times clinical dose based on body surface area or Cmax, respectively) included reduction in bodyweight & food consumption and increase in kidney weight & vacuolation. No adverse effects on liver or any other organ were noted at this dose level (1 mmol/kg/day). At higher dose level (3 mmol/kg/day, 5 or 16-times the clinical dose based on body surface area or Cmax, respectively), in addition to above noted adverse changes, following effects were observed: increased liver weights, decreased (not significant) plasma zinc levels and vacuolation in pancreas. The PK profile of Multihance in monkey (but not that of rat and dogs) is somewhat similar to that in human, so it may be more appropriate to use monkey for species comparison purpose as it relates to extrapolation/potential of adverse effects in humans.

Based on reasons mentioned above, the expanded acute dose study in animals is not necessary. However, it is recommended that the adverse effects seen after repeat-dose administration be reflected in the label.

(B) Repeat-dose study in monkey:
Original comment: In a repeat dose study in monkey, MultiHance (3 mmol/kg) caused vacuolation of islet cells in the pancreas. How does this affect pancreas function?

Sponsor's response: Beta cells in the pancreas (islet cells) are involved in the production of insulin that regulates glucose plasma levels. Therefore, pancreatic function integrity is reflected by normal glucose plasma level. Blood chemistry data shows that at the end of 14 daily administrations, glucose was not significantly different from controls.

Reviewer's comments: This reviewer concurs with the sponsor's response.

V. GENETIC TOXICOLOGY:

(A) In vivo micronucleus test in rats:
Original comment: *In vivo* micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than intravenous administration. Also dose level used in this study was not high enough. Please explain reasons for choosing intraperitoneal route specifically for this particular study (*in vivo* micronucleus assay). We recommend that an *in vivo* micronucleus assay should be conducted using intravenous administration route and higher dose levels of MultiHance.

Sponsor's response: A bridging pharmacokinetic study was conducted to demonstrate that, following intraperitoneal administration, MultiHance is systemically available (RF5921, NDA vol. 1.31). However, if agency feels this to be necessary, the study will be repeated using intravenous injection of MultiHance at MTD in accordance with ICH guidelines.

Reviewer's response: MultiHance was negative in the in vivo rat micronucleus assay at a dose of 5 mmol/kg via ip route. According to the sponsor, this intraperitoneal administration gives Cmax value of 6.4 μmol/L (15 minutes post-injection at 5 mmol/kg dose, study #RF5921, vol. 1.31). For comparison, in human the Cmax value at clinical dose (0.2 mmol/kg) is 1.3 μmol/L. Thus, the intraperitoneal dose tested is approximately 5-times the clinical dose. The intravenous MTD in rats is 4 mmol/kg, which is approximately 16-times the human dose based on Cmax

value. The in vivo micronucleus test should have been carried out at the maximum possible exposure dose level (4 mmol/kg, iv) to evaluate thoroughly the genotoxic potential for MultiHance. It should be also noted that the biodistribution studies after intravenous administration in rat and rabbit showed some accumulation (although low) of free gadolinium (———————) in bone that was persistent. Biodistribution kinetics after intraperitoneal administration was not evaluated.

To address above concerns, an in vivo micronucleus test in rat should be conducted using maximum tolerated dose level for MultiHance (4 mmol/kg, intravenous).

VI. CARCINOGENICITY: N/A

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

(A) Segment 2 study in rats:

Original comment: A Segment 2 study in rats should be conducted with the proposed market formulation (0.5 M) at dose levels where some maternal toxicity is observed.

Sponsor's response: A classical Segment 2 study was conducted in 1990 in rats with the 0.25 M formulation. However, in 1993, ICH S5A reached step 4. In this guideline, the 'most probable option' regarding study design was: a) Fertility and early embryonic development, b) Pre- and postnatal development including maternal function and c) Embryo-fetal development. Pre-postnatal study (BRO/067, NDA vol. 1.26) using the 0.5 M formulation incorporated the study design of the classical Segment 2 following the new ICH guidelines. In this study, MultiHance was administered from Day 6 of gestation to lactation Day 20.

With regard to maternal toxicity, the highest dose tested (2 mmol/kg/day) was selected based on the high dose given in repeat-dose toxicity study. At a dose of 3 mmol/kg, local reaction at the injection site prevented repeat-dosing in 7/18 females (repeat dose study in rats, BRO 57, NDA vol. 1.14).

Reviewer's response: This reviewer concurs with the sponsor's response. In repeat-dose study in rats, there was a dose-dependent increase in the degree of lesions/ulceration found in the vein and perivascular tissue at the injection sites at all dose levels (0.3-3 mmol/kg/day). This kind of local reaction was somewhat more severe in females at 3 mmol/kg/day, since 7/18 females were unable to be dosed on one/two occasions during the 3rd/4th week of study. The highest dose (2 mmol/kg/day) tested in the reproductive study in rats is approximately 2-times the clinical dose based on body surface area (5-times based on Cmax values).

It should be noted that the sponsor did conduct Segment 2 study in rabbits. The NOAEL for this study was established at 0.3 mmol/kg/day. At higher dose levels (0.9 & 2 mmol/kg/day), maternal toxicity (decreased body weight and food consumption) and fetotoxicity (retinal irregularities, additional/fused sternebral centers, offset pelvic girdles) was observed.

Due to reasons mentioned above, Segment 2 reproductive toxicity study in rats at higher dose level is not necessary.

(B) Male fertility study in rats:

Original comment: No effect was observed on male fertility in doses up to 2 mmol/kg. However, in repeat dose study in rats, MultiHance (3 mmol/kg/day) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely. The Segment I reproductive study should have been carried out at higher dose multiples for proper evaluation.

Sponsor's response: The sponsor has not specifically responded to this issue. The highest dose tested (2 mmol/kg/day) is based on the adverse local injection site reaction seen in rats.

Reviewer's comment: The repeat-dose study in rats produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely and it should be reflected in the label. Therefore, no further study is necessary.

VIII. SPECIAL TOXICOLOGY STUDIES:

(A) Local tolerance study:

Original comment: The local tolerance study histological evaluation at 8-days after MultiHance® administration revealed reddening, thickening, inflammatory cell infiltrates, eschare, and larger areas of necrosis. These findings were qualitatively more severe than the Magnevist control, and were not produced by the hyperosmolar control. The study did not include an evaluation at earlier time points. These findings suggest that local extravasation or prolonged intravenous exposure to MultiHance® may lead to thrombosis or phlebitis.

In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

Sponsor's response: Intravenous tolerance can be evaluated also from the repeat-dose study, where the dose of 3 mmol/kg in rats for 28 days caused local damage in almost all animals, without full recovery. In monkeys, repeated dosing (14 days) up to 3 mmol/kg caused no compound-related damage. However, paravasal tolerance and recovery were not assessed in animals.

An extensive local tolerance study will be performed to address this issue and the protocol will be submitted to the Agency for comment.

Reviewer's comment: The local adverse reactions after paravenous administration should be noted as 'warning' in the label (as is the case with other gadolinium agents). The extensive local tolerance study agreed to be performed by the Sponsor will answer the severity of local adverse effects due to rate of infusion and time-period necessary for resolution of these effects.

(B) Effect on coagulation parameters (in vivo study):

Original comment: No effects on coagulation parameters were reported in vivo. However, this study was carried out using 0.25 M MultiHance formulation. Also the study should have been carried out at clinically equivalent or higher doses. In an in vitro study, at higher doses, there were significant increases in PT and PTT and concomitant decreases in fibrinogen, This indicates that MultiHance has potential to affect coagulation parameters. We recommend an in vivo study, using high doses of 0.5 M MultiHance, in order to determine the effects of the drug on coagulation parameters and bleeding time.

Sponsor's response: In vivo study focusing on coagulation parameters was not performed with 0.5 M formulation. However, results from toxicology studies conducted with 0.5 M formulation showed lack of significant effects on coagulation even after multiple administration of MultiHance.

4-week study in rats (BRO/57, vol. 1.14): No effect on PT, aPTT up to 3 mmol/kg (highest dose).
4-week study in dogs (97-3357, vol. 1.16): aPTT increased at 2 mmol/kg and 1 mmol/kg (males only) with recovery. No effects were seen on PT and fibrin.
4-week study in dogs (ESI 143/970956, vol. 1.15): No effects on PT, aPTT, fibrinogen up to 2 mmol/kg (highest dose).
2-week study in monkeys (BRO 92/973656, vol. 1.18): No effects on PT, and aPTT up to 3 mmol/kg (highest dose).

Furthermore, consider that prolongation of coagulation times would have been detected in cases where hemorrhages occur spontaneously (for example, in reproduction studies thus leading to abortion/death of mother or fetuses). In these studies, animals were repeatedly administered before parturition.

Reviewer's comments: The reviewer concurs with the sponsor's response. It should be also noted that in an in vitro study, the effects on PTT, PT and fibrinogen were minimal (~5%) at 6.3 mM dose level. According to the sponsor, this dose corresponds to about three times the maximum plasma levels in clinical setting. At higher doses (13 and 25 mM), somewhat significant increases in PT (13 & 28%, respectively) and PTT (11 & 26%, respectively) and concomitant decrease in fibrinogen (9 & 16%, respectively) were observed. The in vivo study may not be necessary as in vitro study was conducted and repeat-dose studies in various species did not suggest significant effects on coagulation parameters.

IX. CONCLUSIONS AND RECOMMENDATIONS:

Recommendations: The NDA application for MultiHance is deemed **approvable** subject to fulfillment of following pre-clinical studies:

(A) Safety pharmacology study: As stated in our original comments, a comprehensive safety pharmacology study in monkey (as pharmacokinetic profile is similar to humans) should be conducted. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG

monitoring, QT interval etc.) and respiratory parameters. Hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan should be included for comparison purpose.

(B) In humans, gadolinium contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. Conduct *in vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels for MultiHance.

(C) Genotoxicity study: An *in vivo* micronucleus assay using intravenous administration route and higher dose levels of MultiHance® (MTD) should be conducted.

(D) Male fertility study: No effect was observed on male fertility in doses up to 2 mmol/kg. However, in repeat dose study in rats, MultiHance at higher dose level (3 mmol/kg/day) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely and it will be reflected in the label.

(E) Local tolerance study: The local tolerance study histological evaluation at 8-days after MultiHance® administration revealed reddening, thickening, inflammatory cell infiltrates, eschare, and larger areas of necrosis. These findings were qualitatively more severe than the Magnevist control, and were not produced by the hyperosmolar control. The study did not include an evaluation at earlier time points. These findings suggest that local extravasation or prolonged intravenous exposure to MultiHance® may lead to thrombosis or phlebitis.

In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

(F) Due to relatively low safety margin for MultiHance, in particular adverse effects associated with CNS and liver enzyme changes (also seen clinically in some patients), pre-clinical studies in juvenile animals may be necessary depending on the resolution of pediatric clinical studies

We encourage you to submit protocols for the recommended studies in order to resolve any experimental design issues.

X. APPENDIX/ATTACHMENTS: NONE

External Pharmacology/Toxicology Comments For the Sponsor:

(A) Safety pharmacology study: As stated in our original comments, a comprehensive safety pharmacology study in monkey (as pharmacokinetic profile is similar to humans) should be conducted. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG monitoring, QT interval etc.) and respiratory parameters. Hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan should be included for comparison purpose.

(B) In humans, gadolinium contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. Conduct *in vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels for MultiHance.

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In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

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We encourage you to submit protocols for the recommended studies in order to resolve any experimental design issues.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number:	**21-358**
Review number:	02 [01: Original NDA (21-357) review]
Relevant submission, date & review	**21-357**/04-27-01 (Original NDA submission & review)
Sequence number/date/type of submission:	N-000-BP/04-09-02/Responses to Pharmacology & Toxicology comments for the original submission
Information to sponsor:	Yes (X) No ( )
Sponsor and/or agent:	**Bracco Diagnostics Inc., Princeton, NJ**
Manufacturer for drug substance :	Bracco Imaging SpA, Milan, Italy
Reviewer name:	Tushar Kokate
Division name:	DMIRDP
HFD #:	160
Review completion date:	04-24-02
Drug:	
Trade name:	**MultiHance**
Generic name (list alphabetically):	Gadobenate dimeglumine, Gd-BOPTA/Dimeg
Code name:	B19036/7, E7155
Molecular weight:	1058
Relevant INDs/NDAs/DMFs:	NDA: 21-357; IND: 43,779
Drug class:	MRI contrast agent
Indication:	Imaging of CNS
Clinical formulation:	0.5 M Gadobenate dimeglumine (529 mg/ml) Gd-BOPTA: Meglumine: and water for injection: (1970 mOsmol/kg, 6.9-times that of plasma)
Route of administration:	Intravenous

Proposed use: (a) For adults

Dose: 0.1 mmol/kg,

Maximum clinical dose: 0.2 mmol/kg.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I Recommendations:

(A) Recommendation on Approvability: The NDA application for MultiHance is deemed **approvable** subject to fulfillment of pre-clinical studies noted below.

(B) Recommendation for Nonclinical Studies:

(A) Safety pharmacology study: As stated in our original comments, a comprehensive safety pharmacology study in monkey (as pharmacokinetics profile is similar to humans) should be conducted. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG monitoring, QT interval etc.) and respiratory parameters. Hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan should be included for comparison purpose.

(B) In humans, gadolinium contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. Conduct *in vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels for MultiHance.

(C) Genotoxicity study: An *in vivo* micronucleus assay using intravenous administration route and higher dose levels of MultiHance® (MTD) should be conducted.

(D) Male fertility study: No effect was observed on male fertility in doses up to 2 mmol/kg. However, in repeat dose study in rats, MultiHance at higher dose level (3 mmol/kg/day) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely and it will be reflected in the label.

(E) Local tolerance study: The local tolerance study histological evaluation at 8-days after MultiHance® administration revealed reddening, thickening, inflammatory cell infiltrates, eschare, and larger areas of necrosis. These findings were qualitatively more severe than the Magnevist control, and were not produced by the hyperosmolar control. The study did not include an evaluation at earlier time points. These findings suggest that local extravasation or prolonged intravenous exposure to MultiHance® may lead to thrombosis or phlebitis.

In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

(F) Due to relatively low safety margin for MultiHance, in particular adverse effects associated with CNS and liver enzyme changes (also seen clinically in some patients), pre-clinical studies in juvenile animals may be necessary depending on the resolution of pediatric clinical studies.

