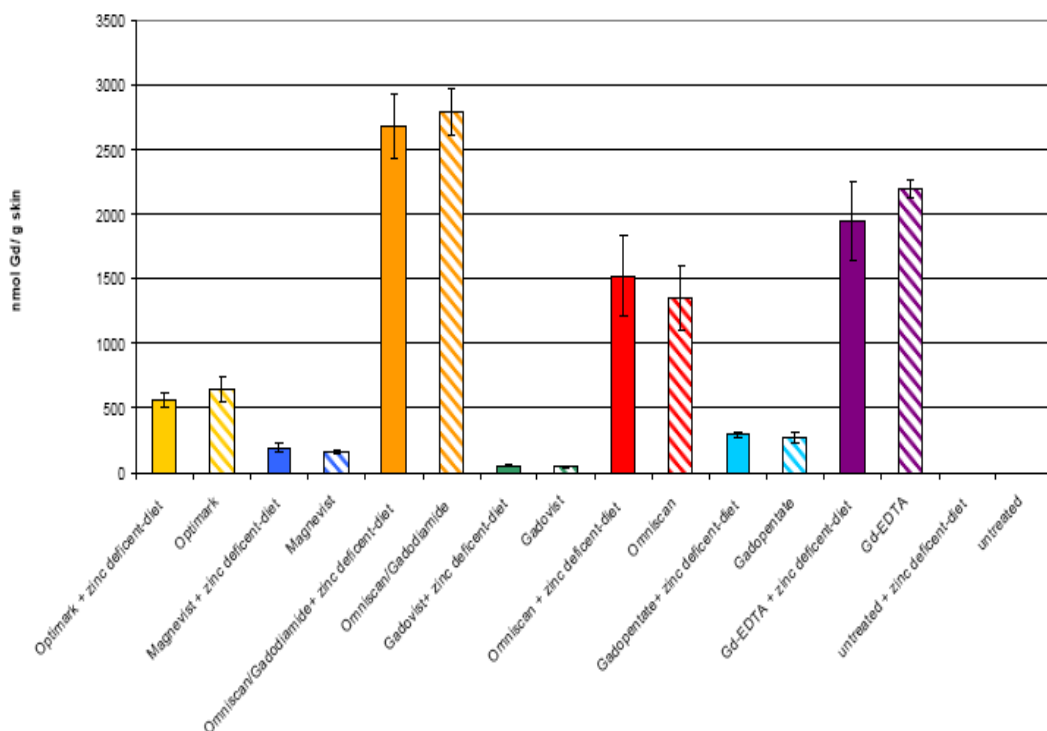
- Microscopic lesions were observed in animals administered Gadopentetate and Zn-depleted diet.
- There were no microscopic findings in animals treated with Gadovist, OptiMARK, Magnevist, or Omniscan regardless of diet type.
- No histopathologic findings were observed in untreated animals fed either diet.

Tissue Gd, Zn and Cu concentrations:

Skin Gd –

- The highest Gadolinium concentrations in all tissue specimens (except the kidney) were obtained in animals treated with Gd-EDTA.
- Gd concentrations were highest in the skin samples with the highest concentrations obtained in animals treated with Gd-EDTA or Omniscan followed by OptiMARK, Gadopentetate and Magnevist.
- The lowest Gd concentrations were in animals treated with Gadovist.
- No remarkable difference was found in skin Gd between rats based on type of diet type.
- The Figure shown below describes the Gd concentration (nmol/g skin) in animals in the different treatments.

Figure 5:



Gd concentration in the femur and liver - Gd concentration in femur and liver varied across treatment groups. Higher values were obtained following treatment with Omniscan and OptiMARK compared to animals treated with Magnevist or Gadovist.

Zn concentration in tissue specimens –

- Zn was evaluated in skin, femur, liver, heart, lung, spleen, muscle and kidney. No remarkable differences in tissue Zn content were found between the treatment groups .
- Except for a lower amount of Zn in the femur, the effect of Zn depletion on tissue concentration of Zn was not apparent.
- No remarkable difference in Zn concentrations was observed between treatment groups in heart, lung, muscle and kidney tissue. There was no detectable influence of zinc depletion on Gd concentration for any of the treatment groups.

Cu concentration in tissues: No differences in Cu levels were detected between treatment groups in the skin, femur, liver, heart, lung, spleen and muscle regardless of the Zn content of the diet. Higher Cu concentrations were observed in the untreated animal groups when compared to the treatment groups.

Zn and Cu concentration in the serum: Feeding the Zn depletion diet resulted in a decreased serum Zn in all the treatment groups.. No changes in Zn levels were found in control animals.

Reviewer's comments:

Depletion of endogenous Zinc ions has been suggested as a possible pathomechanism for nephrogenic systemic fibrosis. Based on the findings of this study, there did not seem to be a compelling evidence for a role of Zn depletion in the occurrence of NSF-like skin lesions in rats following treatment with different GBCAs. A zinc supplementation study by Pietsch et al (2009).

The following deficiencies were identified in this study. a) the basis for zinc depletion in the diet was not established., b) symptoms of identifiable with zinc depletion in animals were not described. If identified and monitored, these symptoms could more clearly serve to distinguish effects attributable to GBCAs from those due to Zn depletion or even deficiency c) sponsor claimed in the results that a decrease in weight due to zinc deficiency was observed but did not provide any evidence as to how this link between zinc deficiency was determined or its implications.

10.1.2.3 Report No. A42495:

Study title: Potential long-term retention of Gadolinium based contrast agents after intravenous administration in rats

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 157
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM06426, KM07218, KM07307
Date of study initiation:	October 2006
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Omniscan, OptiMARK, Magnevist, Prohance, Dotarem and Gadovist, lot #: N/A and % purity: N/A
Animal species/strain/sex per dose:	Rat, Male (M) Han Wistar (CrI:WI), (b) (4)
Age of animals:	~8 weeks (at start of treatment)
Weight:	210-346 g
Doses/Vehicle:	2.5 mmol/kg/GBCA; saline vehicle
Duration/route:	5 consecutive days/ intravenous (tail vein)

Key findings:

- No macroscopic changes were observed in the skin in any treatment group.
- The elimination of Gd from the rat skin decreased considerably In the immediate phase starting after injection and lasting for days to weeks for macrocyclic and linear GBCAs, respectively. T
- The levels of the linear GBCAs Gd were significantly above the level observed in control animals for up to a year after the last Gd injection and were most pronounced in animals treated with Omniscan. Low levels of Gd were observed for the macrocyclic GBCAs in the skin as in control animals.
- The findings of the study indicate long-term retention of GBCAs in the skin.
- Skin Gd retention was longer for the ionic compared to macrocyclic GBCAs.
- The methods used to measure Gd levels could not distinguish between the various forms of Gd.

Objectives: The purpose of the study was to determine the elimination time course of Gd in the skin and evaluate the potential long-term retention of Gd in skin following intravenous administration of different marketed Gadolinium-based contrast agents (GBCAs).

Methods:

- Six animals per group were administered Gadovist, Omniscan, OptiMARK, Magnevist, MultiHance, Prohance, or Dotarem.. Control groups comprised of one group administered saline and another received no treatment (untreated controls).

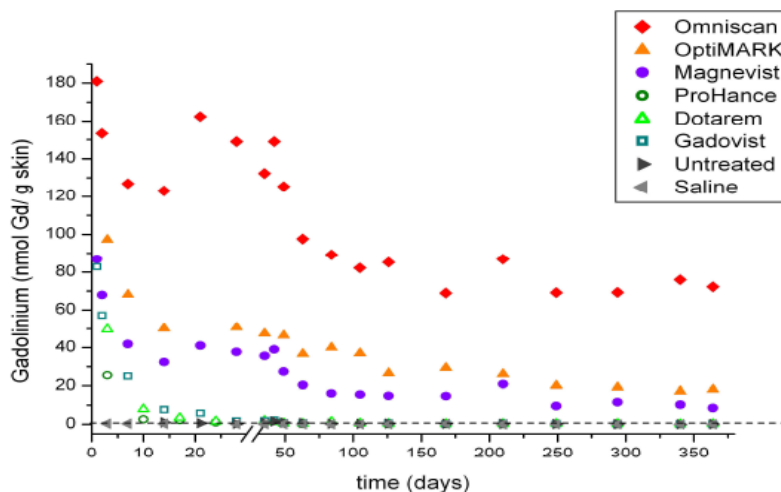
- Gadovist, Omniscan, OptiMARK, Multihance, Magnevist, Prohance and Dotarem were administered intravenously 2.5 mmol/kg body weight daily for five consecutive days.
- Animals were examined daily for macroscopic skin changes.
- Skin biopsies were taken at different time points up to 365 days post-injection.
- Gadolinium concentration in the skin was measured by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS). The ICP-MS method did not distinguish between chelated and free (unchelated) Gd.
- Body weight was determined at the time of each injection.
- To determine the pharmacokinetic parameters of four GBCAs (Gadovist, Omniscan, Magnevist, and Multihance), a single dose of 2.5 mmol/kg of each compound was administered intravenously and arterial blood samples taken at 1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 240 min.
- For histology, skin samples from all animals were fixed formalin, dehydrated, embedded in paraffin and 4-6µm thick sections stained with hematoxylin-eosin for microscopic examination.

Results:**Gd concentration in the skin:**

To monitor skin Gd concentration over time, biopsies were obtained periodically at different intervals over the course of one year from animals treated with Gadovist, Dotarem, Prohance, OptiMARK and Omniscan at a dose of 2.5 mmol Gd/kg for five consecutive days, skin Gd concentration decreased in all the treatment groups over several weeks from a peak of 180 nmol Gd/g of skin (Figure xx).

Results showed that early and long-term deposition of Gd occurred in the skin with most Gd eliminated from the skin within 2 months. A longer-term retention of a smaller portion was also observed with all tested compounds. After one year, Gd concentration was lowest in animals treated with macrocyclic GBCAs (Gadovist group). Intermediate amounts were observed with Magnevist (ionic linear GBCA), while the highest concentrations were obtained with linear nonionic GBCA compounds (Omniscan and OptiMARK)

Figure 6: Gd concentration in skin biopsies taken from animals treated with different GBCAs



The early and long-term skin Gd levels for each GBCA measured over a period of one year is shown in the Table below:

Table 85: Skin Gd levels following treatment with different GBCAs

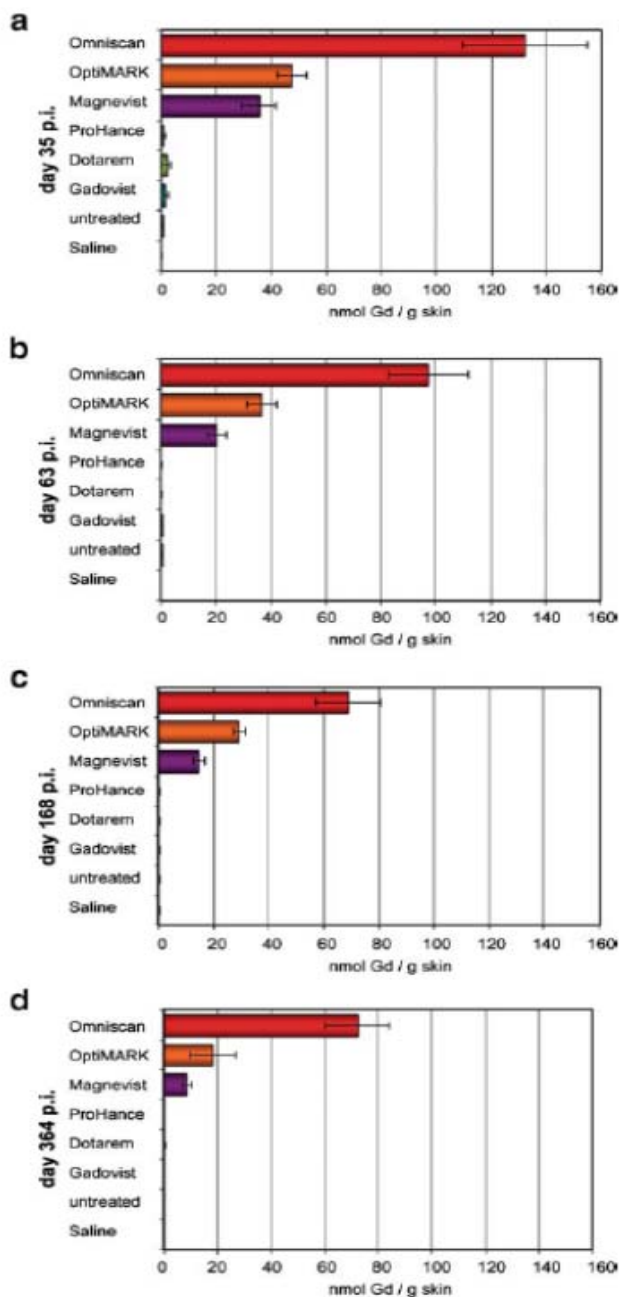
Days p.i	GBCA Structural class	GBCA	Gd conc. in skin (nmol/g skin)
Day 35	Macrocyclic	Gadovist	2 ± 1
		ProHance	1 ± 1
		Dotarem	2 ± 1
	Ionic, Linear	Magnevist	36 ± 6
	Non-ionic, Linear	OptiMARK	47 ± 5
		Omniscan	132 ± 23
Day 364	Macrocyclic	Gadovist	0.06 ± 0.03
		ProHance	0.08 ± 0.02
		Dotarem	0.22 ± 0.17
	Ionic, Linear	Magnevist	9 ± 2
	Non-ionic, Linear	OptiMARK	18 ± 5
		Omniscan	72 ± 12
	Untreated control		0.06 ± 0.03
	Saline-treated control		0.18 ± 0.07
Reviewer's Table constructed from sponsor data			