We encourage you to submit protocols for the recommended studies in order to resolve any experimental design issues.

II Summary of Nonclinical Findings: This amended NDA review for MultiHance is in response to the Sponsor's responses to Pharmacology/Toxicology comments in the original NDA review (#21-357). The draft comments were faxed to the Sponsor on March 13, 2002. The Sponsor's response to these comments was received on April 10, 2002. This amended NDA review is intended to summarize original pre-clinical comments and recommendations, Sponsor's response to these comments and reviewer's response to the Sponsor's comments. Please refer to the original NDA review (NDA#: 21-357) of MultiHance for all the details.

III Administrative:

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/ 4/29/02 - see attached memo BB

Non-Concurrence - _____
(see memo attached)

C. cc: list:

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PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION:

MultiHance (0.5 M gadobenate dimeglumine) is a MRI agent that is proposed for the imaging of ———— CNS. Gadobenate dimeglumine consists of an octadentate chelate of paramagnetic ion Gd^{3+} which, due to its distribution characteristics, is intended as an intravascular MRI contrast agent for hepatic and CNS imaging. Only the anion of the complex contributes to the contrast enhancing effect. The chelation of Gd^{3+} presumably results in substantially less toxicity than the free ion. The drug exits the vascular space and resides primarily in the extravascular space, but is also present within hepatocytes. In animals (rats, dogs & rabbits), MultiHance is excreted mainly through the renal (50-65%) and biliary (35-50%) routes. By contrast, in humans renal elimination (80-96%) is a major route, although some excretion through biliary route (1-4%) is also observed. It should be noted that the elimination kinetics in monkey are somewhat similar to humans, with approximately 80% & 12% renal and bile elimination, respectively.

MultiHance has an osmolarity (1970 mOsmol/kg) that is 6.9 times that of plasma (285 mOsmol/kg), and is hypertonic under conditions of use. The hyperosmolarity of MultiHance is similar to Magnevist (1960 mOsmol/kg), an approved gadolinium type MRI contrast agent. The maximum proposed clinical dose of MultiHance is 0.2 mmol/kg. It is administered as a rapid intravenous infusion or bolus injection followed by 5-ml saline flush.

I. GENERAL PHARMACOLOGY/TOXICOLOGY COMMENTS:

(A) Original comment: Although Pharmacology-Toxicology studies were performed with the ——— 0.5 M formulation, generally the dose multiples that were studied were low. This assessment is based on a body surface area adjustment and the cumulative maximum human dose of 0.2 mmol/kg proposed for CNS imaging.

Sponsor's response: The pre-clinical development of MultiHance started several years ago and ~~dose-multiple~~ criteria used at that time was based on body weight. The concept proposed by ICH for such studies is based on toxicokinetic profile and the maximum tolerated dose (MTD). We performed additional studies using the MTD as the upper limiting dose and using the toxicokinetic profile to determine a safety margin.

For compounds that are rapidly excreted as un-metabolized molecules, such as gadobenate dimeglumine, the Cmax at the MTD represents the threshold of safety for systemic exposure. Since both Cmax and AUC values have been determined in animals and humans for MultiHance, it is more appropriate to use Cmax values to directly compare animal and human exposure. Using this approach, the systemic plasma levels associated with the doses employed in safety pharmacology studies equal or exceed those associated with the human diagnostic dose of 0.2 mmol/kg (0.3 to 10-times the human dose based on Cmax).

Cmax was chosen based on the following observations:

1) Compound-related acute adverse effects of any drugs in animals are related to plasma concentrations being above the threshold of the MTD.
2) Either AUC or Cmax can be chosen, according to kinetics behavior of the compound. As intravenous contrast media do not have a true absorption phase, but only distribution and a rapid elimination through the kidneys and liver, and they are not administered repeatedly (thus no accumulation occurs), AUC is not a good predictor for the adverse effects.
3) The onset and disappearance of adverse reactions in animals seem to mimic the kinetic behavior of MultiHance, from the high initial concentration, to levels below the threshold of toxicity.
4) Cmax is an absolute measurement, which do not imply the time factor, which is different for rats and humans.

Reviewer's comments: We agree that Cmax and AUC values allow direct comparison in terms of animal and human exposure. Both Cmax as well as AUC are important parameters. While Cmax gives the peak measurement, AUC value is a good indication of total extent of exposure, which is important in terms of observed adverse effects and correlation between duration of effects and measured exposure level. Therefore, AUC is an important parameter that can not be ignored. AUC also minimizes the time-point sampling error possible with Cmax. For MultiHance, dose multiples based on AUC were similar to the one calculated by using body surface area. Therefore, dose-multiples were calculated based on body surface area. In addition, for some studies toxicokinetic parameters (Cmax, AUC) were not available, therefore, to allow for uniformity across studies, body surface area was the conservative predictor used for dose-multiple calculations.

II. SAFETY PHARMACOLOGY:

(A) General comment:

Original comment: The dose multiples used for safety pharmacology studies ranged from 0.3 to 3 times the maximum human dose, and were inadequate for establishing clear safety profile of MultiHance. In most of the studies, only one dose was utilized for safety pharmacology evaluation. Evaluation at various dose-levels in the same study is necessary for proper comparison and establishment of a dose-response curve. The identified toxicities of concern ~~were the EEG~~ slowing, motor incoordination, convulsions and death. Adverse effects noted in safety pharmacology studies were attributed to hyperosmolarity solely but most of these studies did not include hyperosmotic control group to attribute these effects to hyperosmolarity. In few studies where hyperosmotic control group was included, some of these effects (slowing of EEG) could not be attributed to hyperosmolality as they were not seen with the active control. Additionally, although MultiHance® permeability through a damaged blood brain barrier was low, the dose level tested was too low to assess the risk (i.e., 0.3 x MHD).

Also, continuous ECG recording was not performed in these studies and effects on ECG parameters such as QT interval were not reported. Therefore, these studies are not sufficient to assess the potential risk and to determine labeling or risk management approaches for drug effects.

To address above concerns, conduct a comprehensive safety pharmacology study in larger species with pharmacokinetic profile similar to humans. This study must be conducted at various dose levels (with high dose-multiples based on body surface area). The study must include a complete battery of CVS (including continuous ECG monitoring, QT interval etc.), CNS (including EEG), renal and respiratory parameters. This study should be conducted in unanesthetized animals with a hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan for comparison purpose.

Sponsor's response: (a) CNS effects: Studies using both intravenous and intrathecal route were performed to investigate the CNS safety of MultiHance. It is true that in most studies a single dose was utilized. The following studies used more than one dose and in the case of study RF1994-1, ascending doses were administered by intracisternal injection, which represents the worst case for CNS toxicity.

The study coded RF5484 (Passage into ischemic brain, vol. 1.10) was conducted by administering 2 or 4 mmol/kg, iv (MTD) to rats with induced cerebral ischemia. The levels of the compound peaked 1 hr after injection (mean: 520 nmol/g tissue for 2 mmol/kg and 740 nmol/g for 4 mmol/kg) and were still higher than normal 3 hr after injection. The maximum attained gadobenate ion concentration in the ischemic region was 3-times higher than the concentration in the contralateral non-ischemic area. Measurements of radiolabeled gadolinium in rat brains with a damaged BBB also gave results comparable to these results. For example, a dose of 0.3 mmol/kg yielded a presumed gadobenate ion concentration in the whole damaged hemisphere of 63 nmol/kg of gadobenate dimeglumine. There was a linear relationship (0.3, 2 and 4 mmol/kg dose levels) for the ratio between the dose level used and the concentration of gadobenate dimeglumine found in the lesioned tissue.

A dose-response relationship was observed in the Irwin behavioral study. In this study, MultiHance was administered by intracisternal route at 0.3, 0.1, 0.06 and 0.03 mmol/kg dose levels. The lowest dose of 0.03 mmol/kg corresponded to a brain concentration of 3.75 micromol/g and resulted in no remarkable activity impairment. This concentration is 5-times higher than obtained after iv injection of the MTD of 4 mmol/kg to rats with cerebral ischemia. Also, in the EEG study by intracerebroventricular route, MultiHance at 0.025 nmol/kg dose ~~results in 3.1~~ micromol/g concentration, which is 5-times higher than that observed after iv injection of the MTD in rats. At this dose level, no effect on EEG was observed.

(b) Hyperosmolarity effect: In animal studies, the adverse effects are a result of the formulation as a whole, of which osmolarity is the most important, but not the only, factor. We agree that in animal studies not all adverse effects are attributable to osmotic load and that the molecule has its own effects.

(c) Continuous ECG recording: We agree that documentation does not comply with current ICH S7B guideline regarding this subject. However, a clinical study was performed at the request of the Agency to evaluate cardiac electrophysiology in patient and non-patient volunteers and demonstrated that in both normal and cardiac compromised patients there are no significant effects on QT. In the light of human data, we are not sure what additional

information would be obtained from the study in dogs. We would like to discuss with the agency the necessity to perform this study.

(d) Renal safety pharmacology study: This study was performed in rats (Study GI996007, vol. 1.11) and no effects on urine volume or electrolytes excretion were observed over a 5-hr collection period at the dose level of 1 mmol/kg.

(e) Effect on respiratory parameters: This study was conducted in anesthetized pigs at 1 mmol/kg dose level.

Based on above responses, we believe the proposed study would provide no additional information. However, we are sensitive to the Agency's concerns and are willing to discuss this further.

Reviewer's comments: (a) CNS effects: The additional information provided by the sponsor with regard to peak gadobenate dimeglumine concentrations in the brain after intracisternal administration helps to compare it with the peak concentration obtained after intravenous administration and adverse effects. We did not ask for a separate CNS study originally but recommended it as part of a comprehensive safety pharmacology study. We concur with the sponsor that additional study evaluating CNS effects for MultiHance is not necessary.

(b) Continuous ECG recording: Animal studies provide data that can not be obtained in humans. For example, higher dose levels can be tested to evaluate if MultiHance has any potential for effect on QT interval. Furthermore, in clinical studies QT/QTc prolongation was noted in some patients. Various cardiac arrhythmias including ventricular arrhythmia was experienced by some patients (no complete details on these patients were provided. Please see clinical review for more details). In clinical setting, gadolinium type contrast agent can cause QT prolongation effects. In view of its clinical importance, continuous ECG data is needed.

(C) Hyperosmolarity effect: As sponsor stated, in the absence of hyperosmotic control group, the adverse effects observed can not be as confirmed solely due to hyperosmolarity and the molecule can have its own effect.

To address above concerns, we recommend as stated in the original reviewer's comment sent to the Sponsor, comprehensive safety pharmacology study in monkey (as its pharmacokinetics profile is similar to humans). This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG monitoring, QT interval etc.) and respiratory parameters. The study should include a hyperosmotic control group (sucrose/mannitol solution). Magnevist® and Optimark or Omniscan should be included for comparison purpose.

(B) In vitro electrophysiology study:

Original comment: In humans, gadolinium contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. Conduct *in vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels for MultiHance.

Sponsor's response: To complete the safety profile on heart studies on APD and HERG channel will be performed.

(c) Original comment: In EEG studies in rats with focal brain ischemia, MultiHance® (4 mmol/kg) caused transient flattening (for 2 min) of EEG in conscious rat. How is it possible to have brain electrical activity completely stopped in a conscious animal even though it is a transient effect? Provide more information on this effect and describe the activity of the rats during this time.

Sponsor's response: The term 'flattening' used in the final report really refers to a reduction in amplitude and frequency, and not an absence of signal.

Reviewer's response: The sponsor provided relevant EEG traces showing reduction in amplitude and frequency (not 'flattening') of EEG at 4 mmol/kg dose level. The NOAEL for EEG effect was 2 mmol/kg, iv (2 or 5-times the clinical dose based on body surface area or Cmax, respectively). Sponsor's response is deemed adequate.

III. PHARMACOKINETICS/TOXICOKINETICS:

(A) Original comment: Stability of gadolinium chelate/complex is of concern. Free gadolinium ion was detected in feces of rats and dogs (~6%). Also, the biodistribution study revealed significant retention of radioactivity in bone (2.7% ID). This retention was attributed to the impurities in the formulation that are not present in the clinical formulation. However, it is possible that this reflects transmetallation of the gadolinium. Such transmetallation would be supported by the finding of ——— in the urine. In order to address the retention in bone of the impurities, provide data to document this conclusion.

Sponsor's response: (a) The compound does not undergo degradation during its passage across the body (as demonstrated in biodistribution studies). Moreover, the gadobenate ion is excreted unchanged with the bile and it is not reabsorbed from the GI tract. The gadobenate ion has been demonstrated to be not stable if stored in feces both at room and 37^0 C temperature (study 36-027, vol. 1.29). the free gadolinium found in the fecal matter is probably due to degradation in the fecal environment and not to transmetallation in the body. Transmetallation in the body would in fact result in Gd deposition in target organs, such as bones, spleen and liver.

There are no concerns about the dissociation in feces, as single dose toxicity study showed that oral administration of doses of 15 and 25 mmol/kg in mice and rats, respectively were unable to cause toxicity (study RF2525 and RF2526, vol. 1.12).

(b) As for study report 36-027, 2.5 microgm equivalent/g is the concentration in the femoral bone and not % of ID. The radioisotopic purity of compound injected in the study was ——— which means ——— could be a radioisotope different from ——— with an affinity constant for the chelating agent BOPTA lower than gadolinium and lower than the affinities of the other circulating metals. Once in the body, this radioisotopic impurity could undergo transmetallation with fast accumulation in bone. This kind of impurity is not present in the

formulation to be marketed because: (1) the gadolinium oxide has a chemical purity higher than ——— (2) The compound for the market is not radiolabeled.

Moreover, such an high concentration of radioactivity in bone at 7 hr to 28 days after administration was found only in the 36-027 study; these results were not confirmed in any further studies, including study in rabbits, where high purity compound was used.

Reviewer's comment: Based on sponsor's response, the free gadolinium detected in the feces (~6%) may be due to degradation in the fecal environment stored at room or 37^0 C temperature. Further documentation to address retention of radiolabeled impurity in bone is not necessary.

IV. GENERAL TOXICOLOGY:

(A) Acute toxicity studies:

Original comment: The application lacks the required expanded acute dose toxicity study evaluating all necessary parameters (such as hematology, clinical chemistry, urinalysis, complete histopathology, etc.). LD_{50} studies can not be substituted for the expanded single dose studies.