Reviewer's Table constructed from sponsor data

In a comparison of Gd concentration in the skin biopsies taken on days 35, 63, 168 and 364 after treatment (Figure xx), skin Gd was consistently in the order

Omniscan>OptiMARK>Magnevist > (ProHance, Dotarem, Gadovist, untreated and saline) groups.

Figure 7: Comparison of skin Gd concentration in biopsies taken on day 35 (a), day 63 (b), day 168 (c) and day 364 (d)



The lowest values for total exposure to Gadolinium was observed after treatment with the macrocyclic compounds (ProHance, Dotarem, Gadovist). Higher values were

observed after treatment with Magnevist (ionic linear) and the highest values were observed after treatment with the non-ionic linear GBCAs (Omniscan, OptiMARK).

Differences were significant between treatment with macrocyclic GBCA Gadovist and the treatment with the non-ionic linear GBCAs Omniscan and OptiMARK. The differences were also significant between treatment with the non-ionic linear GBCA Omniscan and treatment with the ionic linear GBCA Magnevist and macrocyclic GBCA Gadovist treatment.

Histology of the skin and kidney:

- The administration of Gadodiamide for five days was associated with histological lesions of the skin. The findings included dilatation of hair bulbs, acanthosis and superficial mixed cell infiltration. Lymphocytes and dendrite-like cells were observed. Epidermal crusts were present and superficial infiltration of the dermis was noted in 1/5 Omniscan-treated animals.
- Animals treated with Gadovist or Magnevist showed no histological lesions.
- No data was presented for histology of the kidney.

Pharmacokinetic (PK) parameters of GBCAs: The serum Gadolinium levels in the PK study (# KM07307) of GBCAs in rats and PK parameters in the blood are described in the following Tables:

Table 86: Serum Gadolinium levels ($\mu\text{mol Gd/L}$) after administration of 2.5mmol/kg of GBCAs in rats

Time (min)	Omniscan		Magnevist		Gadovist	
	Linear, Nonionic		Linear, Ionic		Macrocyclic, Non-ionic	
	Mean	$\pm\text{SD}$	Mean	$\pm\text{SD}$	Mean	$\pm\text{SD}$
1	13452.2	630.8	14031.1	1678.9	12236.0	686.1
3	8429.9	560.3	8542.7	305.3	7706.8	628.7
5	6521.9	362.5	6690.2	126.6	6318.3	456.1
10	4394.9	635.0	4220.3	152.3	4348.7	50.8
15	3404.0	613.5	3214.2	144.0	3297.3	352.7
30	1834.1	424.3	2005.3	167.2	1811.5	186.9
60	657.2	201.3	790.2	89.6	645.6	198.3
90	236.1	97.5	295.8	18.7	256.6	119.0
120	97.7	50.4	131.3	18.7	102.8	58.6
180	19.1	12.0	28.5	5.0	21.1	14.1
240	6.0	4.8	6.9	2.1	6.6	3.3

Reviewer's Table constructed from sponsor data

Gd concentration for Omniscan, Magnevist and Gadovist decreased progressively with time up to 240 min after the last injection. For each time point the Gd concentration was Omniscan > Magnevist > Gadovist.

Table 87: Pharmacokinetic parameters in blood after the administration of 2.5 mmol/kg of GBCAs in rats

	Omniscan		Magnevist		Gadovist	
	Linear, Nonionic		Linear, Ionic		Macrocyclic, Non-ionic	
	Mean	±SD	Mean	±SD	Mean	±SD
α -t _{1/2} [min]	1.8	0.3	1.9	0.4	1.7	0.3
β -t _{1/2} [min]	19.1	2.0	22.2	1.5	19.4	3.4
AUC [μmol/l*min]	190543.6	31931.4	200248.6	4983.7	184363.4	22985.0
V _c [L/kg]	0.1	0.0	0.1	0.0	0.2	0.0
V _{dss} [L/kg]	0.3	0.0	0.3	0.0	0.3	0.0
Total Clearance [mL/min*kg]	13.5	2.5	12.5	0.5	13.6	1.7

Reviewer's Table constructed from sponsor data

Values for pharmacokinetic (PK) parameters were similar for Omniscan, Magnevist and Gadovist

Conclusions :

- No macroscopic changes were observed in skin in any of the treatment groups during the whole course of the study.
- Gd concentration in the skin decreased gradually over several weeks lasting up to a year following the last injection in all the treatment groups.
- The highest values of Gd following treatment were observed with Omniscan followed by Magnevist.
- Lowest amounts were observed with Gadovist.
- The methods used to measure Gd levels could not distinguish between the various forms of Gd.

Reviewer's comments: I agree with the conclusions from this study.

10.1.3 Studies in Nephrectomized Rats

Summary: Since NSF frequently occurred in association with renal impairment, a rodent model of surgically induced renal impairment (5/6 nephrectomy) was used to evaluate the role of severely diminished renal function on the levels of Gd concentration and its long-term retention in the skin of rats following a 5-day intravenous administration of Gadovist, Magnevist, OptiMARK and Omniscan.

Prior to determining the effect of the GBCAs, an evaluation of the physiological status of the nephrectomized rats was performed. PK parameters and serum Gd levels were also determined in Omniscan-treated nephrectomized in order to evaluate the excretion pattern in the nephrectomy model. Non-nephrectomized rats were used as controls in both studies.

5/6 nephrectomy is a renal ablation procedure involving a two-phase surgical removal of one kidney and two-thirds of the other kidney. This model enables investigation of the influence of pharmacological, nutritive and other factors on functional and morphological renal parameters (Kujal and Vernerova, 2008).

10.1.3.1 Report No. A42496:

Study title: Potential long-term retention of Gadolinium in renally-impaired rats (5/6 nephrectomized) after intravenous administration of Gadolinium based contrast agents

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 55
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM07196, KM07231, and KM08015
Date of study initiation:	April 2008
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Omniscan, OptiMARK, Magnevist or Gadovist lot #: N/A, % purity: N/A
Animal species/strain/sex per dose:	Rat, Males / Han Wistar (CrI: WI) rats were obtained from (b) (4). 6 males per GBCA group
Age of animals:	N/A
Weight:	230-345 g (across studies)
Doses/Vehicle:	2.5 mmol/kg/GBCA; The vehicle used was not described
Duration/route:	5 days/intravenous administration

Key findings:

- It would appear from the findings of this study that 5/6 nephrectomized rats could be used a model for prolonged circulation time of GBCAs as seen in patients with severe renal impairment.
- Surgically-induced severe renal impairment resulted in delayed clearance of the administered GBCAs in the study animals.
- Significant amounts of Gd were observed in the skin following treatment with the non-ionic linear GBCAs, and the lowest Gd values were observed after treatment with the macrocyclic agents.

Study objectives: The purpose of this study was to determine the impact of prolonged circulation time of Gadolinium based contrast agents (GBCAs) resulting from a reduced renal clearance on the Gd concentration and the long-term retention of gadolinium in the skin of rats after administration of different GBCAs. An additional objective was to evaluate 5/6 nephrectomized rats as an animal model for prolonged circulation time of GBCAs seen in renally impaired patients. The data obtained with the 5/6 nephrectomized rats were compared with the data obtained in non-nephrectomized rats in study A42495.

Methods: Report A42496 consisted of three studies, KM07196, KM07231, and KM08015. The following Table describes the protocols in each of these three studies:

Table 88: Study Design (Report No. A42496)

Study #	Protocols
KM07231	Main study
	Measurement of Gadolinium in Organs
	Determination of body weight
	Statistics
KM07196	Pharmacokinetic parameters of GBCAs in 5/6 nephrectomized rats
KM08015	Serum markers in 5/6 nephrectomized rats
Reviewer's Table constructed from Sponsor data	

Groups of renally-impaired Han Wistar Rats (5/6-nephrectomized rats) were administered Omniscan, OptiMARK, Magnevist and Gadovist once daily for five consecutive days into the tail vein at a dose of 2.5mmol Gd/kg body weight. Skin biopsies, taken at different time points, were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Study KM08015 – Determination of Serum markers in 5/6 nephrectomized rats:

In order to monitor the physiological status of the 5/6-nephrectomized rats after surgery, blood samples from five animals was obtained approximately 7 weeks post-surgery to determine serum electrolytes, hematology and serum chemistry parameters. In addition, a histological evaluation of skin samples was performed approximately six weeks after the surgical removal of the kidneys.

The serum level of Gd was determined after a single administration of 2.5mmol Omniscan/kg. In addition, a variety of serum parameters were compared between 5/6 nephrectomized and non-nephrectomized rats. 5/6 nephrectomized rats had been previously evaluated in a pilot study as a model for a prolonged circulation time of GBCAs in rats.

Study KM07196 – Determination of pharmacokinetic parameters of gadolinium-based agents in 5/6 nephrectomized rats:

According to the sponsor, the excretion pattern of the investigated GBCAs (Omniscan, OptiMARK, Magnevist and Gadovist), had been demonstrated to be identical. Therefore, only Omniscan was assessed in evaluating the influence of kidney impairment on excretion. A single 2.5mmol/kg intravenous dose of Omniscan was administered and blood samples collected via the cannulated carotid artery at 1, 3, 5, 10, 15, 30, 60, 90, 120, 240, 360 and 1440 min post-injection. The Gd content in the serum was determined by inductively coupled atomic emission spectroscopy (ICP-AES) at a wavelength of 342.247nm. The pharmacokinetic parameters assessed included elimination $t_{1/2}$, AUC and total clearance.

Study KM07231 - Main study:

5/6 nephrectomized animals were randomized into six per group and were administered Omniscan, Optimark, Magnevist and Gadovist.

The compounds were injected intravenously for five consecutive days at a dose of 2.5mmol Gd/ kg body weight. Skin biopsies were taken under isoflurane narcosis with a biopsy punch and wounds sutured with Vicryl rapideTM.

The Gd concentration in skin samples was determined using Inductively-Coupled Plasma Mass Spectrometry (ICP-MS). As noted previously, ICP-MS could not distinguish between chelated and unchelated Gd. Body weight was determined at each time point of drug injection and biopsy time.

Results:

1. Determination of Serum markers in 5/6 nephrectomized rats:

Compared to non-nephrectomized rats, there was a significant increase in the serum concentration of various clinical chemistry parameters in 5/6 nephrectomized rats. As shown in Table (xx), a significant increase in serum creatinine, BUN, and ALT in nephrectomized compared to non-nephrectomized rats. Of these, creatinine would be an appropriate marker for a successful nephrectomy. Unlike human CKD patients, there was no increase in serum phosphate.

Hematology parameters in nephrectomized rats are not shown.