Additionally, we note that the submitted LD50 studies had a low safety margins for lethality. The lethality and adverse effects were attributed to high osmotic load in animals. However, a positive hyperosmotic control group was not used. Therefore, the effects cannot be attributed to hyperosmolality alone.

We recommend a systematic expanded acute dose study in a large animal with a pharmacokinetic profile that is more consistent with that of humans. This study should be carried out at various dose levels (at least three and higher dose multiples) and hyperosmotic mannitol/sucrose solution & Magnevist should be included as comparative controls. Various toxicity parameters should be evaluated 72-hours post-dosing, and also after 7/14-days recovery period.

Sponsor's response: The pre-clinical section contains 15 acute toxicity studies in mice and rats and 4 MTD studies in rats, rabbits, dogs and monkeys. In addition, sub-acute studies were performed in rats, dogs and monkeys. Based upon the presence in the submission of these studies and in agreement with the draft guidance to Industry (Developing medical imaging drugs and biological products) dated June 19, 2000, we do not believe that any additional substantive information would be provided by an extended acute toxicity study.

Reviewer's response: Most of the studies cited by the sponsor were actually LD50 studies. The expanded acute dose study was recommended based on the low NOAELs (0.4-0.6 or 1-2 times the clinical dose based on body surface area or Cmax, respectively) observed in the repeat dose study. We agree that the acute dose studies are not generally required if proper expanded repeat-dose studies are conducted (as was the case with MultiHance).

It should be noted that in a repeat dose study in monkey, MultiHance was somewhat better tolerated than in rats and dogs. In monkey, only adverse effects noted at 1mmol/kg/day (2 or 7-

times clinical dose based on body surface area or Cmax, respectively) included reduction in bodyweight & food consumption and increase in kidney weight & vacuolation. No adverse effects on liver or any other organ were noted at this dose level (1 mmol/kg/day). At higher dose level (3 mmol/kg/day, 5 or 16-times the clinical dose based on body surface area or Cmax, respectively), in addition to above noted adverse changes, following effects were observed: increased liver weights, decreased (not significant) plasma zinc levels and vacuolation in pancreas. The PK profile of Multihance in monkey (but not that of rat and dogs) is somewhat similar to that in human, so it may be more appropriate to use monkey for species comparison purpose as it relates to extrapolation/potential of adverse effects in humans.

Based on reasons mentioned above, the expanded acute dose study in animals is not necessary. However, it is recommended that the adverse effects seen after repeat-dose administration be reflected in the label.

(B) Repeat-dose study in monkey:
Original comment: In a repeat dose study in monkey, MultiHance (3 mmol/kg) caused vacuolation of islet cells in the pancreas. How does this affect pancreas function?

Sponsor's response: Beta cells in the pancreas (islet cells) are involved in the production of insulin that regulates glucose plasma levels. Therefore, pancreatic function integrity is reflected by normal glucose plasma level. Blood chemistry data shows that at the end of 14 daily administrations, glucose was not significantly different from controls.

Reviewer's comments: This reviewer concurs with the sponsor's response.

V. GENETIC TOXICOLOGY:

(A) In vivo micronucleus test in rats:
Original comment: *In vivo* micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than intravenous administration. Also dose level used in this study was not high enough. Please explain reasons for choosing intraperitoneal route specifically for this particular study (*in vivo* micronucleus assay). We recommend that an *in vivo* micronucleus assay should be conducted using intravenous administration route and higher dose levels of MultiHance.

Sponsor's response: A bridging pharmacokinetic study was conducted to demonstrate that, following intraperitoneal administration, MultiHance is systemically available (RF5921, NDA vol. 1.31). However, if agency feels this to be necessary, the study will be repeated using intravenous injection of MultiHance at MTD in accordance with ICH guidelines.

Reviewer's response: MultiHance was negative in the in vivo rat micronucleus assay at a dose of 5 mmol/kg via ip route. According to the sponsor, this intraperitoneal administration gives Cmax value of 6.4 μmol/L (15 minutes post-injection at 5 mmol/kg dose, study #RF5921, vol. 1.31). For comparison, in human the Cmax value at clinical dose (0.2 mmol/kg) is 1.3 μmol/L. Thus, the intraperitoneal dose tested is approximately 5-times the clinical dose. The intravenous MTD in rats is 4 mmol/kg, which is approximately 16-times the human dose based on Cmax

value. The in vivo micronucleus test should have been carried out at the maximum possible exposure dose level (4 mmol/kg, iv) to evaluate thoroughly the genotoxic potential for MultiHance. It should be also noted that the biodistribution studies after intravenous administration in rat and rabbit showed some accumulation (although low) of free gadolinium) in bone that was persistent. Biodistribution kinetics after intraperitoneal administration was not evaluated.

To address above concerns, an in vivo micronucleus test in rat should be conducted using maximum tolerated dose level for MultiHance (4 mmol/kg, intravenous).

VI. CARCINOGENICITY: N/A

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

(A) Segment 2 study in rats:

Original comment: A Segment 2 study in rats should be conducted with the proposed market formulation (0.5 M) at dose levels where some maternal toxicity is observed.

Sponsor's response: A classical Segment 2 study was conducted in 1990 in rats with the 0.25 M formulation. However, in 1993, ICH S5A reached step 4. In this guideline, the 'most probable option' regarding study design was: a) Fertility and early embryonic development, b) Pre- and postnatal development including maternal function and c) Embryo-fetal development. Pre-postnatal study (BRO/067, NDA vol. 1.26) using the 0.5 M formulation incorporated the study design of the classical Segment 2 following the new ICH guidelines. In this study, MultiHance was administered from Day 6 of gestation to lactation Day 20.

With regard to maternal toxicity, the highest dose tested (2 mmol/kg/day) was selected based on the high dose given in repeat-dose toxicity study. At a dose of 3 mmol/kg, local reaction at the injection site prevented repeat-dosing in 7/18 females (repeat dose study in rats, BRO 57, NDA vol. 1.14).

Reviewer's response: This reviewer concurs with the sponsor's response. In repeat-dose study in rats, there was a dose-dependent increase in the degree of lesions/ulceration found in the vein and perivascular tissue at the injection sites at all dose levels (0.3-3 mmol/kg/day). This kind of local reaction was somewhat more severe in females at 3 mmol/kg/day, since 7/18 females were unable to be dosed on one/two occasions during the 3rd/4th week of study. The highest dose (2 mmol/kg/day) tested in the reproductive study in rats is approximately 2-times the clinical dose based on body surface area (5-times based on Cmax values).

It should be noted that the sponsor did conduct Segment 2 study in rabbits. The NOAEL for this study was established at 0.3 mmol/kg/day. At higher dose levels (0.9 & 2 mmol/kg/day), maternal toxicity (decreased body weight and food consumption) and fetotoxicity (retinal irregularities, additional/fused sternebral centers, offset pelvic girdles) was observed.

Due to reasons mentioned above, Segment 2 reproductive toxicity study in rats at higher dose level is not necessary.

(B) Male fertility study in rats:
Original comment: No effect was observed on male fertility in doses up to 2 mmol/kg. However, in repeat dose study in rats, MultiHance (3 mmol/kg/day) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely. The Segment I reproductive study should have been carried out at higher dose multiples for proper evaluation.

Sponsor's response: The sponsor has not specifically responded to this issue. The highest dose tested (2 mmol/kg/day) is based on the adverse local injection site reaction seen in rats.

Reviewer's comment: The repeat-dose study in rats produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely and it should be reflected in the label. Therefore, no further study is necessary.

VIII. SPECIAL TOXICOLOGY STUDIES:

(A) Local tolerance study:
Original comment: The local tolerance study histological evaluation at 8-days after MultiHance® administration revealed reddening, thickening, inflammatory cell infiltrates, eschare, and larger areas of necrosis. These findings were qualitatively more severe than the Magnevist control, and were not produced by the hyperosmolar control. The study did not include an evaluation at earlier time points. These findings suggest that local extravasation or prolonged intravenous exposure to MultiHance® may lead to thrombosis or phlebitis.

In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

Sponsor's response: Intravenous tolerance can be evaluated also from the repeat-dose study, where the dose of 3 mmol/kg in rats for 28 days caused local damage in almost all animals, without full recovery. In monkeys, repeated dosing (14 days) up to 3 mmol/kg caused no compound-related damage. However, paravasal tolerance and recovery were not assessed in animals.

An extensive local tolerance study will be performed to address this issue and the protocol will be submitted to the Agency for comment.

Reviewer's comment: The local adverse reactions after paravenous administration should be noted as 'warning' in the label (as is the case with other gadolinium agents). The extensive local tolerance study agreed to be performed by the Sponsor will answer the severity of local adverse effects due to rate of infusion and time-period necessary for resolution of these effects.

(B) Effect on coagulation parameters (in vivo study):
Original comment: No effects on coagulation parameters were reported in vivo. However, this study was carried out using 0.25 M MultiHance formulation. Also the study should have been carried out at clinically equivalent or higher doses. In an in vitro study, at higher doses, there were significant increases in PT and PTT and concomitant decreases in fibrinogen, This indicates that MultiHance has potential to affect coagulation parameters. We recommend an in vivo study, using high doses of 0.5 M MultiHance, in order to determine the effects of the drug on coagulation parameters and bleeding time.

Sponsor's response: In vivo study focusing on coagulation parameters was not performed with 0.5 M formulation. However, results from toxicology studies conducted with 0.5 M formulation showed lack of significant effects on coagulation even after multiple administration of MultiHance.

4-week study in rats (BRO/57, vol. 1.14): No effect on PT, aPTT up to 3 mmol/kg (highest dose).
4-week study in dogs (97-3357, vol. 1.16): aPTT increased at 2 mmol/kg and 1 mmol/kg (males only) with recovery. No effects were seen on PT and fibrin.
4-week study in dogs (ESI 143/970956, vol. 1.15): No effects on PT, aPTT, fibrinogen up to 2 mmol/kg (highest dose).
2-week study in monkeys (BRO 92/973656, vol. 1.18): No effects on PT, and aPTT up to 3 mmol/kg (highest dose).

Furthermore, consider that prolongation of coagulation times would have been detected in cases where hemorrhages occur spontaneously (for example, in reproduction studies thus leading to abortion/death of mother or fetuses). In these studies, animals were repeatedly administered before parturition.

Reviewer's comments: The reviewer concurs with the sponsor's response. It should be also noted that in an in vitro study, the effects on PTT, PT and fibrinogen were minimal (~5%) at 6.3 mM dose level. According to the sponsor, this dose corresponds to about three times the maximum plasma levels in clinical setting. At higher doses (13 and 25 mM), somewhat significant increases in PT (13 & 28%, respectively) and PTT (11 & 26%, respectively) and concomitant decrease in fibrinogen (9 & 16%, respectively) were observed. The in vivo study may not be necessary as in vitro study was conducted and repeat-dose studies in various species did not suggest significant effects on coagulation parameters.

IX. CONCLUSIONS AND RECOMMENDATIONS:

Recommendations: The NDA application for MultiHance is deemed **approvable** subject to fulfillment of following pre-clinical studies:

(A) Safety pharmacology study: As stated in our original comments, a comprehensive safety pharmacology study in monkey (as pharmacokinetic profile is similar to humans) should be conducted. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG

monitoring, QT interval etc.) and respiratory parameters. Hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan should be included for comparison purpose.

(B) In humans, gadolinium contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. Conduct *in vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels for MultiHance.

(C) Genotoxicity study: An *in vivo* micronucleus assay using intravenous administration route and higher dose levels of MultiHance® (MTD) should be conducted.

(D) Male fertility study: No effect was observed on male fertility in doses up to 2 mmol/kg. However, in repeat dose study in rats, MultiHance at higher dose level (3 mmol/kg/day) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that effects on male fertility are likely and it will be reflected in the label.

(E) Local tolerance study: The local tolerance study histological evaluation at 8-days after MultiHance® administration revealed reddening, thickening, inflammatory cell infiltrates, eschare, and larger areas of necrosis. These findings were qualitatively more severe than the Magnevist control, and were not produced by the hyperosmolar control. The study did not include an evaluation at earlier time points. These findings suggest that local extravasation or prolonged intravenous exposure to MultiHance® may lead to thrombosis or phlebitis.

In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

(F) Due to relatively low safety margin for MultiHance, in particular adverse effects associated with CNS and liver enzyme changes (also seen clinically in some patients), pre-clinical studies in juvenile animals may be necessary depending on the resolution of pediatric clinical studies

We encourage you to submit protocols for the recommended studies in order to resolve any experimental design issues.

X. APPENDIX/ATTACHMENTS: NONE

External Pharmacology/Toxicology Comments For the Sponsor:

(A) Safety pharmacology study: As stated in our original comments, a comprehensive safety pharmacology study in monkey (as pharmacokinetic profile is similar to humans) should be conducted. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study should include a complete battery of CVS (including continuous ECG monitoring, QT interval etc.) and respiratory parameters. Hyperosmotic control group (sucrose/mannitol solution), Magnevist® and Optimark or Omniscan should be included for comparison purpose.

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In order to address this concern, conduct a more extensive local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance® is proposed for direct bolus or infusion. The study should address the rate of infusion as well.

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We encourage you to submit protocols for the recommended studies in order to resolve any experimental design issues.

To: Florence Houn
Director ODE III

From: John Leighton
Associate Director for Pharmacology/Toxicology, ODE III

Subject: NDA 21-357/21-358
MultiHance (gadobendate dimglumine)

Date: February 19, 2002

Introduction

MultiHance is proposed for MRI contrast enhancement in the CNS ——— The pharmacology and toxicology review indicated that there were a number of deficiencies in the studies submitted by the Sponsor, Bracco Diagnostics, Inc. The Pharmacology/Toxicology team leader, Nakissa Sadrieh, in her Supervisory Pharmacologist Memo, summarized these deficiencies. The corresponding studies to correct these deficiencies are detailed in "II. Safety" section of the draft letter to the Sponsor.

Review of draft comments to the Sponsor

The Division pharmacology reviewer has identified inadequate animal dosing and lack of proper control groups in a number of studies as a detriment to an understanding of the full range of toxicities of gadobenate dimglumine. With few exceptions, I agree with this assessment. The following is an assessment of the Division's recommendations as provided in the draft letter to the Sponsor.

1. While the preclinical safety pharmacology studies are inadequate, they did identify gadobenate toxicities to the central nervous and cardiovascular systems. MultiHance also has a very steep dose-response curve. In addition, the sponsor did not properly control for potential effects of osmolarity. Safety pharmacology studies are usually conducted early in development, usually prior to initial clinical studies. However, for the reasons stated above, the Division's request that additional safety pharmacology studies be conducted in a larger species appears reasonable.

2. The Division's request for an *in vitro* electrophysiological study is appropriate, considering the inadequate clinical monitoring and the potential for this class of agents to cause Qt prolongation.

3. The acute studies provided in the package can provide information of potential risks of overdose but are not substitute for adequate toxicity studies at the clinical dose and schedule. The repeat dose toxicity studies are also not sufficient for this purpose. I support the Division's position that adequate expanded acute studies be conducted and that these studies assess early and delayed toxicities and recovery of toxic effects. These studies are described in ICH guidance. Finally, the wording in number 4 (page 10 of the draft letter) should be changed to "a large animal species....".

4. The Sponsor should repeat the Segment I study in females at appropriate dosing. However, the Division should justify its request to conduct the Segment I study in males. According to the Supervisory Pharmacologist Memo, gadobenate has been shown to result in depletion/degeneration of spermatogenic cells in males and should be labeled accordingly. The adverse effect was still noted after 4 weeks recovery. It is unlikely that the Segment I study in males would provide additional useful information. Additional male fertility studies could focus on the long-term nature of the depletion/degeneration. If the Sponsor should conduct another study in males, then the wording of number 6 should be clarified; assessing fertility parameters in males cannot be conducted at doses that produce maternal toxicity.

5. The *in vivo* micronucleus test in mice should be repeated at appropriate dose levels. This test will provide the only information that will be available regarding the potential for genetic damage in vivo, as carcinogenicity studies will not be conducted for this product. The rationale for this recommendation is slightly different from that stated in the Supervisory Memo. The study should be repeated because the Sponsor failed to demonstrate that gadobendate reached the target site, as described by ICH guidance, rather than the conduct of the study via intraperitoneal injection.

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/s/

John Leighton
2/20/02 10:53:04 AM
PHARMACOLOGIST

Review and Evaluation of Pharmacology and Toxicology Data
Division of Medical Imaging and Radiopharmaceutical Drug Products (HFD-160)

1	**Electronic File Number:**	-------
2	**NDA Number:** Relevant IND:	**21-357** 43,779
3	**Serial Number:** **Date:** **Type of submission:**	000 April 27, 2001 **Original NDA**
4	**Reviewer:**	Tushar Kokate, Ph.D.
5	**Sponsor:**	**Bracco Diagnostics Inc.,** Princeton, NJ 08543
6	**Information to Sponsor:**	**Yes (X)** No ( )
7	**Drug name:**	**MultiHance**
8	**Generic name:**	Gadobenate dimeglumine
9	**Other names**	Gd-BOPTA/Dimeg, B19036/7, E7155
10	**Manufacturer for drug substance:**	Bracco Imaging SpA, Milan, Italy
11	**Molecular weight:**	1058
12	**Chemical formula:**	(4RS)-[4-carboxy-5,8,11-tris(carboxymethyl)--1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato (5-)} gadolinate(2-) dihydrogen compound with 1-deoxy-1-(methylamino)-D-glucitol (1:2) $C_{22}H_{28}GdN_3O_{11}.2C_7H_{17}NO_5$
13	**Drug Class:**	**MRI Contrast Agent**
14	**Route of Administration:**	Intravenous (bolus or rapid infusion)
15	**Strength:**	529 mg of gadobenate dimeglumine/ml

16 Indication:

17 Clinical Dose: (a) Imaging of CNS in adults: 0.1 mmol/kg,

Maximum clinical dose = 0.2 mmol/kg

18 Clinical Formulation: MultiHance for injection is supplied as a sterile, non-pyrogenic solution. Each ml of MultiHance contains 529 mg of gadobenate dimeglumine. During the clinical development of MultiHance, the use of a 0.25 M solution was tested in clinical studies, and in several non-clinical safety and efficacy studies. The formulation for marketing is 0.5 M gadobenate dimeglumine. The sponsor conducted required pharmacology and toxicology studies using 0.5 M clinical formulation. The product will be supplied as a vial with a total volume of 5, 10, 15 or 20 ml.

The clinical formulation (0.5 M) composition: Gd-BOPTA: Meglumine: mg/ml and water for injection: The osmolarity of the solution is 1970 mOsmol/kg. This osmolarity is 6.9 times that of plasma (285 mOsmol/kg). pH of the solution is 6.5-7.5.

19 Studies reviewed within this submission:

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
W19981495 08-01	Imaging study using different solvents	*In vitro* study	K5602--0AZB	0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mM	10
W19972246 08-13	Imaging study in implanted liver metastasis model	Mice	K56019-AZB	0.1 and 0.2 0.2 (0.04 & 0.1)	11
RI263 08-27	Imaging study of liver parenchyma in normal rats	Rats	RG9/89	0.1-0.5 (0.1-0.4)	11
RI259 08-38	Imaging study in rats bearing liver tumors (Walker carcinoma)	Rats	RG9/89	0.25 (0.2)	12
RI1329 08-49	Dynamic imaging study of blood-borne liver metastases	Rats	RG1/90	0.2 (0.2)	13
RI1174 08-59	Characterization of hepatitis in rats by MRI	Rats	RG7/90	0.25 (0.2)	14
RI1164 08-102	Mechanism of MRI signal intensity enhancement in liver	Dogs	RG2/91	0.2 (0.5)	14

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
RI2110-1 08-130	Imaging of CNS in brain tumor bearing rats	Rats	RG8/92	0.1 (0.1)	15
G1996003 09-01	General effects on CNS	Mice	K56091 —9AZB	0.2 & 1 (0.1, 0.4)	18
G97001 09-27	Effect on locomotor activity	Mice	K56091 —9AZB	0.2-1 (0.1-0.4)	18
RF994-1 09-44	Effect on behavior	Rats	RG7/88	0.1-1 (0.1-0.8)	19
RF1150-1 09-56	Effect on locomotor activity	Rats	RG7/88	1 & 2 (0.8 & 1.7)	19
ADE-R002 09-66	Effect on blood brain barrier	Rats	RG2/91	1 (0.8)	19
ADE-R003 09-90	Penetration through damaged BBB	Rats	SP21/91	0.3 (0.3)	19
RF5484 09-128	Passage through BBB with focal brain ischemis	Rats	RG14D/ 95	2 & 4 (1.6 & 3.3)	19
RF2118 09-150	Effect on cerebral electrical activity	Rats	RG7/88	1 (0.8)	20
RF5528 09-168	Effect on EEG	Rats	RG20/9 3	2 & 4 (1.6 & 3.3)	20
RF4791 09-208	Effect on visual evoked potential	Rats	RG4/92	0.9 (0.8)	20
RF1151-1 10-01	Effect on motor coordination	Rats	RG7/88	0.01-0.03 (icv)	20
RF2579 10-14	Conditional taste aversion	Rats	RF2579	0.03 (icv)	20
RF3663 10-30	Effect on evoked potential	Rats	RG02/9	0.03 (icv)	21
RF993 10-178	Effect on BP and ECG	Rats	RG7/88	1 (0.8)	22
RF3334 10-196	Effect on respiratory system	Guinea pigs	RG4/91	1 (1)	22
RF1735 10-243	Effect on CVS	Rabbits	RG7/88	1 (1.7)	22
RF3014 10-277	Effect on renal function	Rabbits	RG2/91	1 (1.7)	23
RF2953 10-292	Effect on respiratory system	Rabbits	RG2/91	1 (1.7)	23
B19036/7 10-314	Effect on CVS and respiratory system	Pigs	RG4/91	1 (3.6)	23
RF5470 10-356	Effect on CVS and maximum tolerated dose	Micropigs	RG14D/ 95	1-8 (3-14)	23

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
RF5474 11-01	Effct on CVS in ischemia model	Micropigs	RG14D/ 95	1-3 (2-6)	24
RF1157 11-39	Effct on papillary muscle	*In vitro* study	RG7/88	30 mM	24
RF1162 11-51	Effct on g. pig atria	*In vitro* study	RG7/88	30 mM	24
RF6646 11-64	Effect on cardiac function (Lagnedorff preparation)	*In vitro* study	RG14D/ 95	20 micromol	24
G1996007 11-83	Effct on watre & electrolyte metabolism	Rats	RG14D/ 95	0.2-1 (0.2-0.8)	25
G1996005 11-127	Effect on contractile responses of smooth muscle	*In vitro* study	K56019 AZB	0.01-1 mM	25
G1996004 11-147	Effect on intestinal transit	Mice	K56019 AZB	0.2 & 0.1 (0.4)	25
RF1433 29-209	PK study in rats	Rat	RG7/88	0.1-0.5 (0.1-0.4)	30
36-027 29-239	PK parameters & distribution study	Rat	RG9/95	0.25 (0.2)	31
ADE-R91006 29-340	PK study in rats	Rat	RG2/91	1 (0.8)	32
9896 30-247	PK parameters in dogs	Dogs	RG9/92	1 (2.5)	33
36-029 30-332	PK parameters in dogs	Dogs	RG9/95	0.25 (0.6)	35
RF5631 30-363	PK study in monkeys	Monkey	RG15D/ 95	1-8 (2-13)	36
36-030 31-24	PK study in mice	Mice	RG9/95	0.1 (0.04)	38
ADME002 31-076	Distribution & elimination in rats	Rats	RG9/88	0.25 (0.2)	38
ADE-R92001 31-119	Whole body autoradiography study	Rats	RG1390	1 (0.8)	40
IRI9858 31-402	Distribution & excretion study	Rabbits	RG9/92	1 9 (1.7)	41
ADER004 31-158	PK study in pregnant rats	Rats	RG1/91	0.5 (0.4)	43
ADER92002 31-288	Whole body autoradiography in pregnant rats	Rats	RG1390	0.5 (0.4)	44
ADER91005 31-237	Transfer of contrast ageny via milk	Rats	RG2/91	0.5 (0.4)	45
RF1619 31-49	Binding to plasma proteins	*In vitro* study	RG7/88	0.2-10 mM	46

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
RF1768 31-64	Binding to rabbit plasma and human serum albumin	*In vitro* study	RG9/89	0.2-10 mM	46
RF1432 32-20	Biliary and urinary excretion study	Rats	RG7/88	0.1-0.5 (0.1-0.4)	47
RF4648 32-47	Biliary & urinary excretion in mutant rats	TR⁻ Mutant Rats	RG4/92	0.25 (0.2)	48
36-028 32-89	Enterohepatic circulation study	Rats	RG9/95	0.25 (0.2)	49
36-031 32-10	Effect on hepatic drug metabolizing enzymes	Rats	RG9/95	0.1-0.5 (0.1-0.4)	50
RF946-1 12-01	LD50 study (1 ml/min)	Mice	RG7/88	5-6.3 (2-3)	55
RF2784 12-10	LD50 study (0.2 ml/min)	Mice	RG4/91	6.5-8.4 (3-4)	55
RF954-1 12-22	LD50 study (6 ml/min)	Rats	RG7/88	3-8 (3-7)	55
RF2785 12-31	LD50 study (1 ml/min)	Rats	RG4/91	8-13 (7-10)	55
RF3213 12-193	LD50 study (neonatal rats)	Rats	RG4/91	6-10 (4-8)	56
RF1050-1 12-217	LD50 study (icv)	Mice	RG7/88	0.3-0.7 (icv)	56
RF1161-1 12-226	LD50 study (icv)	Rats	RG7/88	0.2-0.4 (icv)	56
S98610 12-43	Acute study: renal tubular vacuolation effect	Rats	K56019 AZB	0.1-2 (0.1-2)	57
RF946-1 12-01	Limited acute toxicity study	Dogs	RG7/88	2 & 6 (5 & 15)	57
BRO54/962122 12-256	Maximum tolerated dose (MTD) in rats	Rats	RG14D/95	4-6 (3-5)	58
RF5531 13-16	MTD (acute) study	Rabbits	RG7/88	4-8 (7-13)	60
LSR-RTC215 13-45	MTD (acute) study	Dogs	RG4/91	1-4 (3-10)	60
BRO53/962175 13-187	MTD (acute) study	Monkey	RG4/91	2-8 (5-20)	61
BRO57/963792 14-01	Repeat dose study	Rats	B5/20	0.3-3 (0.3-2.5)	61
97-3357	Repeat dose study	Dogs	B9/20	0.25-2 (0.6-5)	65

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
16 & 17-01					
BRO92/97365 6 18-01	Repeat dose study	Monkey	B5/20	0.25-3 (0.4-5)	68
RF4511 21-193	Histamine release fro mast cells study	*In vitro* study	RG4/92	125 & 250 mM	77
936161 21-212	Antigenicity study	G. pigs	K2Y018 ZZA	0.25 & 1 (0.3 &2)	78
RF2681 21-103	Complement activation study	*In vitro* study	RG9/89	25×10^{-4} M-- 4×10^{-2} M	79
880376 28-01	Ames test	*In vitro* study	RG7/88	1-5000 microgm	81
920876 28-41	Ames test (E. coli)	*In vitro* study	RG2/92	50-5000 microgm	82
880377 28-71	Ames test (S. cerevisiae)	*In vitro* study	RG7/88	125-5000 microgm	82
880379 28-100	Chromosome aberration in human lymphocyte cells	*In vitro* study	RG7/88	1-1000 microgm	83
880378 28-122	Unscheduled synthesis in HeLa cells	*In vitro* study	RG7/88	10-5000 microgm	84
910466 28-149	Chinese hamster lung cells mutagenicity assay	*In vitro* study	RG4/91	10-5000 microgm	84
880380 28-182	Micronucleus assay	Rats	RG2/89	5 mg/kg (ip)	85
BRO70972236 22-01	Male fertility study	Rats	B5/20	0.3-2 (0.3-1.7)	88
BRO71970104 22-158	Female fertility & embryo-fetal development (Segment I)	Rats	B5/20	0.3-2 (0.3-2)	89
BRO56962671 24-19	Segment II reproductive toxicity study (preliminary)	Rabbit	B5/20	0.5-3 (1-5)	90
BRO58963667 24-57	Segment II reproductive study	Rabbit	B5/20	0.3-2 (0.5-3.3)	91
BRO67970081 26-199	Segment III reproductive study	Rats	B5/20	0.3-2 (0.3-2)	92
900092 23-01	Fertility study in male & female rats (0.25 M)	Rats	B5/20	0.4-1.5 (0.3-1.3)	93
RF2735 21-120	Local tolerance (iv)	Rabbit	RG2/91	0.5 ml	97
RF27321 21-139	Local tolerance (paravenous)	Rabbit	RG2/91	0.3 ml	98
703915 21-154	Muscular irritation study	Rabbit	K56019 AZB	1 ml	99

Study # Vol #-page #	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
RF5435 21-01	Hemolytic potential (human blood)	*In vitro* study	RG15D/ 95	10:1 (v/v)	100
RG4862 21-13	Effect on human erythrocytes	*In vitro* study	RG15D/ 95	13-30% v/v	100
RF1812 21-47	Effect on coagulation parameters (in vivo)	Rats	RG9/89	0.1-0.25 (0.1-0.2)	101
RF1776 21-88	Effect on coagulation parameters (in vitro)	*In vitro* study	RG9/89	6-25 mM	101

20 **Studies not reviewed within this submission:** Most of the studies that utilized 0.25 M formulation of MultiHance were not reviewed, since intended strength of clinical formulation is 0.5 M, and all required pharmacology & toxicology studies were conducted (and reviewed) using 0.5 M formulation. Follwing is the list of studies that utilized 0.25 M formulation that were not reviewed. In addtition, studies conducted using oral route of administration were not reviewed, since the intended clinical route of administration is intravenous.