Table 89: Serum chemistry markers in Nephrectomized and Non-nephrectomized Rats (Mean \pm SD)

Serum chemistry parameters	5/6 Nephrectomized rats		Non- nephrectomized rats	
	Mean	\pm SD	Mean	\pm SD
AST (unit/L)	95.3	25.1	76.7	14.0
ALT (unit/L)	61.7	12.4	40.8	4.6
ALP (unit/L)	191.8	35.9	214.3	35.7
Glucose (mg/dL)	213.8	39.0	224.8	40.5
Cholesterol (mg/dL)	85.5	14.8	52.2	9.0
BUN (mg/dL)	52.8	7.7	18.3	2.6
Total protein (g/100mL)	6.0	0.2	5.8	0.2
Creatinine (mg/dL)	0.9	0.1	0.5	0.0
Calcium (mg/dL)	2.7	0.2	2.6	0.1
Sodium (mmol/L)	143.8	1.7	144.7	0.5
Potassium (mmol/L)	6.3	0.9	5.3	0.4
Chloride (mmol/L)	100.8	1.7	104.0	0.6
Phosphorus (mg/dL)	6.5	0.2	6.4	0.8

Reviewer's Table constructed from sponsor data; SD = standard deviation

2 Determination of PK parameters in Omniscan-treated nephrectomized rats:

: Blood samples were obtained at different time points up to 1440 minutes from Omniscan-treated nephrectomized and non-nephrectomized rats. Omniscan (2.5 mmol/kg) was administered as a single i.v dose. The results (Table 5) showed a prolonged exposure (AUC) and β -t_{1/2} in nephrectomized rats compared to intact controls. The total clearance (i.e., the glomerular filtration rate) was 2.71 mL/min*kg in 5/6-nephrectomized rats compared to 7.76 mL/min*kg in non-nephrectomized rats.

Compared to non-nephrectomized rats, the exposure and the plasma half-live in 5/6 nephrectomized rats were prolonged compared to healthy rats

Although the change in the α t_{1/2} was not apparent, β t_{1/2} was prolonged by a factor of 2.and the AUC was elevated by a factor of 3 in 5/6-nephrectomized rats. The total

clearance (i.e., the glomerular filtration rate) was 2.71 mL/min*kg in 5/6-nephrectomized rats compared to 7.76 mL/min*kg in non-nephrectomized rats. PK data are described in the following Tables:

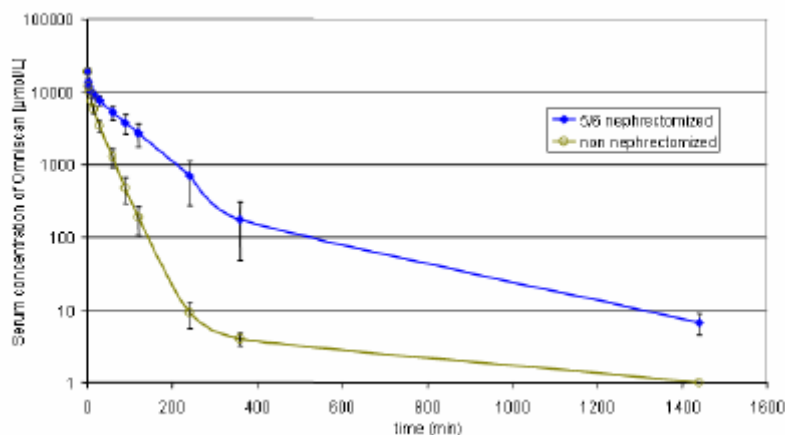
Table 90: PK parameters in 5/6 nephrectomized vs. non-nephrectomized rats

Parameter	5/6 nephrectomized		Control (non-nephrectomized)	
	Mean value	±SD	Mean value	±SD
α - $t_{1/2}$ (min)	1.42	0.13	1.35	0.12
β - $t_{1/2}$ (min)	58.77	13.29	19.89	2.26
AUC (μ mol/L*min)	962919	245422	321706	53894
Vc (L/kg)	0.10	0.01	0.10	0.00
Vd (L/kg)	0.21	0.01	0.0	0.00
Total Clearance (mL/min*kg)	2.71	0.69	7.76	1.20
Sponsor's Table reconstructed by Reviewer, SD=standard deviation				

A prolonged Gd presence in serum was observed in the 5/6-nephrectomized rats as compared with non-nephrectomized control rats:

Table 91: Gadolinium values in the serum after injection of a single i.v. Omniscan injection of 2.5 mmol/kg in 5/6 nephrectomized and non-nephrectomized rats (Mean ± SD)

Time p.i (min)	5/6 Nephrectomized rats		Non-nephrectomized rats	
	Mean (n=6)	±SD	Mean (n=6)	±SD
1	12373.3	698.0	11810.5	711.1
3	8596.9	575.9	7501.8	589.9
5	7305.9	580.9	6261.6	570.2
10	6149.1	507.3	4679.5	522.3
15	5653.0	454.9	3651.5	497.4
30	4741.8	660.2	2109.8	391.4
60	3286.6	737.1	803.8	232.9
90	2354.3	709.7	296.1	114.0
120	1692.4	605.5	116.4	50.1
240	438.4	266.6	5.8	2.3
360	109.3	79.4	2.5	0.5
1440	4.2	1.3	LLOQ	LLOQ
Reviewer's Table constructed from sponsor data				

Figure 8: Serum clearance of Omniscan in 5/6 nephrectomized (blue) vs. non-nephrectomized (green) rats**Table 92: Serum chemistry markers in Nephrectomized and Non-nephrectomized Rats (Mean \pm SD)**

Serum chemistry parameters	5/6 Nephrectomized rats		Non- nephrectomized rats	
	Mean	\pm SD	Mean	\pm SD
AST (unit/L)	95.3	25.1	76.7	14.0
ALT (unit/L)	61.7	12.4	40.8	4.6
ALP (unit/L)	191.8	35.9	214.3	35.7
Glucose (mg/dL)	213.8	39.0	224.8	40.5
Cholesterol (mg/dL)	85.5	14.8	52.2	9.0
BUN (mg/dL)	52.8	7.7	18.3	2.6
Total protein (g/100mL)	6.0	0.2	5.8	0.2
Creatinine (mg/dL)	0.9	0.1	0.5	0.0
Calcium (mg/dL)	2.7	0.2	2.6	0.1
Sodium (mmol/L)	143.8	1.7	144.7	0.5
Potassium (mmol/L)	6.3	0.9	5.3	0.4
Chloride (mmol/L)	100.8	1.7	104.0	0.6
Phosphorus (mg/dL)	6.5	0.2	6.4	0.8

Reviewer's Table constructed from sponsor data

Gadolinium in the skin: Differences in the skin Gd concentrations were observed between the four investigated GBCAs. For the non-ionic linear compounds, Omniscan and OptiMARK, high Gd concentrations were maintained in the skin over the observation period of up to 168 days post-injection. For the ionic linear compound, Magnevist, comparatively lower Gd retention in the skin was observed over time. For the macrocyclic compound, Gadovist, the Gd values in the skin were lower than Gd values in the skin in Omniscan and OptiMARK treated animals. These results are described in the following Table:

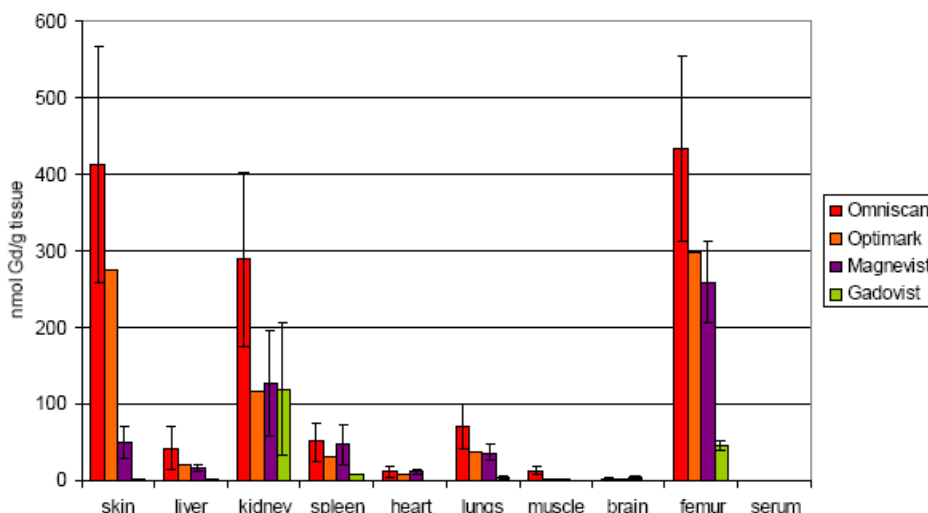
Table 93: Exposure of the skin to Gadolinium in 5/6 nephrectomized and non-nephrectomized rats

GBCA	Model (5/6n or n-n)	Skin Gd (mol Gd /g skin	
		d0 - d172	d63 - d172
Omniscan	5/6n	92.55± 25.49	52.90± 15.93
	n-n	17.37± 2.50	9.11± 1.22
OptiMARK	5/6n	37.99± 7.67	20.91± 4.36
	n-n	7.27± 0.29	4.04± 0.27
Magnevist	5/6n	15.89± 6.97	8.95± 4.44
	n-n	4.47± 0.31	1.85± 0.23
Gadovist	5/6n	2.25± 0.54	0.41± 0.34
	n-n	0.98± 0.16	0.15± 0.10

Reviewer's Table constructed from sponsor's Table; The Gd concentration in the skin from day0 - day172 was compared with that in the skin from day 63 – day 172; 5/6N, n-n = 5/6 nephrectomized, non-nephrectomized

In 5/6 nephrectomized animals, skin Gd values after treatment with linear GBCAs were at all time points significantly higher than values observed with non-nephrectomized rats.

Gadolinium, Zinc and Copper in various tissues: The amounts of Gadolinium, Zinc and Copper were measured in various organs on day 209 post-injection. The results for Gadolinium are shown in Figure 5. Omniscan and Optimark had the highest amounts of Gd in the skin, femur and liver. Gadovist was associated with the least amounts of tissue Gadolinium.

Figure 9: Gd concentration in different organs

No differences in Zn and Cu concentrations were found in the various tissues.

Macroscopic skin changes in 5/6 nephrectomized rats: Macroscopic skin lesions were observed in 3/12 animals treated with Omniscan beginning on day 5 after the last injection. The skin lesions were comparable to the NSF-like lesions noted in non-nephrectomized rats and Omniscan-treated rats in previous studies. Skin lesions occurred in animals with high skin Gd. As noted earlier, high skin Gd was observed in animals that were treated with the linear non-ionic GBCAs (Omniscan and Optimark). Skin lesions were not seen in the other treatment groups, including Gadovist.

Conclusions:

- A prolonged circulation time of administered Gadolinium-based contrast agents due to a delayed clearance was demonstrated in 5/6 nephrectomized rats indicating a surgically induced renally-impaired rats can be used a model for prolonged circulation time of GBCAs, as seen in patients with severe renal impairment. Exposure and plasma half-life were prolonged in the nephrectomized rats when compared to their non-nephrectomized controls.
- Although some characteristics of the pathophysiology of renally-impaired patients such as reduced GFR and high serum urea were reflected in 5/6-nephrectomized rats, there was no evidence of increased phosphate levels as demonstrated in renally-impaired patients
- The highest amount of Gd was observed in the skin after treatment with the non-ionic linear GBCAs, whereas the lowest Gd values were observed after treatment with the macrocyclic agents.

Reviewer's comments: Overall, I agree with the conclusions. The study was conducted to evaluate a possible animal model of NSF. The findings appear to support the use of 5/6 nephrectomized rats as a model for prolonged circulation time of GBCAs as seen in renally-impaired patients resulting in delayed clearance of the administered GBCAs in the five-sixth nephrectomized rats. The PK data obtained in 5/6 nephrectomized rats relative to the corresponding PK findings in naïve rats appear to provide important supportive evidence for the relevance of this model. I do not however agree that there was a two-fold increase in α - $t_{1/2}$ in nephrectomized rats compared to non-nephrectomized controls. While not specifically stated in the study report, it appears reasonable to expect skin lesions to appear earlier in renal-impaired rats compared to normal rats.