Study # (Vol #-page #)	Study Type	Species
RI516 (8-146)	Cardiac imaging study	Rats
RI1332 (8-159)	Cardiac imaging study	Rats
RF3013 (10-48)	Brain levels of monoamines after conrast administration	Rats
RF5138 (10-69)	Effect on dopamine release (icv)	Rats
RF5481 (10-107)	Effect on neuron metabolic function (icv)	Rats
RF5484 (10-131)	Effect on visual evoked potential	Rabbit
RF2221 (11-162)	Effect on liver function (0.25 M)	Rats
RF5606 (11-221)	Effect on ACE enzyme (0.25 M)	*In vitro* study
RF4117 (11-236)	Effect on captopril activity (pharmacodynamic interaction)	Rabbit
RF4086 (11-271)	Effect on isosorbide dinitrate activity (pharmacodynamic interaction)	Rabbit
RF4302 (11-338)	Effect on dobutamine activity (pharmacodynamic interaction)	Rabbit
RF1277 (12-132)	Limited acute toxicity study (0.25 M): LD50 study	Mice
RF1757 (12-142)	Limited acute toxicity study (0.25 M): LD50 study	Mice
RF1513 (12-152)	Limited acute toxicity study (0.25 M): LD50 study	Rats
RF1758-1 (12-161)	Limited acute toxicity study (0.25 M): LD50 study	Rats
RF2525 (12-171)	Limited acute toxicity study: LD50 study (oral administration)	Mice
RF1477 (12-246)	Limited acute toxicity study (0.25 M): LD50 study (icv)	Mice
216-092-005 (18-269)	Prliminary repeat dose study (0.25 M)	Rats
232-092-008 (19-01)	Repeat dose study in rats (0.25 M)	Rats

Study # (Vol #-page #)	Study Type	Species
219-092-006 (20-01)	Repeat dose study in dogs (0.25 M)	Dogs
36-032 (21-029)	Transfer into blood cells (0.25 M)	*In vitro* study
900094 (24-132)	Preliminary Teratology study (Segment II) (0.25 M)	Rats
900090 (25-01)	Segment II teratoogy study (0.25 M)	Rats
RF5603 (26-01)	Priliminary embryo-fetal toxicity study (0.25 M)	Rabbit
900091 (26-80)	Segment II reproductive toxicity study (0.25 M)	Rabbit
900093 (27-01)	Segment III reproductive toxicity study (0.25 M)	Rats
RF1829 (28-204)	HPLC assay method (validation study)	*In vitro* study
RF1830 (28-219)	HPLC assay for bile and urine determination (validation study)	Rats
RF1855 (28-284)	[redacted] spectrophotometric assay (validation study)	*In vitro* study
RF1733 (28-309)	Stability if B19036/7 in bilogical fluids	*In vitro* study
BRO65/970894 (28-350)	Validation of bioanalytical method for rat plasma	Rats
ESI144963528 (29-01)	Validation of bioanalytical method for dog plasma	Dogs
BRO63963785 (29-67)	Validation of bioanalytical method for rabbit plasma	Rabbit
BRO91972871 (28-135)	Validation of bioanalytical method for Monkey plasma	Monkey
RF5921 (31-01)	Plasma levels after intraperitoneal injection	Rats
RF1761 (32-183)	Biliary transport after intravenous infusion (0.25 M)	Rabbit
RF1606 (32-266)	Enterohepatic circulation	Rabbit

21 **Disclaimer-use of sponsor's material:** Some of the information contained in this review is taken from the sponsor's IND submission.

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22 Introduction: MultiHance (0.5 M gadobenate dimeglumine) is a MRI agent proposed for the imaging of the CNS. Gadobenate dimeglumine consists of an octadentate chelate of paramagnetic ion Gd^{3+} which, due to its distribution characteristics, is intended as an intravascular MRI contrast agent for hepatic and CNS imaging. The chelation of Gd^{3+} presumably results in substantially less toxicity than the free ion. The drug exits the vascular space rapidly and resides primarily in the extravascular space, but is also present within hepatocytes. In animals (rats, dogs & rabbits), MultiHance is excreted mainly through the renal (50-65%) and biliary (35-50%) routes. In humans, renal elimination (78-96%) is a major route, although some excretion through biliary route (1-4%) is also observed.

MultiHance has an osmolarity (1970 mOsmol/kg) that is 6.9 times that of plasma (285 mOsmol/kg), and is hypertonic under conditions of use. The hyperosmolarity of MultiHance is similar to Magnevist (1960 mOsmol/kg), an approved gadolinium type MRI contrast agent. The maximum clinical dose of MultiHance is 0.2 mmol/kg. It is administered as a rapid intravenous infusion or bolus injection followed by 5-ml saline flush.

23 Table of contents:

24 Pre-clinical efficacy (imaging) studies:

Imaging studies using 0.5 M and 0.25 M gadobenate dimeglumine were conducted in normal animals and in animal models of disease to assess its potential efficacy as a contrast medium for MRI and to compare with Magnvist. Imaging studies were conducted in liver, brain and heart. [redacted] since MultiHance is indicated for imaging of CNS [redacted] All CNS [redacted] imaging studies were reviewed.

24.1 Comparison of *in vitro* MR signal intensity between dimeglumine gadobenate and dimeglumine gadopentetate:

Study number: W19981495 Report date: August 28, 1998
Study located in Vol. 08, page 01 Batch # K56020AZB
Certificate of analysis: No GLP: No
Site: [redacted]
Doses: 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mM

Experimental design: The intensity of MR signals between 0.5 M MultiHance and 0.5 M Magnevist was compared in various solvents using T1- (spin-echo, fast spin-echo and gradient-echo) and T2 (spin-echo)-weighted images. Five kinds of solvents were used: phosphate-buffered saline, bovine serum albumin (1.75%, 3.5% and 7%) and rat plasma. MR imaging was performed on a [redacted] apparatus.

Results and conclusions: MultiHance and Magnevist produced a bell-shaped dose-response curve with T1-weighted images in all solvents. Gadobenate diemglumine and Magnevist enhanced the signal intensity in T1-weighted images under all MRI conditions, starting at low concentration (0.01 mM and 0.03 mM, respectively). For both compounds, the signal intensity reached at peak at 1 mM and reduced at higher concentrations (3 and 10 mM). The signal intensity was slightly higher for gadobenate dimeglumine than for Magnevist at lower concentrations (<1 mM). However, the peak intensity at 1 mM was identical. In T2-weighted images, MultiHance enhanced the signal intensity up to 0.1 mM concentration and Magnevist up to 0.3 mM concentration. At higher concentrations, the signal intensity decreased sharply due to T2 reduction (nearly zero at 10 mM). According to the sponsor, the weakening of the signal intensity in T1-weighted images in the high concentration range may be due to accentuated T2 reduction.

24.2 [redacted]

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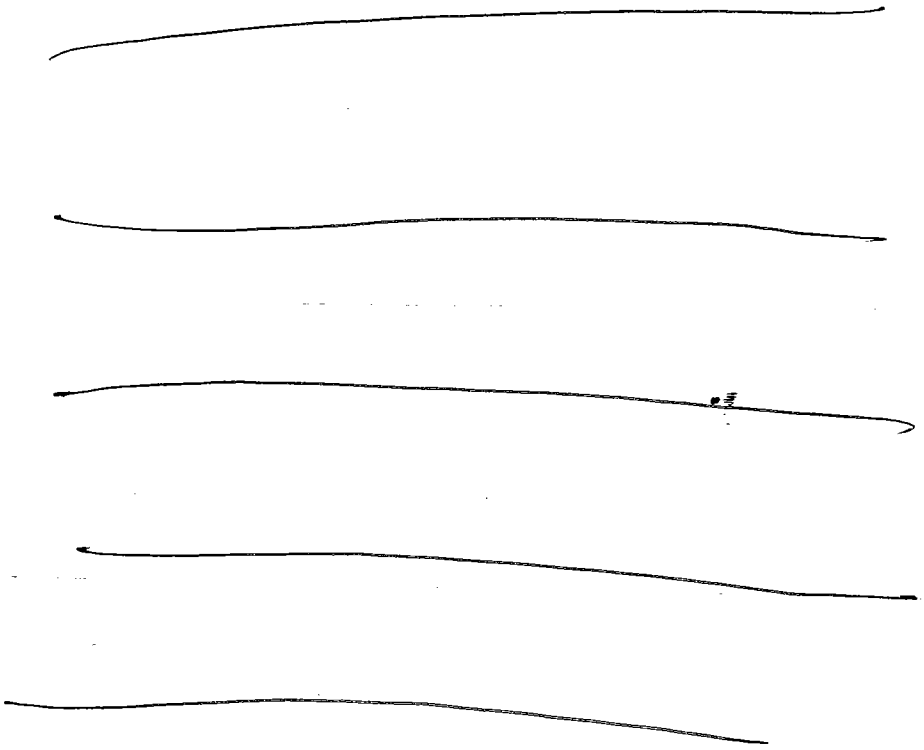
_____ § 552(b)(5) Deliberative Process

_____ § 552(b)(5) Draft Labeling



24.8 Conventional and magnetization transfer (MT) MRI of brain tumor bearing rats with gadopentetate dimeglumine and gadobenate dimeglumine:

Study number: RI 2110-1 Report date: April 11, 1997
Study located in Vol. 08, page 130 Batch # RG8/92
Certificate of analysis: No GLP: No
Site: Bracco SpA, Milano, Italy
Species: Sprague-Dawley male rats (175-275 g)
Doses: 0.1 mmol/kg (0.1-times the clinical dose, mg/m^2)

Experimental design: This study was conducted to compare 0.25 M MultiHance and 0.5 M Magnevist with regard to their ability to increase conspicuity of brain neoplasms in a rat model using conventional Spin Echo (SE) and Magnetization Transfer Spin Echo (MT-SE) imaging at 2T. Cerebral tumors were induced by stereotaxic injection of C6-glioma live cells into the brain of five male rats. Each rat received 0.1 mmol/kg MultiHance followed by the same dose of Magnevist after three hours. T1-weighted images were acquired at 2.5 min intervals prior to and

up to 3 hours post-dosing. Enhancement of lesion and of brain lesion to brain ratio (L/B) and MT ratio (MTR) were calculated from normalized signal intensities.

Results and reviewer's comments: In conventional SE imaging, tumor enhancement peaked after 7.5 min at 64% and 87% for Magnevist and MultiHance, respectively. The stronger lesion enhancement led to a higher L/B ratio, with peak values 1.6 and 1.4 for MultiHance and Magnevist, respectively. In MT-SE imaging, peak L/B values were 2.6 (at 15 min) for MultiHance and 2 (at 15 min) for Magnevist. The signal imaging enhancements in MT-SE imaging was 149% and 93% for MultiHance and Magnevist, respectively. The MTR (% loss of SI following MT) of normal brain did not change after administration of either contrast agents (48%). The MTR of the tumors, which amounted to 37% and 36% prior to contrasts, decreased to 16% and 25% for MultiHance and Magnevist, respectively indicating enhanced ability of MultiHance in suppressing the MT effect in the lesion. The sponsor concluded that in conventional SE imaging MultiHance increases lesion conspicuity somewhat more than Magnevist. Also MT was more effective after MutliHance than after Magnevist administration. The CNS imaging was carried out using 0.1 mmol/kg dose; no dose-response relationship was evaluated.

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Summary of pre-clinical efficacy/imaging studies:

Imaging studies using 0.5 and 0.25 M gadobenate dimeglumine were conducted in normal animals and in animal models of disease in order to assess its potential efficacy as a contrast medium for MRI and to compare with Magnevist.

. CNS imaging studies (# RI2110-1) were conducted in a rat model of implanted brain tumors. MultiHance (0.1 mmol/kg) increased brain lesion conspicuity somewhat more than Magenvist (0.1 mmol/kg) in conventional SE imaging (87% versus 64% enhancement over pre-contrast).

In summary, pre-clinical imaging studies

In rats, MultiHance increased sensitivity of contrast enhanced MRI of CNS over that achieved with Magnevist.

25 Safety Pharmacology:

Safety pharmacology studies were conducted in mice, rats, rabbits, guinea pigs and micropigs to evaluate the potential effects of MultiHance on cardiovascular, central nervous, renal and respiratory systems. *In vivo* studies were conducted in healthy animals and in animal models of clinical disease. These studies were carried out using 0.5 M (clinical formulation) and 0.25 M formulations of MultiHance. Only studies that were carried out with 0.5 M formulation are reviewed here. These studies with 0.5 M formulation includes all the required safety pharmacology studies.

(A) 25.1-25.13: Neuropharmacological effects:

Several studies were conducted to evaluate potential effects of 0.5 M MultiHance on central nervous system function. The parameters evaluated in these studies include effects on behavior, spontaneous locomotor activity & motor coordination, pentobarbital-induced anesthesia, pentylenetetrazol-induced seizures, conditioned taste aversion, EEG and blood brain barrier. The effects were evaluated using both intravenous and intraventricular administration (in terms of 'worse case' effect of the contrast agent in patients with damaged BBB). The table below summarizes in detail the experimental design, dosing regimen and results obtained using various dose levels of MultiHance.

Study title (study #, vol.: page #, Lot #) and species	**Dose levels (mmol/kg) and experimental design**	**Findings**	**NOAEL: mmol/kg (human dose multiple, mg/m2)**
General effects on CNS (G1996003, 09:01, K56019AZB) Male ICR mice (8/group)	Dose: 0. 0.2 and 1, iv Parameters: Spontaneous locomotor activity, pentobarbital (45 mg/kg, ip)-induced anesthesia, PTZ (75/150 mg/kg, ip)-induced convulsions, acetic acid (0.7%, ip)-induced writhing and body temperature (10-120 min). Observation period was 10-30 min.	Increased locomotor activity at 0.2 but not 1 mmol/kg. Prolongation of pentobarbital-induced anesthesia. No effect on body temperature, acetic acid-induced writhing and PTZ-induced seizures.	Locomotor activity: <0.2 Pentobarb anesthesia: 0.2 (0.1) Body temp., PTZ-seizures, acetic acid writhing: 1 (0.4)
Effects on locomotor activity & pentobarbital anesthesia (G97001, 09:27, K56019AZB) Male ICR mice (8/gr.)	Dose: 0.2, 0.5 & 1, iv Parameters: Locomotor activity, pentobarbital-induced anesthesia test.	No remarkable effects on spontaneous locomotor activity or pentobarbital-induced anesthesia.	Locomotor activity: 1 (0.4) Pentobarb anesthesia: 1 (0.4)

Effect on behavior (RF994-1, 09:44, RG7/88) CD (SD)BR male rats (4/group)	Dose: 0 & 1, iv. 0, 0.03, 0.06, 0.1 & 0.3, intracisternal. Comparison with same doses of Magnevist. Irwin behavioral test.	No remarkable effects after iv injection. Intracisternal: Decreased spontaneous activity, impaired motor coordination at 0.06 dose level. Convulsions and death (1/4) at 0.1 & 0.3 mmol/kg. Effects were similar for both MultiHance & Magnevist.	Behavioral effects NOAEL: Iv: 1 (0.8) Intracisternal: 0.03 mmol/kg
Effect on motor coordination (RF1150-1, 09:56, RG7/88). CD (SD)BR male rats (10/group)	Dose: Dose: 0, 1 & 2, iv Comparison with same doses of Magnevist. Rotorod test	No effect on motor coordination noted for both MultiHance and Magnevist at doses of 1 or 2 mmol/kg.	Motor coordination NOAEL: 2 (1.7)
Effect on blood brain barrier (ADE-R002, 09:66, RG2/91) Sprague-Dawley male rats (5/group)	Dose: 0 & 1, iv. Comparison with same doses of Magnevist. Measuring radioactivity in brain hemispheres of 14C-Sucrose administered iv (after 30-min).	No significant increase/ alteration in blood brain barrier permeability at 1 mmol/kg dose for both compounds.	BBB permeability NOAEL: 1 (0.8)
Penetration through damaged BBB (ADE-R003, 09:90, SP21/91) Sprague-Dawley rats (5/group)	Dose: 0.3, iv. Comparison with Magnevist. Measurement of brain radioactivity of 153Gd-labeled MultiHance or Magnevist, injected iv. BBB permeability was altered by hyperosmolar L(+)-arabinose.	Less than 0.2% of the injected dose of MultiHance or Magnevist passed through the damaged BBB.	No significant penetration through the damaged BBB at 0.3 mmol/kg dose (0.3 times human dose).
Passage of Gd ion into brain submitted to photochemical-induced ischemia (RF5484, 09:128, RG14D/95). CD (SD)BR male rats (4-7/group)	Dose: 2 and 4, iv Brain focal ischemia was induced photochemically. Measurement of gadobenate ion concentration in brain & plasma using HPLC.	The gadobenate ion was detected in both control and ischemic rat brains. 30-min after injection, mean ion concentration was 40-70 & 150-300 nmol/g for 2 & 4 mmol/kg doses, respectively. The ion concentration in ischemic area was higher than for the non-ischemic areas.	---
Effect on cerebral electrical activity (RF2118, 09:150, RG7/88) CD (SD)BR male rats	Dose: 1 (iv) & 0.025 (icv) Comparison with same doses of Magnevist. Measurement (chronic) of EEG	No remarkable effect of both compounds on duration of waking and sleeping periods or on REM sleep. No spikes reported. Some	NOAEL for effect on EEG: Iv: 1 (0.8) Icv: <0.025

(4-5/group)	activity (wake, nonREM, REM & spikes).	depressant activity seen with the intraventricular injection of both drugs.	
Effect on EEG in rats with focal brain ischemia (RF5528, 09:168, RG20/93) CD (SD)BR male rats (7-10/group)	Dose: 2 & 4, iv Brain focal ischemia was induced photochemically. EEG recorded in conscious animals by chronic cortical electrodes. Effect of hyperosmotic manitol solution (1.6 M) was also studied.	Ischemic lesion involved most of the right parital cortex and caused reduction in amplitude of EEG. MultiHance at 2 mmol/kg dose produced no modification of EEG traces. At 4 mmol/kg dose, 2/10 rats showed transient (2 min) flattening of EEG tracings in both hemispheres. Also slowing of EEG signal during the quite wake noted. No such effects were seen with hyperosmotic mannitol solution.	NOAEL for EEG effect in focal brain ischemia model: 2 mmol/kg (1.7)
Effect on visual evoked potentials in focal ischemia model (RF4791, 09:208, RG4/92) CD (SD)BR male rats (10/group)	Dose: 0.9 mmol/kg (Magnevist at same dose level). Flash visual evoked potentials (FEP) were recorded from the focal brain ischemic rat model by cortically implanted electrodes.	No significant effects of MultiHance or Magnevist on visual evoked potentials were noted in a rat model of focal brain ischemia.	NOAEL for effects on evoked potential: 0.9 (0.8)
Effect on motor coordination after ics injection. (RF1151-1, 10:01 RG7/88) CD(SD)BR male rats (10/group)	Dose: MultiHance (0.01, 0.02 & 0.03, intracisternal). Magnevist (0.015-0.07, ics). Rotorod test for motor coordination.	Both compounds dose-dependently impaired motor coordination after intracisternal injection. The ED50 values for motor incoordination were: 0.02 (MultiHance) & 0.04 (Magnevist) mmol/kg.	NOAEL for motor coordination after ics injection not established (incorodination at lowest dose).
Conditional taste aversion test (RF2579, 10:14, 2/91) CD (SD)BR male rats (10/group)	0 & 0.025, icv (Magnevist at similar dose). Conditioned taste aversion, (refusal to drink sucrose solution), indicative of general malaise state	No aversive effect noted for both compounds.	NOAEL for taste aversion effect: 0.025, icv
Effect on evoked potential after intraventricular injection (RF3663, 10:30, RG02/92) CD (SD)BR male rats (10/group)	Dose: 0.025, icv (Magnevist at similar dose) Flash evoked visual potentials.	No effect of both compounds on evoked potentials after 0.025 mmol/kg intraventricular administration.	NOAEL for evoked potential after icv injection: 0.025

Reviewer's comments: The NOAELs for various CNS parameters (intravenous administration) ranged from 0.2-2 times the clinical dose. However, in general, the dose multiples (0.1-2 times the clinical dose based on body surface area) used in above studies for evaluating CNS effects were too low for proper evaluation. It is not clear why higher doses were not tested, since the maximum tolerated dose in rats is 4 mmol/kg (3.3-times clinical dose). In general, the CNS effects of MultiHance were reported to be comparable to that of Magnevist.

Intravenous administration of MultiHance (1 mmol/kg or less, 0.8 times clinical dose) did not affect motor coordination, body temperature, pentylenetetrazol-induced seizures, acetic acid-induced writhing or general behavior in rats. However, studies conducted using intraventricular administration ('worse effect' scenario in patients with damaged BBB) suggest that MultiHance (<0.1 mmol/kg, <0.1-times clinical dose) has potential to produce hypoactivity, impaired motor incoordination, convulsions and death.

The NOAEL for adverse effects on EEG was established at 2 mmol/kg (1.7 times clinical dose) after intravenous administration in animal model of focal brain ischemia. However, at higher dose level (4 mmol/kg) slowing of the EEG signal during the quite wake was observed. In addition, transient flattening (for 2 min) of EEG was noted at this dose level (4 mmol/kg, 3.3 times the clinical dose). The sponsor needs to explain this EEG flattening effect in more detail. This reviewer is not sure how brain electrical activity is totally stopped in a conscious animal even though it is a transient effect. These adverse effects on EEG/spontaneous cortical electrical activity (slowing of signal and transient flattening) are not due to hyperosmolarity of the solution, since such effects were not seen with the hyperosmotic mannitol solution.

MultiHance (1 mmol/kg, 0.8 times human dose) did not alter the BBB permeability in normal rats, and in rats with damaged BBB less than 0.3% of the injected dose (0.3 mmol/kg, 0.3 x clinical dose) passed through the damaged BBB. The effects of MultiHance on blood brain barrier permeability in BBB damaged animals should have been studied at higher dose levels for meaningful comparison.

(B) 25.14-25.27 Cardiovascular, Respiratory, Renal and Gastrointestinal Effects:

The sponsor conducted several *in vitro* and *in vivo* studies in rats, micropigs, guinea pigs and rabbits to evaluate potential effects of MultiHance (0.5 M) on cardiovascular, respiratory, renal and gastrointestinal systems. These studies are summarized in detail in the table below:

Study title (study #, vol.: page #, Lot #) and species	Dose levels (mmol/kg) and experimental design	Findings	NOAEL: mmol/kg (human dose

			multiple, mg/m2)
Effect on blood pressure, ECG and heart rate (RF993, 10:178, RG7/88) Anesthetized CD (SD)BR male rats (5/group)	Dose: 0 & 1 (1 ml/min), iv Magnevist (1 mmol/kg, iv) Parameters: Arterial BP, ECG and heart rate. BP and heart rates were recorded continuously for 30 min. Values for BP and HR were determined at 4 & 2 min pre-dosing and 2, 4, 6, 8, 10, 20 & 30 min post-dosing. ECG was recorded for 10 sec at the same times above (not continuous).	Transient reduction in heart rate (7% compared to baseline) within 2 min post-injection. No significant variation in BP (maximum 15% decrease for <2 min). Magnevist produced 22% transient drop in BP and 6% decrease in heart rate. No significant variations in ECG parameters noted for both drugs.	NOAEL for CVS effects: 1 mmol/kg (0.8 times clinical dose). No continuous ECG recording. Also parameters such as QT interval were not evaluated.
Effect on intratracheal pressure (RF3334, 10:196, RG/4/91) Anesthetized male HA SPF guinea pigs (7/group)	Dose: 0 & 1, iv (3 ml/min) Also mannitol (2 mmol/kg) & Magnevist (1 mmol/kg) were tested for comparison. Intratracheal pressure measurements for 60 min to determine bronchoconstriction potential.	Although not significant, mild (9-14% maximum) increases in the intratracheal pressure seen over the time as compared to baseline values. The effects were comparable to the hyperosmotic mannitol solution.	NOAEL: 1 mmol/kg (1.0 times clinical dose)
Effects on cardiac output and total peripheral resistance (RF1735, 10:243, RG7/88) Anesthetized HY/— male rabbits (5/group)	Dose: 0 & 1 (MultiHance and Magnevist), iv Parameters: Mean arterial pressure, heart rate, cardiac output and stroke volume. Observation period: 30 min. Recording times: 1, 2, 4, 6, 8, 10, 15 & 30-min post-dosing.	No remarkable effects on MAP or heart rate. Transient but significant increases (within 1-2 min) in cardiac output (35%) and stroke volume (37%), which reversed within 10 min. Also transient decrease in peripheral resistance (24%). Magnevist produced similar but somewhat more prominent effects.	NOAEL: < 1 mmol/kg (1.7 times clinical dose). Transient but significant effects on CO, SV and total peripheral resistance
Effect on renal artery flow (RF3014, 10:277, RG2/91) Anesthetized HY/ — male rabbits (5/group)	Dose: 0 & 1 (MultiHance & Magnevist), iv (3 ml/min) Renal artery blood flow measurements for 30 min post-injection.	Early (within 1 min) transient increase in renal artery flow (maximum 29% increase compared to baseline) seen within one minute, which returned to baseline values by 10 min. The effects were comparable with Magnevist. Blood pressure and heart rate changes minor (<10%)	NOAEL: < 1 mmol/kg (1.7) Transient but significant increase in renal blood flow.