10.1.4. Other NSF-related Studies**10.1.4.1 Report No. A39927:**

Study title: A systemic toxicity study in rats (M) with daily intravenous administration of gadodiamide (ZK 117439) over periods of 1 to 8 days to investigate the pathomechanism of skin lesions

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 447
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	TXST20070142
Date of study initiation:	September 11, 2007
GLP compliance:	Yes (x), No (), Signed
QA report:	Yes (x), No (), Signed
Drug, lot #, and % purity:	Gadodiamide, lot #: N/A, % purity: N/A
Animal species/strain/sex per dose:	Rat, Male Wistar Hsd CpB:WU, (b) (4) / 7M per group except 9M in satellite group 7 for toxicokinetics)
Age of animals:	~10 weeks (at start of treatment)
Weight:	261 - 324 g
Doses/Vehicle:	2.5 mmol/kg (groups 4-7) /CaCl ₂ -fortified normal saline control vehicle
Duration/route:	Duration of injection is described in the treatment table below/intravenous administration

Key findings: Six hours after the administration of 2.5 mmol/kg gadodiamide there was an increase in monocytes, an increase in serum calcium and aspartate aminotransferase (AST) but a decreased albumin fraction. Kidney tubular vacuolation commencing at the 6h time point was marked by day 8. A significant increase in several serum cytokines and peptides was observed at the early time point of 6h post treatment. The cytokines included interleukin 1 α , 7, 10, inducible protein 10, lymphotactin, monocyte chemoattractant proteins (MCP), macrophage inflammatory proteins (MIP), TNF- α , tissue inhibitor of matrix metalloproteinase, osteopontin, and VEGF. There were further changes in serum biochemistry on days 4 and 8. Alterations of the skin were noted in the back, hind limbs, flank and abdomen at day 3 necropsy. The skin changes were maintained up to the 8 day time point at which time there was also hematuria in one animal, decreased food consumption that was associated with decreased body weight and signs of impaired body condition. A notable decrease was observed in the weight of the thymus, spleen and kidneys. Gd serum analysis showed a rapid distribution and quantitative excretion within 24 h. Traces of Gd were found in liver, femur and skin after the first administration, increasing with the number of injections

Objectives: The purpose of this study was to elucidate the underlying mechanism of the skin lesions described in association with the occurrence of NSF in patients with severe kidney impairment. The study was conducted using gadodiamide intravenously administered to rats at various time-points.

Methods

Study Design: The experimental design for the study is summarized in the following Table.

Table 94: Treatment Schedule (Report A39927)

Group	No. of rats [#]	Test article	Dose (mmol/kg)	Vol. (mL/kg)	(Number of administrations/day of sacrifice)
1	7	Vehicle*	0	5	1/1 (6h p.a)
2	7	Vehicle*	0	5	3/4
3	7	Vehicle*	0	5	8/8
4	7	Gadodiamide	2.5	5	1/1 (6h p.a)
5	7	Gadodiamide	2.5	5	3/4
6	7	Gadodiamide	2.5	5	8/8
7	9**	Gadodiamide	2.5	5	1/2

[#] = male rats; * 0.025 M CaCl₂ dissolved in 0.9% (w/v) NaCl solution; ** = Satellite animals for toxicokinetic examinations and were sacrificed without necropsy; Reviewer's Table modified from sponsor's Table using Sponsor's data; p.a = post-administration

Three groups of 7 male Wistar rats (groups #4-6) each received a daily intravenous injection of 2.5 mmol/kg gadodiamide via the tail vein. The first group (#4) was treated once followed by necropsy 6 h post injection. The second group (#5) was treated for three days followed by necropsy on day 4. A third group (#6) was treated for 8 days followed by necropsy on day 8. Each treatment group (#4-6) was paralleled by a control group of 7 male rats (i.e., groups #1-3 in parallel with groups #4-6, respectively). The control groups were treated with 0.025 M CaCl₂ in 0.9 % NaCl (w/v). CaCl₂ was added to the vehicle in order to account for the high CaCl₂ content in the gadodiamide formulation. The injection volume of the gadodiamide and the control solution was set to 5 mL/kg. One further group of nine male rats received a single intravenous dose of 2.5 mmol/kg gadodiamide, from which serum was obtained at 2, 20 min and 1, 2, 6 and 24 h for toxicokinetic analysis.

The serum samples were analyzed for Gd content with inductive-coupled plasma – optical emission spectrometry (ICP-OES). While this method determines the content of Gd in the serum, it was not able to differentiate between chelated, precipitated or free Gd ions. The effects of gadodiamide administration were assessed based on clinical parameters, namely mortality, general observations, food and water consumption, and body weight, as well as hematology, biochemical parameters and urinalysis.

Following sacrifice, the animals were examined macroscopically, organ weights determined and histopathological examination performed for major organs and tissues. Besides conventional Hematoxylin/Eosin stain for the microscopic slides, von Kossa

stain was applied to skin and kidneys tissue in order to detect mineralization (calcium/gadolinium deposits). The following were conducted in addition to the above:

Skin samples and whole blood were taken for gene expression analysis (Report of gene expression analysis was not described in Report A39927).

Gd content was determined in skin, liver and femur using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Metabolic profiling was performed on urine (pre-values and day 5) and terminal serum samples based on NMR measurement.

Cytokines and serum peptides were determined in the terminal plasma at all necropsy time points with a multiplexed fluorescent bead technology.

Immunohistochemistry for smooth muscle actin (myofibroblasts), factor XIIIa (dermal dendrocytes), CD34 and collagen (circulating fibrocytes), CD1a/b (dermal Langerhans cells), tumor growth factor, osteopontin, CD3 (T-cells), 68-IB-3 (B-cells) and ED1-1 (macrophages) was applied to skin samples to identify and characterize dermal infiltrates (the results of the immunohistochemistry part of the study are reported separately under study number TOXT 0079121).

Electron microscopy of skin samples of macroscopically affected and unaffected areas. Specific regions of interest with electron dense material were further analyzed by electron dispersive X-ray (EDX) analysis, which allows an element-specific detection of gadolinium in the micrographs.

The in-life phase, necropsy and the analysis of the conventional toxicological parameters of the study were performed under GLP. The processing and determination of all additional parameters were undertaken at institutions not compliant under GLP rules.

Results:

Mortality: No death was reported at the tested dose of 2.5 mmol/kg gadodiamide.

Clinical observations: The following clinical findings were reported based on the administration of 2.5 mmol/kg gadodiamide.

Scab formation in the back, hind limbs, flanks and abdomen was noted beginning on day 3. Skin reddening and swelling at the flanks were observed from day 6 onwards. Skin fissures in the flank were noted in one animal on day 8. Hematuria was observed on day 5 and 6 in one animal of group 6 (8 days of treatment). A slight swelling of the head and ruffled fur were noted on days 7 and 8 and emaciation was seen on day 8 (day of sacrificing) in most of the animals.

Food consumption: A statistically significant decrease in food consumption was observed in treated animals of groups 5 (3 administrations) and 6 (8 administrations).

Water consumption: No treatment-related effect was observed.

Body weight: A significant decrease in body weight gain was noted in group 5 animals (between day 1 and day 3) and in group 6 animals (between day 1 and day 8).

Hematology: A treatment-related increase in monocytes namely 61%, 84% and 218% was observed on days 1, 4 and 7, respectively. There was also an increase in neutrophil (240%), eosinophil (111%), basophil (114%) and large unstained cell (95%) counts on day 7.

Clinical chemistry: The Table below summarizes the treatment-related effects of 2.5 mmol/kg of gadodiamide on serum chemistry. Other parameters not indicated in the Table did not reveal treatment-related effects.

Table 95: Serum Chemistry data (Report A39927)

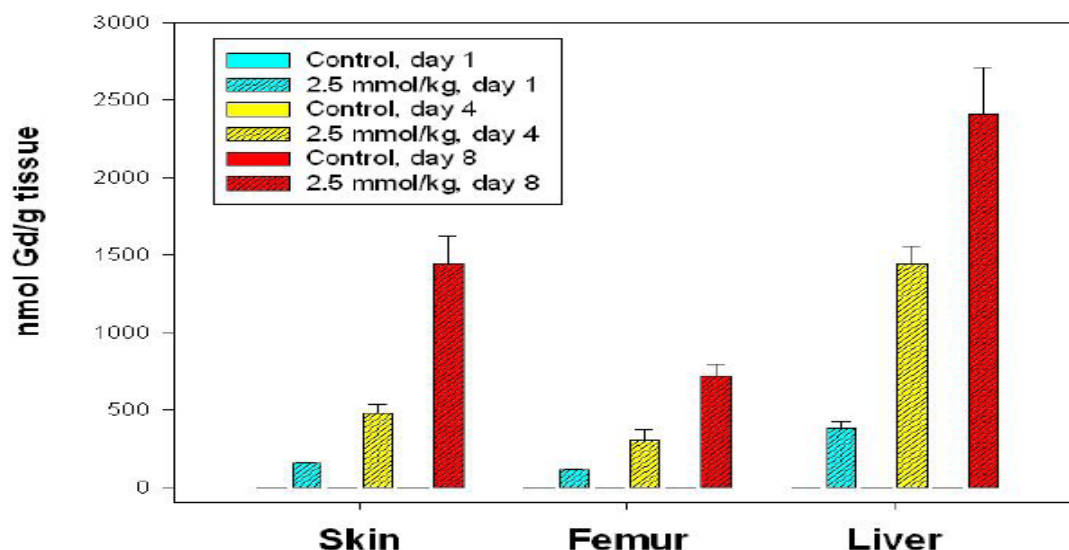
Parameter	% Change (+/-) post-treatment relative to controls		
	Day 1	Day 4	Day 8
Calcium	+26		
AST	+24; lesser on D4, D8		
ALT		+17	
ALP		-25	-40
Glucose		-10	-15
Cholesterol		+43	+43
Albumin fraction	-3	-7	-16
Relative α -globulin			+15
Relative α -globulin			+32
Reviewer's table constructed from sponsor's data. AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, +/- = increase or decrease, D4, D8 = day 4, day 8			

Urinalysis: An increase in urinary blood score of 2.5 vs. 1.5 relative to controls was observed on day 7. All other urinary parameters were not dose-related.

Cytokine/serum analysis: The cytokines or peptides that increased after treatment with gadodiamide were pro-inflammatory chemokines, acute phase proteins or peptides regulating extracellular matrix processes. Monocyte chemotactic proteins MCP-1 and MCP-3, the macrophage inflammatory proteins MIP-1 β and MIP-2, the tumor necrosis factor TNF- α , the extracellular matrix regulators tissue inhibitor of metalloproteinase type 1 (TIMP-1), osteopontin and the vascular epithelial growth factor (VEGF) The cytokines/serum peptides showed highly significant changes relative to the controls at all three time points.

Gd determination in skin and tissues: Gadolinium content was determined in serum, skin, femur and liver. The concentration in serum showed a rapid distribution and elimination with a terminal elimination half-life of 3 h and an almost quantitative excretion within 24 h. The area under the curve (AUC) for the single dose of 2.5 mmol/kg gadodiamide was calculated with 0.49 mol*min/L. Gd was detectable in skin, femur and liver after the first dose. The content in the analyzed tissues increased linearly with time with highest contents in the liver followed by the skin. The Gd content in the controls was below the limit of quantification for all tissues and time points.

Figure 10: Gadolinium content in tissues after treatment of rats with 2.5 mmol/kg gadodiamide at different time points



Necropsy findings: All animals survived to the end of the scheduled test period. After 3 days of treatment, scab formation and focal skin reddening were observed in 3/7 animals. These findings were confirmed microscopically as crusts and ulcerations. At 8 days post-treatment, all animals showed emaciation, decreased body weight, scab formation (7/7), focal skin thickening (6/7) that correlated histologically with ulceration, inflammation and acanthosis.

Microscopic findings: The observed microscopic findings noted post days 3 and 8 were limited to the kidneys and skin. Tubular vacuolation was observed in the kidney. Skin changes were ulceration, crust formation, acanthosis, dermatitis, and dermal infiltration. Microscopic changes were not detectable in the control and group 4 (post-6h sacrifice) animals.

Organ weights: Compared to the controls, the absolute liver weight in animals of group 6 (post 8 days sacrifice) was decreased but there was little change in relative organ weight. There were no histological hepatic changes observed, the liver weight change.

There was a reduced absolute and an increased significant relative kidney weight at 8 days post-treatment. A decreased absolute and relative thymus weight was observed after 8 days of treatment. A decrease in spleen weight (absolute and relative) was also observed. In other organs, differences in group mean absolute and/or relative weights between control and treated groups in all other organs was not statically significance and not treatment-related.

Conclusions: The sponsor concluded from this study that daily intravenous administration of 2.5 mmol/kg gadodiamide administration resulted in macroscopic skin lesions occurring after three administrations. Lesions were accompanied by changes in clinical chemistry, hematology, a finding that tended towards the presence of inflammatory processes. Gd was detectable in skin and other tissues after the first administration and time-dependent changes in kidneys and impaired general condition were observed. At 6 hours after the first administration, renal tubular vacuolation was observed. At the end of the study, the animals were emaciated and incidence and severity of skin and kidney lesions was increased. A significant increase in serum concentration of some cytokines or serum peptides was observed already 6 h after the first administration point.

Reviewer's comments: I agree with the conclusions.

10.1.4.2 Report No. A47234:

Study title: The role of residual Gadolinium in the induction of Nephrogenic Systemic Fibrosis-like lesions in rats

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 66
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM08151
Date of study initiation:	August 2008
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadodiamide (Gd-DTPA.BMA), lot #: N/A, % purity: N/A
Animal species/strain/sex per dose:	Rat, Males / Han Wistar (CrI: WI) rats were obtained from (b) (4). 6 male per study group
Age of animals:	N/A
Weight:	240-270g
Doses/Vehicle:	2.5 mmol/kg; 0.9% NaCl (saline)
Duration/route:	For duration (refer to study design)/intravenous

Key findings:

- Rats were intravenously injected with Gd-DTPA-BMA (Gadodiamide: the drug substance of Omniscan) without the excess ligand present in Omniscan at a dose of 2.5 mmol/kg body weight using different time intervals.
- Gd-DTPA-BMA resulted in an increase of Gd levels in skin tissue. Each injection resulted in a similar increase in skin Gd level. The shorter the intervals between injections, the more severe the changes associated with the occurrence of skin lesions.
- The findings appeared to suggest that the occurrence of NSF-like skin lesions in rats is influenced not only by the amount of Gadolinium accumulated in skin tissue, but also by the time frame, within which Gd accumulation occurs in skin tissue.

Objectives: The aim of this study was to investigate the influence of different time intervals between the injections of Gadodiamide on the total Gd accumulation in the skin and the potential development of NSF-like skin lesions in rats.

Methods: Rats were injected intravenously with Gd-DTPA-BMA, Gadodiamide (the drug substance of Omniscan) without the excess ligand present in Omniscan at a dose of 2.5 mmol/kg body weight using different time intervals as shown in the following Table:

Group	Gadodiamide (2.5 mmol/kg Injection protocol)	Description
1	1 injection only	Negative control
2	3 daily applications on 3 consecutive days	Positive control
3	3 applications with intervals between the injections of 14 days	Test
4	3 applications with intervals between the injections of 28 days	Test
5	3 applications with intervals between the injections of 56 days	Test
6	3 applications of the same volume 0.9% saline on 3 consecutive days	Saline control

Gadolinium levels in the skin and organs: Gd levels were determined in skin biopsies by ICP-MS

Macroscopic changes and histology: The methods and scoring for the assessment of macroscopic and microscopic skin changes were described in the review of Report 47233.

Results:

Gadolinium concentration in the skin: Each injection of Gd-DTPA-BMA resulted in a similar increase of the Gd levels in the skin tissue. Values for this increase were per injection between 99 -137nmol Gd/ g of skin with an average of 117±11nmol Gd/g of skin.

The similarity was also reflected by the comparable total Gd concentration observed following the third Gd-DTPA-BMA injection. Differences between the experimental groups were observed in regards to the extent, time point of onset and number of animals affected with skin lesions.

56 days after the last injection, similar total skin Gd concentrations were observed irrespective of the injection interval.

The shorter the intervals between the injections, the more severe the changes associated with the occurrence of skin lesions.

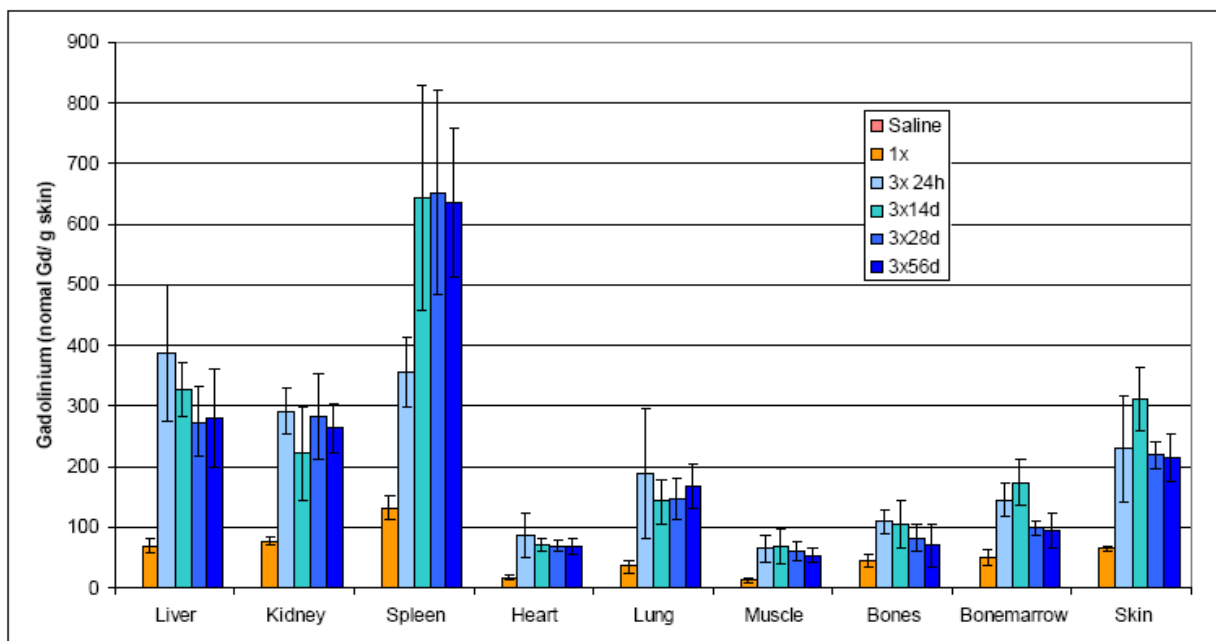
Gadolinium concentration in different tissues: Gd concentrations observed 56 days after the 3rd Gd-DTPA-BMA injection in different organs were similar between the different treatment groups. However, the concentration of total Gd amounts varied in the different organs.

The highest Gd concentration was observed in the spleen (356–652 nmol Gd/ g tissue), followed by liver (274–388 nmol Gd/ g tissue), kidney (222–292 nmol Gd/ g tissue) and skin (215–312 nmol Gd/ g tissue). The lowest Gd concentrations were observed in muscle (54–68 nmol Gd/ g tissue), heart (69–87 nmol Gd/ g tissue) and bones (72–110 nmol Gd/ g tissue).

Table 96: Gadolinium concentration in different tissues (Report A47234)

Organ/Tissue	Gd conc. (nmol/g tissue)
Spleen	356–652
Liver	274–388
Kidney	222–292
Skin	215–312
Muscle	54–68
Heart	69–87
Bones	72–110

Figure 11: Gd concentration in different tissues 56 days after the last of 3 injection of Gd-DTPA-BMA



Gd values in the control group with only one single injection of Gd-DTPA-BMA were correspondingly lower compared to all treatment groups, which received three injections.

Macroscopic changes:

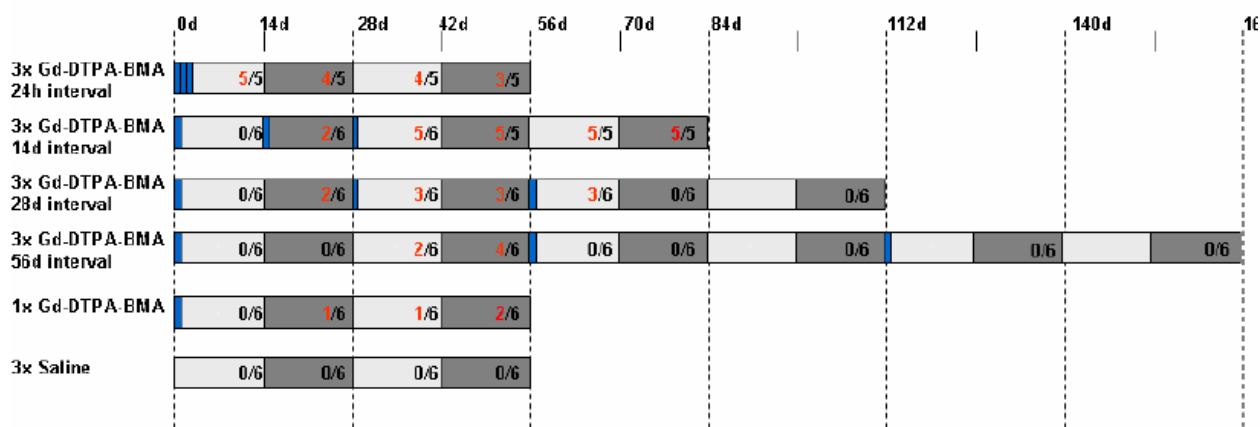
24 h interval group: All animals receiving 3 injections at an interval of 24 hours developed macroscopic skin changes as from 3 days after injection at which time none of the animals from other treatment groups had developed skin changes. The most severe macroscopic skin changes were observed in this group. In this group, skin lesions were present 14 days post injection (mean severity score of 3.2), at 56 days p.i and at necropsy but with decreased severity.

14-day interval group: Macroscopic skin lesions developed after the second injection in 4/6 animals. All animals had lesions by the third injection. After the last injection, the mean severity score was 2.8. There were lesions in all animals at necropsy (56 days after last injection).

28-day interval group: In the group with intervals of 28 day between each injection, 2 of 6 animals developed macroscopic skin changes before the second injection. Before the third injection 3 of 6 animals developed skin changes with a mean severity score of 2.2. After the third injection, neither the number nor the severity of skin lesions increased. 24 days after the last injection skin lesions were no longer detectable.

56-day interval group: In the group with intervals of 56 days between each injections 4 of 6 animals developed skin changes before the second injection with a mean severity of 1.3. The next two injections did not result in further skin lesions. The initial skin lesions disappeared over time in all animals and lesions were no longer detectable 24 days after the last injection.

In the group with a single injection 2 of 6 animals developed macroscopic skin lesions with a mean severity of 1.5 at the necropsy time point 56 days p.i. The first lesion was observed on day 35 p.i. No animal in the saline control group developed skin changes.



Microscopic skin changes: The intravenous administration of 2.5mmol/kg Gadodiamide at different intervals was associated with differences in extent of microscopic skin lesions. The incidence and severity of the findings were higher in group 3 (3x14 d) and 2 (3x 24 h) than in group 1 (1x) and 4 (3x28 d). There were no differences between group 5 (3x 56 d) and group 6 (saline).

Body weight: There were no changes in body weigh across the groups.

Conclusions: Data from this study appeared to suggest that the occurrence of NSF-like skin lesions in rats is influenced not only by the amount of Gadolinium accumulated in skin tissue, but also by the time frame, within which Gd accumulation occurs in skin tissue.

Reviewer's comments: I agree with the conclusion. However, while it is unlikely that a single patient will receive consecutive administrations of this single-use contrast agent in the clinical context of MRI diagnostic use, it is not uncommon for administration of a gadolinium-based contrast agent to occur months apart. The identified risk of Nephrogenic Systemic Fibrosis is considered serious enough even when a single dose is administered to an at-risk renaly-insufficient patient in which symptoms of this debilitation fibrotic condition may manifest weeks to months following a single administration. Expectedly, NSF risk will be increased in multi-dosed patients.