Effect on pulmonary artery flow (RF2953, 10:292, RG4/91) Anesthetized HY, —\ male rabbits (6/group)	Dose: 0 & 1 (MultiHance & Magnevist), iv (3 ml/min) Measurement of pulmonary blood flow for 30 min post-administration.	Transient but significant increase in pulmonary blood flow (maximum 49% as compared to baseline) at the end of administration. The effect returned to baseline level by 10 min. Effects were similar to Magnevist.	NOAEL: < 1 mmol/kg (1.7) Transient significant increase in the pulmonary blood flow.
Effects on CVS and respiratory system (no study number, 10:315, RG4/91) Anesthetized pigs (4 male + 4 female./group)	Dose: 0 & 1 (Magnevist at same dose), iv, 10 ml/min Parameters: Heart rate, BP, pulmonary arterial pressure, cardiac output, stroke volume, ECG, respiratory rate, tidal volume, inspiratory flow, pulmonary resistance etc. Recording time points: 1, 2, 5, 10, 15, 30, 60 & 90 min	There were no remarkable effects on any of the respiratory parameters studied. Also no significant effects noted on heart rate and BP (maximum 5% changes). Short-lasting slight increase in pulmonary arterial pressure. Transient but significant increases in cardiac output (28%) and stroke volume (23%), which reversed within 30 min. No significant effect on ECG (not continuous recording and no evaluation of QT interval were evaluated). Effects similar in proportion as noted with Magnevist.	NOAEL: < 1 mmol/kg (3.6 times human dose) Transient but significant changes in CO & SV.
Maximum tolerated dose, CVS parameters and toxicokinetics (RF5470, 10: 356, RG14D/95) Anesthetized Yucatan micropigs (5 males)	The MTD was determined by injecting increasing doses in the range from 1 to 8 mmol/kg, with 2-hr washout period between doses. CVS parameters: Heart rate, BP, left ventricular end systolic pressure for 60 min. Blood samples: 0, 1, 3, 5, 10, 15, 30, 60, 90 & 120 min for plasma detection of gadobenate ions.using HPLC method.	Transient but not significant effects on various CVS paramters at doses up to 4 mmol/kg. At 6 mmol/kg, significant decreases in heart rate, increase in LVEDP. Animal at 8 mmol/kg dose level died 60 min after injection. (due to severe hypotension). The gadobenate ion Cmax at 4 mmol/kg dose was 26.3 mmol/L	Maximum tolerated dose: 4 mmol/kg (14 x clinical dose) Cmax: 26.3 mmol/L at 4 MTD. (Human: 2.3 mmol/L at 0.3 mmol/kg)
CVS effect in pig model of myocardial ischemia (RF5474, 11:1, RG14D/95) Anesthetized Yucatan micropigs (3/group)	1, 2, & 3 mmol/kg, iv and 1.6 M mannitol (2, 4 & 6 ml/kg). CVS parameters: Heart rate, BP, LVP, CO, SV and systemic vascular resistance for 60 min. Drug was administered before	At 2 to 3 mmol/kg dose levels, transient dose-dependent decreases in heart rate, BP, LVESP and systemic vascular resistance, with associated transient increases in CO and SV (effect similar to that	NOAEL for pig myocardial ischemic model: 1 (3.6 times human dose).

	ischemia and post-ischemia (1 hr washout period) at same dose levels. Ischemia induced by20-min occlusion of coronary artery.	seen in healthy animals). Mannitol did not affect heart rate, BP. Other CVS effects similar but significantly less marked as compared to MultiHance.	
In vitro study: Effect on rat papillary muscle (RF1157, 11:39, RG7/88)	Concentration. 30 mM (Magnevist at same concen.). Effects on force of contraction of isolated rat cardiac muscle.	Both drugs produced minor but significant reduction in the contractile force (16-20%) of the rat papillary muscle as compared to the basal contraction force.	--
In vitro study: Effect on guinea pig atria (RF1162, 11:51, RG7/88)	Concentration: 30 mM To evaluate effect on cardiac rhythmic activity of isolated guinea pig atria.	Mild but significant decrease in both amplitude (19%) and frequency (10%) of spontaneous atrial contractions for both MultiHance and Magnevist.	--
In vitro study: Effect on cardiac function of isolated ischemic heart of rat (RF6646,11:64, RG14D/95 Langendorff heart preparation (rat)	Concentration: 20 micromol per organ (heart) Isolated hearts from rats were perfused with the Lagendorff solution and ischemia was produced by a ligation of descending coronary artery for 20 min. Left ventricular pressure and heart rate were monitored at 30 s and 2-min after treatment.	Under pre-ischemic, ischemic or post-ischemic status, both MultiHance and Magnevist caused quick short-lasting depressive action on cardiac activity (decrease in left ventricular pressure and heart rate). Mannitol produced similar but less prominent effect on the preparation.	---
Effects on water and electrolyte metabolism (G1996007, 11:83, RG14D/95) Male Sprague-Dawley rats (8/group)	Dose: 0.2 & 1 mmol/kg, iv Urine samples were collected in individual metabolic cages from 0-5 hr post-dosing. The urine volume and electrolyte concentrations (sodium, potassium and chloride) were determined.	No remarkable effects on urine volume or urinary electrolyte excretion were noted over a 5-hr observation period at both dose levels (0.2 & 1 mmol/kg).	NOAEL for effect on water and electrolyte metabolism: 1 (0.8)
In vitro study: Effect on contractile responses of isolated ileum (G1996005, 11:127, K56019AZB)	Concentration: 10, 100 micro molar, 1 mM Contractile responses induced by acetylholine, barium chloride and histamine in the isolated ileum of rat and guinea pig.	No remarkable effects on contractions induced by various agonists in smooth muscle of the isolated rat/guinea pig ileum.	---

Effect on intestinal transit in mice (G1996004, 11:147, K56019AZB) Male — mice (8/group)	Dose: 0.2 & 1 mmol/kg, iv Ten minute after injection, charcoal suspension was administered orally and rate of intestinal transit of charcoal was determined after 30 minute.	No prominent effect on intestinal transit was noted at 0.2 or 1 mmol/kg dose levels.	NOAEL: 1 (0.4)

Reviewer's comments: In general, the maximum dose levels tested for evaluating various CVS, respiratory and renal parameters were too low (1 mmol/kg, 0.8 to 3-times the human dose depending on the species utilized for the studies) to adequately establish the potential adverse effects of MultiHance on these systems. In addition, in most studies only one dose was utilized for evaluation. Evaluations at various dose-levels in the same study are necessary for proper comparison and establishment of a dose-response curve.

MultiHance at highest dose-level tested (1 mmol/kg) produced transient but significant changes in various CVS (significant effects: increased cardiac output and stroke volume, decreased peripheral resistance, increased renal blood flow, not significant but noticeable: decreased heart arate and blood pressure) and respiratory (increased intratracheal pressure and pulmonary blood flow) parameters. These transient effects returned to baseline values within 10 minutes. Effects were reported to be similar to Magnevist. These transient effects may be partly due to the hyperosmolarity of the solution. However, inclusion of positive control (hyperosmotic mannitol solution) in these studies would have been more confirmative. It should be also noted that in some of the studies mannitol was included as a positive control (such as CVS effects in pig model of ischemia), however, in these studies mannitol produced

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less prominent effects than MultiHance. So these transient changes can not be solely explained by hyperosmolarity of the solution. At the maximum dose level tested (1 mmol/kg, 0.8 times clinical dose), MultiHance did not affect urine output or electrolyte excretion.

No significant effects on ECG were reported at the maximum dose level tested [1 mmol/kg: 1.7 (rat) & 3.6 (pig)-times human dose]. However, the ECG recording was not continuous and effects on various ECG parameters such as QT interval were not reported.

In an *in vitro* study, MultiHance and Magnevist produced minor but significant reduction in the contractile force of rat papillary muscle (16-20%). Both drugs caused decrease in amplitude (19%) and frequency (10%) of spontaneous atrial contractions in guinea pigs and produced transient depressant cardiac activity (decrease in LVP and heart rate) in the Langendorff heart preparation.

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Summary of Safety Pharmacology Studies:

Safety pharmacology studies were conducted *in vitro* and *in vivo* in normal animals and in animal models of clinical diseases. MultiHance was tested for effects on CVS, CNS and target organs such as heart, liver and kidneys. The sponsor attributed most of the observed adverse effects to hyperosmolality and to the volume of the injected solutions. However, it should be noted that appropriate hyperosmolar control group (such as hyperosmotic mannitol/sucrose solution) were not included in most of the studies for comparison purpose. Therefore, this reviewer can not agree with this conclusion. In few studies, where hyperosmotic mannitol solution was included as a positive control, it caused qualitatively less prominent effects than MultiHance.

Neuropharmacological effects: Several studies (total 13) were conducted to evaluate potential effects of 0.5 M MultiHance on central nervous system function. The parameters evaluated in these studies included effects on behavior, spontaneous locomotor activity & motor coordination, pentobarbital-induced anesthesia, pentylenetetrazol-induced seizures, acetic acid-induced writhing, conditioned taste aversion, EEG and blood brain barrier. The effects were evaluated using both intravenous and intraventricular administration (in terms of 'worse case' effect of the contrast agent in the patients with damaged BBB). Intravenous administration of MultiHance (1 mmol/kg or less, 0.8 times clinical dose) did not affect motor coordination, body temperature, pentylenetetrazol-induced seizures, acetic acid-induced writhing or general behavior in rats. However, studies conducted using intraventricular administration suggest that MultiHance (icv: <0.1 mmol/kg, <0.1-times clinical dose) has potential to produce hypoactivity, impaired motor incoordination, convulsions and death.

MultiHance (1 mmol/kg, 0.8 times human dose) did not alter the BBB permeability in normal rats; and in rats with damaged BBB, less than 0.3% of the injected dose (0.3 mmol/kg, 0.3 x clinical dose) passed through the damaged BBB.

The NOAELs for various CNS parameters after intravenous administration ranged from 0.3-2 times the clinical dose. The adverse CNS effects were comparable to Magnevist. However, it should be noted that, in general, dose multiples used (0.3-1 mmol/kg, 0.1-3 times clinical dose) in these studies were too low for proper evaluation of neurotoxicity of MultiHance.

The NOAEL for adverse effects on EEG was established at 2 mmol/kg (1.7-times clinical dose) after intravenous administration in animal model of focal brain ischemia. However, at higher dose level (4 mmol/kg) slowing of the EEG signal during the quite wake was observed. In addition, transient flattening (for 2 min) of EEG was noted at this dose level (4 mmol/kg, 3.3 times the clinical dose). This reviewer is not sure how brain electrical activity is totally stopped in a conscious animal even though it is a transient effect. **The sponsor needs to explain this EEG flattening effect in more detail.** These adverse effects on EEG/ spontaneous cortical

electrical activity (slowing of signal and transient flattening) are not due to hyperosmolarity of the solution, since such effects were not seen with the hyperosmotic mannitol solution.

Cardiovascular, renal and respiratory effects: These studies were conducted in rats, rabbits and pigs. MultiHance at highest dose-level tested (1 mmol/kg) produced transient but significant changes in various CVS (significant effects: increased cardiac output and stroke volume, decreased peripheral resistance, increased renal blood flow, not significant but noticeable: decreased heart rate and blood pressure) and respiratory (increased intratracheal pressure and pulmonary blood flow) parameters. These transient effects returned to baseline values within 10 minutes. Effects were reported to be similar to Magnevist. These transient effects may be partly due to the hyperosmolarity of the solution. However, inclusion of positive control (hyperosmotic mannitol solution) in these studies would have been more confirmative. At the maximum dose level tested (1 mmol/kg, 0.8 times clinical dose), MultiHance did not affect urine output or electrolyte excretion.

The sponsor conducted a separate study to evaluate CVS effects in pig model of myocardial ischemia that included mannitol as a positive control. In this study, mannitol produced similar transient effects (noted above) like MultiHance (1-3 mmol/kg dose levels, NOAEL: 1 mmol/kg, 3.6-times human dose), but these effects with mannitol were less prominent than MultiHance. So these transient changes can not be solely explained by hyperosmolarity of the solution.

ECG effects: MultiHance effects on ECG were studied in pigs and rats (not a separate study, combined with above CVS studies). No significant effects were reported at the maximum dose level tested [1 mmol/kg: 1.7 (rat) & 3.6 (pig)-times human dose]. However, the ECG recording was not continuous and effects on various ECG parameters such as QT interval were not reported.

The sponsor conducted *in vitro* studies for evaluation of MultiHance (30 mM) effects on rat papillary muscle, Langendorff heat preparation and guinea pig atria. MultiHance and Magnevist produced minor but significant reduction in the contractile force of rat papillary muscle (16-20%). Both drugs caused decrease in amplitude (19%) and frequency (10%) of spontaneous atrial contractions in guinea pigs and produced transient depressant cardiac activity (decrease in LVP and heart rate) in the Langendorff heart preparation.

The potential effects of MultiHance on contractile responses induced by acetylcholine, histamine and barium chloride were studied in isolated ileum of rat and guinea pig. MultiHance (0.01-1 mM) had no effect on these contractions, suggesting no significant effects on smooth muscle of the ileum *in vitro*. Additionally, MultiHance (0.2-1 mmol/kg) produced no effect on charcoal intestinal transit in mice.

Safety pharmacology studies for MultiHance had some major deficiencies that are summarized below:

1) In general, dose-multiples used (0.3-3 times clinical dose, mg/m^2) for safety pharmacology studies were inadequate for establishing clear safety profile of MultiHance in terms of its effect on CVS, CNS, renal and respiratory parameters.
2) There was no continuous ECG recording utilized for these studies. No report of evaluation of any findings on various ECG parameters such as QT interval.
3) The sponsor attributed most of the adverse effects to hyperosmolarity of the solution but did not include hyperosmotic control group (mannitol/sucrose solution) in most of the studies to attribute these effects to hyperosmolarity.
4) In most of the studies, only one dose was utilized for safety pharmacology evaluation. Evaluation at various dose-levels in the same study is necessary for comparison and establishment of a dose-response curve.

It is recommended that the sponsor conduct a comprehensive general safety pharmacology study in bigger species (monkeys or dogs) to address various deficiencies mentioned above. This study should be carried out at various dose levels (with high dose-multiples). The study should include complete battery of CVS (including continuous ECG monitoring, QT interval etc.), CNS (including EEG), renal and respiratory parameters. This study should be carried out in unanesthetized animals; and hyperosmotic control group (sucrose/mannitol solution) and Magnevist should be included for comparison purpose.

The sponsor also needs to conduct a study to evaluate effects of MultiHance (clinically equivalent and higher doses) on blood brain barrier (BBB) permeability in BBB damaged animal models.

In humans, gadolinium class of contrast agents can cause prolongation of QT interval resulting in cardiac arrythmias. We have been requesting *in vitro* electrophysiological studies evaluating effects on cardiac action potential (purkinje fibers) or potassium channels for gadolinium contrast agents. **It is recommended that the sponsor conduct such an *in vitro* electrophysiological study for proper evaluation of MultiHance effect on QT interval.**

26 Pharmacokinetic studies:

The pharmacokinetic studies were performed in rats, rabbits, dogs and monkeys after single or repeat-dose intravenous administration of MultiHance. A study was also conducted in a mutant strain of rats that has defective ATP-dependent bile canalicular membrane organic anion transporter (cMOAT), to examine mechanisms of bile transport of gadobenate dimeglumine. Pharmacokinetic (ADME) studies were performed using radioisotopic methods (labeled gadobenate dimeglumine), spectroscopic methods (X-ray fluorescence analysis) and chromatographic methods (HPLC, TLC). All analytical assays were validated, and validation data was provided.

26.1 Blood kinetics of B19036/7 after intravenous administration in rat:

Study number: RF1433 Report date: June 12, 1989
Study located in Vol. 29, page 209 Batch # RG7/88
Certificate of analysis: No GLP: No
Site: Bracco Industria Chimica, Italy
Species: CD (SD)Br male rats (222-280 g)
Doses: 0.1, 0.25 and 0.5 mmol/kg (0.1, 0.2 and 0.4 x clinical dose, mg/m^2)

Experimental design: Blood kinetics of MultiHance was determined after intravenous administration to male rats (5/time point/dose). Plasma samples were collected at 4, 7.5, 15, 30, 45, 60, 75, 90, 120 and 240-min post-dosing. The assay was performed by using HPLC.