10.1.4.3 Report No. A47235:**Study title: Determination of cytokines after the single intravenous administration of Gd-DTPA-BMA in rats**

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 16
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM08152
Date of study initiation:	June 2008
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadodiamide (Gd-DTPA.BMA), lot #: N/A, % purity: N/A
Animal species/strain/sex per dose:	Rat, Males/Han Wistar (Crl: WI) rats were obtained from (b) (4). 6 males; 2 groups: Test and control
Age of animals:	N/A
Weight:	255-276 g
Doses/Vehicle:	2.5 mmol/kg; 0.9% NaCl (saline)

Key findings: Elevated levels of cytokines were observed in all the organs/tissues examined 6h after the single intravenous administration of gadodiamide (Gd-DTPA-BMA; 2.5mmol/kg). The greatest level of cytokine increase 6h after Gadodiamide treatment occurred with MCP-1 and MCP-3. The organs with the most obvious changes in cytokine expression were the bone marrow and the spleen. No specific organ was identified as the source of elevated cytokine expression.

Objective: Preclinical studies have demonstrated an elevation of serum cytokines involved in inflammatory processes following the administration of Gadodiamide. The purpose of this study was to determine the origin of the cytokines suggested to play a role in the pathology of the skin changes.

Study Design: Han-Wistar rats were injected intravenously with a single dose of 2.5 mmol/kg BW of Gadodiamide-DTPA-BMA (drug substance of Omniscan without the excess Gd-free ligand). The same volume of saline was administered as control. Animals were scarified 6h post-injection and organs (bone marrow, lung, skin, spleen, kidney), and blood leucocytes were obtained. Cytokines (TIMP1, Osteopontin; VEGF, MCP1, MCP3, MiP1 β , MiP2) and serum peptides were determined from blood samples.

Results: Compared to the saline control group, elevated levels of cytokines were observed in all the organs/tissues examined 6h after the single intravenous administration of gadodiamide (Gd-DTPA-BMA; 2.5mmol/kg). The greatest level of cytokine increase 6h after Gadodiamide treatment occurred with MCP-1 and MCP-3. Compared to saline administration, increased MiP1 β was significant only in bone marrow while Mip2 levels were significantly higher in bone marrow and spleen.

Osteopontin levels were higher in the lung and spleen. $\text{TNF}\alpha$ showed a low significance in leukocytes. Overall, the organs with the most obvious changes in cytokine expression were the bone marrow and the spleen.

Conclusions: No specific organ was identified as the source of elevated cytokine expression.

Reviewer's comments: Although I agree with the conclusions of this study, the interpretative value of the data should be taken in the overall context of the usefulness and limitations of using cytokines as biomarkers of safety in preclinical studies. Cytokines are known to show robust modulation in the setting of inflammatory processes (Tarrant, 2010), immune response and tissue repair - processes that are key factors in many toxicities. The interpretation of cytokine data can however presents challenges such as a lack of tissue-specific or toxicity-specific expression and the complexity of cytokine expression in multi-organ involvement. In view of this, an acceptable and broad interpretation that can be given to the findings of this study is that the presence of Gd in the skin, organs and tissues might be a trigger for inflammatory reactions in the locations where Gd is sequestered following its systemic administration. Elevated cytokine levels in tissues appear therefore to be an important signal of ongoing acute inflammation in tissues in response to the presence of Gadolinium.

10.1.4.4 Report No. A47233:

Study title: Potential toxic effects of lanthanoids complexes after multiple intravenous administration in rats

Volume # and Page #:	Module 4.2.3.7.7.1, pages 1- 89
Conducting laboratory and location:	Bayer Schering Pharma, Germany
Study #:	KM08150
Date of study initiation:	August 2008
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Praseodymium (Pr), Europium (Eu), Gadolinium (Gd), Holmium (Ho) and Lutetium (Lu) as DTPA-BMA complexes were tested in each in a group of six animals. Ca- DTPA-BMA and saline were used as controls.
Animal species/strain/sex per dose:	Rat, (M) Han Wistar (CrI:WI) (b) (4)
Age of animals:	N/A
Weight:	244-271g
Doses/Vehicle:	2.5mmol/ kg B.W
Duration/route:	intravenous/5 consecutive daily injections/compound tested

Key findings:

- Similar to Gadolinium, macroscopic and microscopic changes were observed in animals treated with lanthanoids.
- Microscopically, Gd-, Eu- and Ho-DTPA-BMA were associated with microscopic skin lesions including ulceration of epidermis, formation of sero-cellar crusts after Gd-DTPA-BMA (1/6), Eu-DTPA-BMA (4/6) and Ho-DTPA-BMA (1/6) treated animals. Epidermal acanthosis was observed 6/6 animals in the Gd-DTPA-BMA and Eu-DTPA-BMA groups and in 4/6 of the Ho-DTPA-BMA treatment group. Increased infiltration of inflammatory cells was present in most animals.
- There was an elevation of cytokine levels six hours after the administration of Pr-, Gd-, Lu-, Eu- and Ho-DTPA-BMA. Early induction of cytokine expression could suggest a systemic response to the lanthanoids instead of a local reaction to potential lanthanoid deposits.
- Lanthanoid release was similar in Lu and Gd-DTPA-BMA complexes in human serum at 37°C. A release of 28% and 31% of Gd and Lu, respectively were observed. A lower release (37%) was observed from the Ho-DTPA-BMA complex.
- Similar differences were observed in skin lanthanoid concentration after the administration of different lanthanoid-DTPA-BMA. There were no remarkable changes in lanthanoid concentration in the skin during the period of observation.
- The highest lanthanoid concentrations were observed in the kidneys 21 days after the administration of the three Lanthanoid-DTPA-BMA complexes. No lanthanoid accumulation could be detected in any organ in the Ca-DTPA-BMA and saline treated animals.
- Based on the findings of this study, the sponsor concluded that three of the four tested lanthanoid complexes did cause NSF-like skin reactions in animals. According to the sponsor, these results suggested a potential of the entire class of lanthanoids to have the potential to trigger NSF-like skin lesions rather than only some of specific elements of this series.

Objective: The aim of this study was to evaluate whether other elements of the lanthanoids series of the periodic Table - of which Gadolinium is a member, possessed a similar potential to be associated with the induction NSF-like skin lesions. In this study, NSF-like skin lesions were induced with the injection of non-linear Gadolinium compounds (Gadodiamide and Gadoversetamide) formulated without excess ligand.

Methods:

- Lanthanoids: Five lanthanoids, namely Praseodymium (Pr), Europium (Eu), Gadolinium (Gd), Holmium (Ho) and Lutetium (Lu) as DTPA-BMA complexes were tested each in a group of six animals. Ca- DTPA-BMA and saline were used as controls. Praseodymium (Pr), Europium (Eu), Gadolinium (Gd), Holmium (Ho) and Lutetium (Lu) were selected for this study because they were considered possibly well suited for use as x-ray absorbing elements in CT

contrast agents due to their high atomic number and may possess a similar potential as Gd in terms of association with induction of NSF

Table 97: Ionic radius and stability in Log of DTPA-Lanthanoid complexes used in Report A47233

Lanthanoid	Atomic Number	Ionic Radius*	Stability (Log)
Lanthanum	58	122	19.54
Car	58	107	20.04
Praseodymium	59	106	21.15
Neodym	60	104	21.69
Promethium	61	106	ND
Samarium	62	100	22.44
Europium	63	98	22.49
Gadolinium	64	97	22.46
Terbium	65	93	22.81
Dysprosium	66	91	22.92
Holmium	67	89	22.88
Erbium	68	89	22.83
Thulium	69	104	22.08
Ytterbium	70	113	22.07
Lutetium	71	85	ND
Reviewer's Table contracted from sponsor's data; Lanthanoids used in this study are highlighted; * units of the ionic radius were not specified in the Report			

- Induction of NSF-like lesions: NSF like skin lesions were induced in the rats with non-ionic linear compounds (gadodiamide and gadoversetamide) formulated without excess ligand. The biodistribution of the Gd following the injection of these non-ionic compounds formulated without excess ligand is comparable to that of the marketed formulation of non-ionic and other GBCAs, but is significantly different from that of Gd-EDTA, which also induces NSF-like lesions in an animal model. The test compounds were injected intravenously (i.v.) via the tail vein at a dose of 2.5mmol/ kg body weight on five consecutive days and the following parameters recorded, namely, macroscopic appearance of the skin; potential accumulation of lanthanoids in the skin and various organ tissues; expression of cytokines in the serum and histology of the skin
- Evaluation of skin lanthanoids: To evaluate lanthanoids in the skin, biopsies were taken under isoflurane (3.5-4%) anesthesia. The lanthanoids concentration in skin serum and serum were determined by ICP-MS. As noted in previous reports, bound and unbound Gd could not be distinguished by the ICP-MS analytical method
- Release of lanthanoids from DTPA-BMA: To assess the release of the different lanthanoids from the DTPA-BMA, lanthanoid release was determined in vitro,

under physiological conditions, in pooled human serum as described in the report A42715. DTPA-BMA (the ligand in gadodiamide) was used for the formulation of the new lanthanoid complexes

- Assessment of cytokines: Cytokines and serum peptides were analyzed in samples obtained from blood
- Necropsy: At scheduled necropsy (21 days post-injection), the rats were killed by exsanguination under isoflurane anesthesia and the skin examined for gross lesions. For histology, skin samples were stained with H/E and evaluated by grades numbered 1-5. Macroscopic findings were also scored in 5 grades of measure as shown in the Table below:

Table 98: Macroscopic/Microscopic lesion Grading

Grade	Lesion Evaluation Grade	
	Macroscopic lesions	Microscopic lesions
1	minimal, very few singular lesions	minimal, very few, very small
2	slight, few, small singular lesions	slight, few, small
3	few large lesions, moderate number of small lesions	moderate, moderate number, moderate size
4	marked, many large lesions	marked, many, large
5	massive, extensive number of large lesions of extensive size	massive, extensive number, extensive size
Reviewer's Table constructed with sponsor's data		

- Evaluation of body weight: Body weight was determined at each time point of drug injection and biopsy time points

Results:

Body weight: Compared to saline control group, all the animals treated with all the tested lanthanoid compounds showed a decreased body weight gain. This was most prominent following treatment with Praseodymium-, Lutetium- and Ca-DTPA-BMA in the first 10 days post-injection.

Mortality: All animals (6/6) administered Praseodymium-DTPA-BMA died. Dystrophy, vacuolated Kupffer cells and lymphoid cell infiltration was observed in the liver of 2 necropsied animals. Alveolar histiocytosis and congestion were observed in the lung and an increased number of foam cells were noted in the spleen. Three deaths occurred following Ca-DTPA-BMA administration. On postmortem, microscopy revealed liver dystrophy, degeneration of kidneys – manifested as tubular necrosis/degeneration/regeneration and casts. Alveolar histiocytosis and lung congestion were also present.

Macroscopic skin changes and histology:

- Macroscopic changes were observed after the last injection in 6/6 animals treated with Gd- and Eu-DTPA-BMA with a mean severity score of 2.7 and 3.3, respectively. At the end of treatment, 5/6 rats in the Ho-DTPA-BMA group had skin changes with a severity score of 1.2. None of the animals in the Lu-, Ca-DTPA-BMA and saline groups had any observed skin changes.

Test article	No. of animals with lesions	Mean severity score
Gd-DTPA-BMA	6/6	2.7
Eu-DTPA-BMA	6/6	3.3
Ho-DTPA-BMA	5/6	1.2
Lu-DTPA-BMA	0/2	-
Ca-DTPA-BMA	0/3*	-
Saline	0/6	-

Reviewer's table constructed with sponsor's data. * = 3/6 animals in the Ca-DTPA-BMA group died during the observation period (day 3 after first injection – day 21)

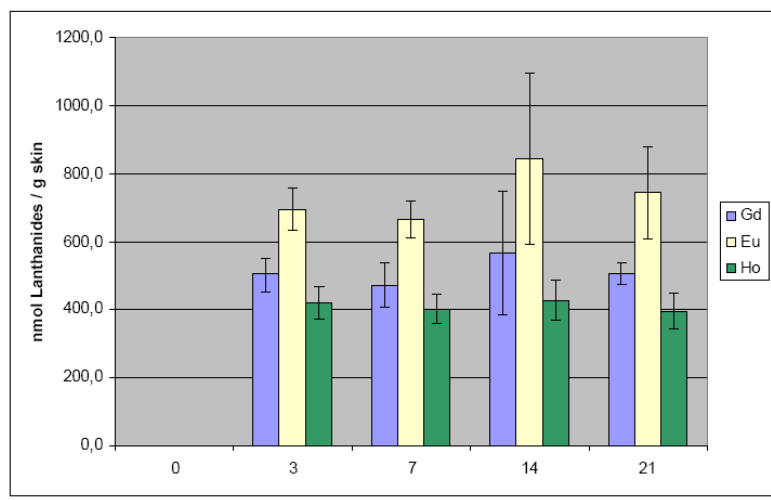
Microscopic changes: The skin was evaluated for histological changes at the end of the treatment period. Gd-, Eu- and Ho-DTPA-BMA were associated with microscopic skin lesions including ulceration of epidermis, formation of sero-cellar crusts after Gd-DTPA-BMA (1/6), Eu-DTPA-BMA (4/6) and Ho-DTPA-BMA (1/6) treated animals. Epidermal acanthosis was observed 6/6 animals in the Gd-DTPA-BMA and Eu-DTPA-BMA groups and in 4/6 of the Ho-DTPA-BMA treatment group. Increased infiltration of inflammatory cells was present in most animals. However, no similar findings were observed in the skin of the Ca-DTPA-BMA and saline treated animals.

Determination of cytokines: Six hours after the administration of Pr-, Gd-, Lu-, Eu- and Ho-DTPA-BMA, there were increased levels of MCP-1, MCP-3, Mip-1beta, Mip-2, osteopontin, Timp-1, TNF-alpha were observed compared to saline treated and the Ca-DTPA-BMA groups. There was no remarkable increase in CRP, TNF and VEGF levels compared to saline and Ca-DTPA-BMA treated group. A lower induction of cytokines was observed after Gd-DTPA-BMA compared to the other lanthanoid complexes. Osteopontin was elevated after Pr-DTPA-BMA compared to the other treatment groups. After 6 hours following the application of Ca-DTPA-BMA, there was no increase in cytokines compared to the saline treated group.

Release of Lanthanoids in vitro and in vivo: Lanthanoid release was similar in Lu and Gd-DTPA-BMA complexes in human serum at 37°C. A release of 28% and 31% of Gd and Lu, respectively were observed. A lower release (37%) was observed from the Ho-DTPA-BMA complex. However, a higher release of 37% was noted from Eu-DTPA-BMA complex. Lastly, a very high lanthanoid release of 82% was observed from Pr-DTPA-BMA complex.

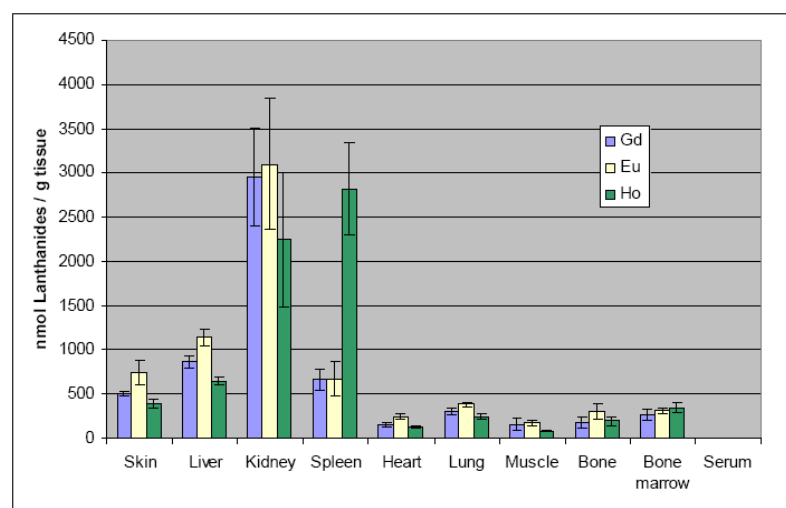
Lanthanoid concentration in the skin: Similar differences were observed in skin lanthanoid concentration (Figure shown below) after the administration of different lanthanoid-DTPA-BMA. There were no remarkable changes in lanthanoid concentration in the skin during the period of observation.

Figure 12: Lanthanoid concentration in the skin before and 3, 7, 14 and 21 days after administration of Lanthanoids



Lanthanoid concentration in body tissues:

Figure 13: Lanthanoid concentration in the different tissues 21 days after administration



21 days after injection, the highest lanthanoid concentrations (2200-3100 nmol Ln/g kidney) was observed in the kidneys after the administration of the three Ln-DTPA-BMA complexes. No lanthanoid accumulation could be detected in any organ in the Ca-DTPA-BMA and saline treated animals.

Conclusion: Gadolinium is one of the elements of the lanthanoid (or lanthanide) series of fifteen chemically similar elements. The potential of other lanthanoids for producing toxic effects was compared to that of Gadolinium with a focus on the development of NSF-like skin lesions in rats.

There was an early induction of cytokine expression following the administration of all lanthanoid complexes except the calcium complex. Cytokines play a role in fibrosis. This includes regulation of intracellular and matrix processes. Cytokines are also pro-inflammatory or could function as chemo-attractants for macrophages and monocytes. In particular, members of the subfamily of monocyte chemoattractant proteins, such as MCP-1 (CCL-2) and MCP-3 are known to be overexpressed by systemic sclerosis fibroblasts and in skin lesions of systemic sclerosis patients. Early induction of cytokine expression could suggest a systemic response to the lanthanoids instead of a local reaction to potential lanthanoid deposits.

In summary, the data suggests that the macroscopic skin changes are an acute effect to lanthanoids or the lanthanoid complex, an effect that was possibly triggered by the acute expression of cytokines.

Reviewer's comment: I agree with the conclusions of the sponsor. The results of this study suggested a very likely possibility of the lanthanide series of elements to trigger NSF-like lesions. This is evidenced by the early induction of cytokine expression following the administration of lanthanides. Since it is known that cytokines play a role in the induction of fibrosis and intracellular and matrix-based processes, cytokines are known to be essential in the induction of cellular processes essential not only to the initiation of fibrosis but also its progression. As noted by the sponsor, important cytokines with a possible morpho-pathogenic significance include the monocyte chemoattractant proteins (MCP) MCP1 (CCL-2) and MCP-3. These cytokines are known to be overexpressed by fibroblasts and skin lesions in patients with systemic sclerosis.

10.2 Antigenicity

10.2.1 Report No. A20948

Study title: Gadobutrol (ZK 135079 / SH L562BB) - Antigenicity study in female dogs with once daily intravenous administration over 4 weeks followed by a 7 day treatment free period and subsequent intravenous challenge

Volume #, and Page #:	Module 4.2.3.7.1, 233 pages
Conducting laboratory and location:	Bayer Schering Pharma, Berlin, Germany
Study #:	TXST20040066
Date of study report:	November 27, 2008
Date of study initiation:	March 18, 2008
GLP compliance:	Yes (x), No ()
QA report:	Yes (x), No ()
Drug, lot #, and % purity:	Gadobutrol (SH 562 BB)
Substance Batch number(s):	ZK135079, batch #: 69713703, purity: 100%
Formulation Batch number(s):	SH 562 BB, batch #: N21007
Animal species/strain/sex per dose:	Dog/Beagle/Female/4 per dose; (b) (4)
Age:	Age at Start: 12 months
Weight:	6.0-6.6 kg
Doses/Vehicle:	0 and 3 mmolGd/kg SH 562 BB/0.9% NaCl, batch #: 3433A191, purity: N/A
Duration/route:	Daily, 4 weeks/intravenous
Protocol deviation:	Blood samples for histamine determination 5 min after administration on day 37 were actually taken 6 min after administration for 2 animals and 7 min after administration for 1 animal

Key findings: Based on the results, there was no indication of potential induction of anti-gadobutrol specific immune response that could result in type I hypersensitivity reactions.

Study rationale/objective: In the repeat-dose toxicity study in dogs (A10548), reddening of gingival, other mucosa surfaces, and inner ears was observed in varying numbers of animals treated with SH L562 BB at 1 and 3 mmolGd/kg. These observed findings which started after treatment might point to induction of a SH L562 BB-specific immune response. This study investigated if SH L562BB induced a specific immune reaction that could result in type I hypersensitivity reactions.

Study Design:

- Four females dogs were administered daily via the i.v route 3.0 mmolGd/kg gadobutrol (SH L562 BB) for 4 weeks (prechallenge period). Four dogs were administered physiological saline i.v as control
- After a 1-week treatment-free period, dogs in both groups were challenged i.v with gadobutrol (3.0 mmolGd/kg) to intensify potential immunological reactions (challenge period)
- Clinical signs, histamine liberation, activation of the compliment system and induction of anti-SH 562 BB specific antibody formation were evaluated during the prechallenge and challenge periods. Blood pressure and heart rate were analyzed during the challenge period

- Histamine levels were determined in EDTA plasma samples during the challenge period (day 1, 10 min post administration at 8, 15 and 22). In the challenge period, histamine levels were determined before challenge on day 37 and 5-7-, 15-, 30-min and 24h post challenge. Histamine quantitation was performed by acylation and subsequent tracer-mediated competitive ELISA method.
- Complement total hemolytic activity (CH50) was determined in the prechallenge period on day 1, and on days 8, 15, 22 and 29. In the challenge period, determination was made at day 37 before challenge and 1h and 24h post challenge on day 47.

Results:

- Clinical Examination: Reddening of the inner ear skin, gingival, flews and conjunctiva and retching reported during the 4-week treatment period
- Mortality: No treatment-related mortality
- Food consumption: Slight decrease in food consumption in treatment group in week 4
- Body weight: No treatment-related effect
- Blood pressure and heart rate: a non-significant, treatment-related decrease in systolic blood pressure and heart rate occurred after challenge on day 37
- Histamine plasma levels: There was no elevation in histamine level in the prechallenge and challenge periods
- Complement hemolytic activity: There was no alteration in complement hemolytic values during prechallenge and challenge periods
- Anti-ZK 135079 antibody formation: No anti-ZK 135079 antibody formation was observed in the prechallenge and challenge periods.

Conclusions: The result of this study indicated after treatment with gadobutrol, (SH L562 BB/ZK 135079) there was no alteration in histamine plasma levels and complement hemolytic activity or evidence for anti-ZK-135079 antibody formation in the prechallenge and challenge periods. There was no indication of potential induction of anti-gadobutrol specific immune response that could result in type I hypersensitivity reactions.

Reviewer's comments: I agree with the findings and conclusions of the study.

10.3 Impurities

11 Integrated Summary and Safety Evaluation

Gadobutrol, is an intravenous, single use, paramagnetic, gadolinium-based contrast agent (GBCA) for magnetic resonance imaging (MRI). The gadolinium ion (Gd^{3+}) in Gadobutrol is bound in a neutral and stable macrocyclic complex. Gadobutrol is intended for use in adults and children ages two years and older at a recommended standard adult dose of 0.1 mmol/kg (0.1 mL/kg or 3.7mmol/m²) for the detection and visualization of disrupted blood brain barrier (BBB) and/or abnormal vascularity of the central nervous system.

As a GBCA, the efficacy of Gadobutrol is based on its paramagnetic (relaxivity) effect. It acts by shortening the T_1 (spin-lattice) and T_2 (spin-spin) relaxation times of surrounding water protons to produce its signal-enhancing effect.

In-vitro and *in-vivo* primary pharmacodynamic studies were conducted to demonstrate the relaxivity of Gadobutrol. The result of these studies indicated that Gadobutrol produced a more dominant T_1 effect compared to T_2 and that its relaxivity in visualizing well-perfused *in-vivo* tumors in rodent models was comparable to gadopentetate dimeglumine.

Nonclinical single-dose pharmacokinetic (PK) iv studies were conducted using unlabeled or labeled (¹⁵³Gd)-Gadobutrol. A linear PK was observed in the rat, pregnant rabbit, dog and monkey with elimination half-life ($t_{1/2}$) ranging from 13 to 59 minutes indicative of a rapid elimination. Similarly, a short $t_{1/2}$ of 1.82 h was obtained at the clinical dose of 0.1 mmol/kg in human adults. Drug exposure, measured by the area under the plasma concentration versus time curve (AUC) increased dose proportional after a single-dose administration of Gadobutrol in the species studied.

Gadobutrol has a low plasma protein binding with less than 5% bound to plasma proteins. Gadobutrol did not appear to penetrate the blood-brain barrier and radioactivity was lowest in the CNS (brain and spinal cord). The rat tissue and whole-body distribution studies suggested a low-level uptake of Gd in bone tissue following a single intravenous dose of Gadobutrol. The bone Gd is then slowly released and excreted within 24 hours. Measured radioactivity at 0.25 hours post dose was highest in the kidneys but less in the plasma where labeled Gd concentration decreased rapidly to lower limit of quantification 3-6 hours post-dose. Gadobutrol is not metabolized and only the unchanged drug was present in urine samples of rats and dogs. In rats, over 90% Gadobutrol was excreted primarily via the renal route while fecal excretion accounted for about 1- 2%. After repeated administration of (¹⁵³Gd)-Gadobutrol using 0.1, 0.5 and 2.5 mmol/kg once daily for 5 days, there was a dose-proportional accumulation of radioactivity in rats sacrificed two days after the last dose at which time the highest radioactivity concentration was obtained in the kidneys.

The impact of Gadobutrol on vital organs (cardiovascular, central nervous, respiratory and renal systems), was evaluated using in-vivo safety pharmacology studies. The findings revealed a safety profile similar to that of approved GBCAs. In an *in-vitro* study of the hERG channel in CHO cells, Gadobutrol caused a slight inhibition of the potassium current comparable to that of two other GBCAs Omniscan, Prohance, and the non-ionic MR x-ray contrast agent, Imeron. This result may indicate a slight potential of Gadobutrol to prolong the QT interval. However, Gadobutrol did not significantly affect repolarization of action in the isolated guinea pig papillary muscle at doses up to 50 mmol/L. In a cardiovascular safety pharmacology study conducted in conscious telemetered dogs, intravenous Gadobutrol, administered at 0.1, 0.5 or 2.5 mmol Gd/kg (or 0.54x, 2.7x and 13.5x the intended human dose), did not appear to have notable effects on blood pressure, PR interval and the QRS duration. Based on an increase in QT and QTcQ intervals at the high dose (13.5x MHD), a NOAEL of 0.5 mmol Gd/kg (or 2.7x MHD) was established.

Cardiovascular safety, also addressed in clinical studies, indicated that Gadobutrol, up to 0.5 mmol Gd/kg, had no significant effect on QT interval. No other safety signals were identified in CNS, pulmonary or renal pharmacology studies.

Nephrogenic systemic fibrosis (NSF) is a fibrosing disease, primarily identified in the skin and subcutaneous tissues but also known to involve other organs, such as the lungs, esophagus, heart, and skeletal muscles. In a series of exploratory, non-GLP studies that evaluated the potential for NSF following Gadobutrol administration, potential skin lesions and changes in skin and tissue concentrations of gadolinium were evaluated in animals administered Gadobutrol or other gadolinium-based contrast agents (GBCAs) having different structural and ionic formations. In addition, since NSF frequently occurred in association with renal impairment, a rodent model of surgically induced renal impairment (5/6 nephrectomy) was used to evaluate the role of severely diminished renal function on the plasma, skin and tissue levels of Gd in rats following 5-day repeated administration of Gadovist, Magnevist, OptiMARK or Omniscan. Findings showed prolonged presence of Gd in the serum of nephrectomized rats compared with intact controls rats.

It was concluded from preclinical studies that there was a potential for Gd skin deposition following the use of GBCAs; that the propensity for skin deposition appeared highest with the linear, nonionic Gd agents and lesser with the linear, ionic and the macrocyclic compounds and that accumulation of Gd in skin and tissues appeared higher in the 5/6 nephrectomized model compared to non-nephrectomized rats

The toxicity profile of Gadobutrol was comparable to that of approved GBCAs as summarized in the table below.

Table 99: NOAELs, Safety Margins and prominent findings in Toxicity Studies

Toxicity	Species	NOAEL	Safety Margin	Prominent Findings
Single-Dose	Rat	6.0 mmol Gd/kg	10x	Renal tubular vacuolation
	Dog	Not established		Renal tubular vacuolation
	Neonate Rat	Not established		Renal tubular vacuolation
Repeat-Dose	Rat	Not established		Renal tubular vacuolation
	Dog	0.3 mmol Gd/kg/day	1.6x	Renal tubular vacuolation
Reproductive Toxicity				
Fertility and Early Embryonic Devt.	Systemic Toxicity (rat)	2.2 mmol Gd/kg/day	3.6x	Mortality at high dose (12.2x MHD)
	Reproductive Performance (rat)	7.5 mmol Gd/kg/day	12.2x	No effect on mating performance
Rat Embryofetal Devt.	Systemic Toxicity	5.0 mmol Gd/kg/day	8.1x	Mortality at high dose (16.2x MHD)
	Embryotoxicity			↑ Incidence of skeletal variations at 12.2x MHD
Rabbit Embryofetal Devt.	Maternal toxicity	Not established		Pale, discolored kidneys
	Embryotoxicity	Not established		Skeletal variations
Monkey Embryotoxicity study	Maternal toxicity	0.75 mmol Gd/kg/day	2.4x	No maternal toxicity but abortions at higher of 2 doses
	Embryotoxicity	2.5 mmol Gd/kg/day	8.1x	Embryolethal at the higher dose but no teratogenicity
Prenatal and postnatal Devt. In Rat	Maternal effect (F ₀)	0.6 mmol Gd/kg/day	1x	Severe clinical signs and tremors at mid and high doses
	Pre- and Postnatal development of F ₁ generation	2.2 mmol Gd/kg/day	3.6x	Litter loss at high dose (12.2x MHD)

	Late effect, including fertility testing, of F ₁ generation	7.5 mmol Gd/kg/day	12.2x	No adverse effects observed up to and including high dose
--	--	--------------------	-------	---

Dev. = Development; ↑ = increased

Genotoxicity assessment of gadobutrol was negative for all assays conducted. Sponsor submitted a request for waiver of carcinogenicity. The waiver was granted by agency.

Overall Conclusions and Recommendations

Overall Conclusion: Based on the nonclinical studies reviewed in this submission, there appears to be no significant safety concerns with the proposed indication of Gadobutrol as an MRI contrast agent to detect and visualize areas with disrupted blood brain barrier and/or abnormal vascularity at a recommended adult dose of 0.1 mmol/kg (0.1 mL/kg or 3.7mmol/m²).

Recommendation: The approval of NDA 201-277 is recommended from a nonclinical perspective.

12 Appendix/Attachments

References

- Aime, S and Caravan, P (2009). Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. J Magn Reson Imaging. 30(6):1259-67).
- Grobner, T (2006). Gadolinium – a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? Nephrol Dial Transplant. 21(14):1104-1108.
- Frenzel T, Lengsfeld P, Schirmer H, Hutter J, and Weinmann, H-J (2008). Stability of Gadolinium-Based Magnetic Resonance Imaging Contrast Agents in Human Serum at 37°C. Invest Radiol, 43: 817–828
- Kuo, PH (2008). Gadolinium-containing MRI contrast agents: important variations on a theme for NSF. J. Am Coll Radiol, 5(1): 29-35).
- Kujal, P and Vernerova, Z (2008). 5/6 nephrectomy as an experimental model of chronic renal failure and adaptation to reduced nephron number. Cesk Fysiol., 57(4):104-109. [Article in Czech]

- Pietsch H, Perring C, Lengsfeld P, Walter J, Stegmann-Hartmann T, Golfier S, Frenzel T, Hutter J, Weinmann HJ and Sieber MA (2009). Evaluating the role of zinc in the occurrence of fibrosis of the skin: a preclinical study. *J Magn Reson Imaging*.30(2):374-383
 - Port M, Idee JM, Medina C, Dencausse A, Corot C (2008). Stability of gadolinium chelates and their biological consequences: new data and some comments. *Br J Radiol.*, 81(963):258-9.
 - Rampe D, Roy ML, Dennis A, Brown AM. (1997). A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.*, 417(1):28-32.
 - Rohrer M, Bauer H, Mintorovitch J, Requardt M, Weinmann H-J (2005). Comparison of Magnetic Properties of MRI Contrast Media Solutions at Different Magnetic Field Strengths. *Invest Radiology*, 40(11):715-724.
 - Sieber MA, Lengfeld P, Frenzel T, Golfier S, Schmitt-Willich H, Siegmund F, Walter J, Weinmann HJ, and Pietsch H (2008). Preclinical investigation to compare different gadolinium-based contrast agents regarding their propensity to release gadolinium *in-vivo* and to trigger nephrogenic systemic fibrosis-like lesions. *Eur Radiol*, 18(10): 2164-2173.
 - Sieber MA, Lengsfeld P, Walter J, Schirmer H, Frenzel T, Siegmund F, Weinmann HJ, Pietsch H (2008). Gadolinium-based contrast agents and their potential role in the pathogenesis of nephrogenic systemic fibrosis: the role of excess ligand. *Magn Reson Imaging*. 27(5):955
- Tarrant JM (2010). Blood cytokines as biomarkers of *in-vivo* toxicity in preclinical safety assessment: considerations for their use. *A Review, Toxicol Sci.*, 117(1):4-16.
- Tweedle MF, Wedeking P, Telser J, Sotak CH, Chang CA, Kumar K, Wan X, Eaton SM (1991). Dependence of MR signal intensity on Gd tissue concentration over a broad dose range. *Magn Reson Med.*,22(2):191-4; discussion 195-196.

.

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

/s/

OLAYINKA A DINA
02/15/2011

ADEBAYO A LANIYONU
02/15/2011

Supervisory Pharmacologist Memo

NDA: 201-277
Drug: Gadovist 1.0® (Gadobutrol)
Sponsor: Bayer HealthCare Pharmaceuticals, Inc.

Gadobutrol, is an intravenous, single use, paramagnetic, macrocyclic contrast agent for magnetic resonance imaging (MRI). It is intended for use in adults and children ages two years and older for the detection and visualization of disrupted blood brain barrier (BBB) and/or abnormal vascularity of the central nervous system.

Dr. Olayinka Dina conducted the Pharmacology/Toxicology primary review of the NDA and concluded that the nonclinical studies conducted support safety and efficacy. Dr. Dina recommended approval from pharmacology/Toxicology perspectives. He proposed changes in the label.

I concur with Dr. Dina's recommendations.

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

/s/

ADEBAYO A LANIYONU
02/15/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 201,277 Applicant: Bayer HealthCare Stamp Date: May 20, 2010
Pharmaceuticals Inc.

Drug Name: Gadovist NDA/BLA Type: NDA

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		Carcinogenicity studies were not conducted. Waiver submitted in section 1.12.5
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	Yes		Four formulations, including the to-be-marketed formulation of the drug product were used in the nonclinical studies. These formulations were considered by the sponsor to be comparable. However, the comparability of the formulations is a review issue
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	Yes		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	Yes		Statements to the effect that pivotal pharm/tox studies were performed with the GLP regulations was made in the nonclinical overview – page 7/28 in module 2.4
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Yes		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		2 impurities (b) (4) were characterized in respect of repeat-dose toxicity after intravenous injection in rats and independently in <i>in vitro</i> Genotoxicity studies (Ames and chromosome aberration test) as noted in 4.2.3.7.6 (AK84, AL74, AM22, AL58, AM21 & AM73) As stated in the submission, due to changes in the manufacturing process, these impurities are no longer seen in the to-be-marketed formulation (SH L562 BB). However, qualification of new impurities that may arise due to the manufacturing process is a review issue
11	Has the applicant addressed any abuse potential issues in the submission?	Yes		Abuse potential was addressed in 1.16
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		Yes	

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION
FILEABLE? __Yes__**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Olayinka A. Dina, Ph.D	7/2/2010
Reviewing Pharmacologist	Date
Adebayo Laniyonu, Ph.D.	7/2/2010
Team Leader/Supervisor	Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-201277	ORIG-1	BAYER HEALTHCARE PHARMACEUTICA LS INC	GADOBUTROL INJECTION

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

/s/

OLAYINKA A DINA
07/05/2010

ADEBAYO A LANIYONU
07/05/2010