Results: The plasma kinetics showed a bi-exponential profile. The volume of distribution values indicated that the compound is distributed extracellularly. Clearance values suggest that the drug is rapidly eliminated from the plasma compartment. The relevant PK parameters are presented in the table below:

Parameters	0.1 mmol/kg	0.25 mmol/kg	0.5 mmol/kg
Cmax (µg/ml) at 4 min			
Half-life (min)			
α-phase	8	5	7
β-phase	23	22	20
Vol. of distribution (ml/kg)	222	165	202
Clearance (ml/min/kg)	14	12	12

26.2 Blood concentration, tissue distribution, metabolism and excretion after a single intravenous administration of 153Gd-BOPTA/Dimeglumine in rats:

Study number: 36-027 Report date: Aug 25, 1998
Study located in Vol. 29, page 239 Batch # RG9/95
Certificate of analysis: Yes GLP: No
Site: [redacted]
Species: Sprague-Dawley male rats (249-307 g)
Doses: 0.25 mmol/kg (0.2 x clinical dose, mg/m^2)

Experimental design: Rats (3/time point) received a single iv injection of 0.25 mmol/kg (2.59 MBq/kg) 153Gd-labeled gadobenate dimeglumine. Plasma, kidney and liver samples were collected at 1 and 24 hrs after dosing. Urine was collected 8 and 24 hrs post-dosing, and feces were collected 24 hrs after dosing. In addition, plasma samples were collected at 5, 15, 30 min and 1, 2, 4, 6, 8 and 24 hrs time points. Samples were analyzed for 153Gd-labeled gadobenate dimeglumine and free 153Gd using TLC method. The distribution of radioactivity into blood cells was calculated from the plasma and blood radioactivity and hematocrit. Radioactivity was measured using a gamma counter.

Results: The blood concentration of radioactivity was highest (Cmax: 326 µg equivalent/ml) at 5 min post-dosing (first time point), followed by a rapid elimination with 5.9 µg/ml at 1.5 hrs post-dosing. The blood concentration of radioactivity was undetectable from 2-hours post-dosing. The blood elimination half-life was 0.25 hrs and AUC was 149 µg x hr/ml.

The radioacitivity in tissues increased rapidly in parallel with the decrease in plasma levels. The organs related to elimination (GI tract, liver, kidney and bladder) showed high radioactivity until 8-hours post-dosing (500-1600 µg/g at 0.25 hrs). The femoral bone showed low (2.5 µg/g) but constant concentration from 8 hrs to 28 days post-dosing. According to the sponsor, this radioactivity in bone may be related to the radioactive impurities [redacted] present in the formulation. Sponsor states that the clinical formulation does not contain radioactive impurities.

The concentrations of intact drug in plasma, liver and kidney were 370, 602 and 1630 µg/ml or µg/g at 15 min post-dosing, and free gadolinium was not detected in plasma. The distribution of radioactivity in the blood cells was approx. 1-2% of ID at 0.25-1 hr post-dosing.

Excretion: Most of the radioactivity was excreted in urine and feces by 24 hours (98%). At 168 hours, total urinary and fecal excretion was 61% and 38%, respectively. Only intact gadobenate ion was detected in urine. However, in feces, approximately 6% was present as a free gadolinium (31% as intact gadobenate ion).

Reviewer's comments: The blood kinetics for MultiHance seem to be rapid with 98%

excretion by 24-hrs. Significant elimination (38%) occurs through the feces. Free gadolinium ion was detected in the feces (6%), indicating it may dissociate from the gadolinium complex. In addition, there seems to be some accumulation in the femoral bone (1.5% ID, free gadolinium ion) that remained persistent up to 28 days post-dosing (last time-point). Sponsor has attributed this accumualtion in bone to radiactive impurities, which are not present in the clinical formulation. However, no specific data was provided to support this contention. It should be noted that the dose used in the present study is too low (0.2-times clinical dose) compared to the clinical dose.

26.3 Blood concentration, tissue distribution, metabolism and excretion after a single intravenous administration of 153Gd-BOPTA/Dimeglumine in rats:

Study number: ADE-R91006 Report date: Sept 28, 1994
Study located in Vol. 29, page 340 Batch # RG2/91
Certificate of analysis: Yes GLP: No
Site: [redacted]
Species: Sprague-Dawley male rats (Avg. Wt. 212 g)
Doses: 1 mmol/kg (0.8 x clinical dose, mg/m^2)

Experimental design: This study was conducted to determine the blood kinetics, biodistribution and elimination of 153Gd-gadobenate dimeglumine in rats (5/group) after administration of single and repeated (once daily for 5 days) intravenous doses (1 mmol/kg, 0.7 MBq/animal). The rats were sacrificed at the following time points after the first or last (repeat dose) treatment: 5, 15, 30 min, 1, 2, 4, 7, 24 and 168 hrs. Blood and selected organs (liver, kidneys, adrenals, brain, lungs, spleen, heart, spleen, intestines, stomach, pancreas, thyroid gland, testis, bone, skin and muscle) were taken from all animals at above time points for analysis. For the elimination studies, urine and feces were collected at 4, 8, 12, 24, 36, 48 and every 24 hr up to 168 hrs after first injection (single administration). For repeat dose administration, samples were collected at 4, 8, 12 and 24 hr after each dose. In addition, a 36-hr sample was collected after the last repeat dose injection on day 5.

Results: Similar tissue level decays of radioactivity were observed after single and repeat dose administration, although levels of radioactivity after repeat dose were somewhat higher than those measured after single injection. The drug was rapidly eliminated from blood with half-life of elimination of 10 min for both single and repeat dose administration. 5-min after injection, plasma levels were 2.6 µmol Gd/ml and 3.1 µmol Gd/ml for the first and fifth dose, respectively. By 4-hr, both plasma levels were <0.002 µmol Gd/ml. studies. The table below summarizes the PK parameters for blood and plasma:

Parameters	1st dose		5th dose	
	Plasma	Blood	Plasma	Blood
Half-life (min)	10	10	9.5	10
$AUC_{0\text{-infinity}}$ (μmol Gd/ml. min)	55	33	61	37
Vd (ml)	58	97	50	87

The radioactivity was mainly observed in the liver, kidneys, intestines, bone, skin and muscle; and decreased rapidly within first 24-hrs. After 168 hr, residual radioactivity in the body accounted for approximately 0.3% of ID for single and repeat dose. This residual activity was mainly seen in liver (0.015 & 0.08% ID after 1st and 5th dose, respectively), kidney (0.06 & 0.33% ID), intestine (0.02 & 0.09% ID), bone (0.07 & 0.24% ID) and skin (0.004 & 0.14% ID).

Excretion: Urinary cumulative (0-168 hr) elimination of radioactivity amounted to 70% and to 62% of ID after single and repeat dose administration, respectively. Fecal elimination was 30% and 31% of ID, respectively. More than 90% of the drug was eliminated within first 24-hrs. There was no trend of accumulation in any of the tissues after repeat dose administration.

Reviewer's comments: Unlike previous study in rats (#36-027), low radioactivity was detcted in the bone). It is not clear if any free gadolinium was detected in the feces. Similar to previous study, major routes of elimination seem to be kidney and GI tract.

26.4 The metabolism and pharmacokinetics of 153Gd-BOPTA/Dimeg in the dog following a single intravenous administration:

Study number: 9896 Report date: Nov 7, 1994
Study located in Vol. 30, page 247 Batch # RG9/92
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: Male beagle dogs (8.7-9.4 kg)
Doses: 1 mmol/kg (2.5 x clinical dose, mg/m^2)

Experimental design: This study was conducted to determine the blood kinetics, metabolic fate and excretion 153Gd-BOPTA/Dimeg in dogs (n=3) after single dose intravenous administration (1 mmol/kg, 23 MBq/animal). Blood samples were collected before administration and at the following times post-administration: 5, 15, 30, 45 minutes, and 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hours. Level of radioactivity was determined using a gamma counter in plasma, whole blood, urine and feces collected up to 168 hr (7 days) post-dose. The metabolic profile in plasma, urine and feces was characterized using a radio-HPLC chromatography for selected samples. Analysis was carried out for plasma samples (at 15 min and 1 hr), urine (0-6 hr) and feces (0-24 hr).

Results: The table below shows mean values for various PK parameters after single dose (1 mmol/kg) intravenous administration of MultiHance in dogs:

Cmax (at 5 min, first time-point)	3.43 μmol equivalent/ml
Distribution half-life (α)	26 minutes
Elimination half-life (β)	175 minutes
$AUC_{0\text{-}8\ hrs}$	2.85 μmol x hr x ml^{-1}
Volume of distribution	281 ml/kg
Clearance	5.9 ml/min/kg (353 ml/hr/kg)

Mean level of total radioactivity decreased to 0.8 μmol equivalents/ml at 1hr, and 0.03 μmol/ml at 8-hrs post-dosing. Plasma levels were below the limit of detection by 24 hr post-dosing. Levels of radioactivity in whole blood were generally paralleled those to plasma, although at lower level. The Cmax value (at 5 minutes post-dosing) for the whole blood was 2.4 μmol/ml. The subsequent decrease in blood levels was similar to that for plasma. Plasma level decay of radioactivity suggested a bi-exponential profile (half-lives shown in the table above). Clearance suggested that the radioactivity eliminated quickly from the plasma and distributed rapidly into extracellular space

Majority of radioactivity was recovered by 24 hr (77%: 51% in urine and 24% in feces). The cumulative radioactivity recovered by 168 hrs was 84% (83% by 48 hrs) with 51% in urine and 30% in feces. Chromatographic analysis (HPLC and TLC) of samples of urine, feces and plasma resolved a single radiolabeled component which co-chromatographed with 153Gd-BOPTA/Dimeg (retention time: 5.5 min), suggesting that gadobenate dimeglumine was not metabolized and excreted unchanged in urine and feces.

Reviewer's comments: The total cumulative radioactivity recovered was 84% by 168 hr (last time point). Remaining 16% was not accounted. Whether this remaining amount (16%) is eventually eliminated or retained in the body (as a free gadolinium ion?) can not be established from this study. This is a concern, since there was some retention in bone in rats in an earlier study (# 36-027).

The study indicates significant amount (30%, approximately one-third of total excretion) of excretion through feces, suggesting biliary elimination component for MultiHance is substantial.

The sponsor analyzed only selected samples of plasma (15 min and 1 hr post-dosing), urine (0-6 hr) and feces (0-24 hr) for evaluating potential for metabolism of MultiHance *in vivo*. Although sponsor reported, majority of gadobenate dimeglumine is eliminated as unchanged by 24 hr, the

study would have been more confirmative if all samples were analyzed for evaluating metabolism of MultiHance.

26.5Blood concentration and excretion after a single intravenous administration of 153Gd-BOPTA/Dimeg in dogs:

Study number: 36-029 Report date: Aug 24, 1997
Study located in Vol. 30, page 332 Batch # RG9/95
Certificate of analysis: Yes GLP: Yes
Site: ———
Species: Male beagle dogs (10-10.7 kg)
Doses: 0.25 mmol/kg (0.6 x clinical dose, mg/m^2)

Experimental design: The study design was similar to the previous PK study in male dogs (study # 9896), except that the dose used in this study was lower (0.25 mmol/kg versus 1 mmol/kg in previous study), and this study was carried out at a different testing facility. Also in this study (unlike previous study), it is clearly stated that TLC method was used for analysis of 153Gd-labeled gadobenate dimeglumine and free 153Gd ion.

Results: The table below shows values for various PK parameters after single dose (0.25 mmol/kg) intravenous administration of MultiHance in dogs:

Cmax plasma (at 5 min, first time-point)	———
Elimination half-life (β)	90 minutes
$AUC_{0\text{-}8\ hrs}$	489 µg x hr/ml

The urinary excretion of radioactivity was 49%, 57% and 58% ID by 8, 24 and 168 hrs, respectively. Fecal excretion was 30 and 43% by 24 and 168 hrs, respectively. Total cumulative radioactivity recovered in urine and feces was 101% after 7-days (168 hrs) of post-dosing. All radioactivity in plasma (0-8 hrs) and urine (0-24 hrs) was associated with the parent drug (153Gd-BOPTA/dimeg) and no free 153Gd was detected. However, in feces, approximately 25% of radioactivity (6.2% ID) in 24 hr was associated with free 153Gd.

Reviewer's comments: Similar to previous PK study in rats (# 36-027), free gadolinium ion was detected (———) in feces samples collected after 24 hrs post-dosing. The stability of gadobenate dimeglumine complex *in vivo* is of concern.

26.6HPLC assay of gadobenate ion in plasma, urine, bile and feces samples of monkeys

following gadobenate dimeglumine intravenous administration:

Study number: RF5631 Report date: July 25, 1996
Study located in Vol. 30, page 363 Batch # RG15D/95
Certificate of analysis: Yes GLP: No
Site: [redacted]
Species: Cynomolgus monkeys (Avg. wt.: 2 kg)
Doses: 1-8 mmol/kg (1.7-13.3 x clinical dose, mg/m^2)

Experimental design: This study was done in conjunction with the preliminary MTD study described in the toxicology section (study # BRO/53962175). Total two male and two female cynomolgus monkeys received gadobenate dimeglumine at doses ranging from 1 to 8 mmol/kg, according to various experimental schedules (dose-escalation PK study). For Phase I study, two animals received single doses of 2, 4, 6, 7 and 8 mmol/kg, with a one-day wash-out period between each doses. Only urine samples were collected up to 24 hrs. For Phase II study, the test drug was administered to two monkeys at doses of 2, 3, 4 and 6 mmol/kg, once per day with a two-day wash-out period between doses. Urine and feces samples were collected up to 48 hrs. Blood samples were collected at 4, 8, 24, 48 and 72 hours after injection (last time-point for 4 and 6 mmol/kg dose levels only). For Phase III of the study, test compound was given to two anesthetized animals at a dose of 1 mmol/kg. Blood samples were collected at 1, 3, 15, 30, 60, 120, 180, 240 and 480 minutes after injection. Bile and urine were collected at various time-points up to 480 minutes after injection. For Phase IV of the study, two animals received 6 mmol/kg and blood samples were collected up to 480 minutes after dosing. Analysis of all samples was carried out by HPLC.

Results: This study was deemed invalid for the reasons described in the 'reviewer's comments' section below. The results of this study are briefly summarized below:

Phase I of the study: The recovery of gadobenate ion in urine (up to 24-hr sampling) was between 50% to 80% of ID for various dose levels (2, 4, 6, 7 and 8 mmol/kg) of gadobenate dimeglumine injected. There was no dose-dependent effect in terms of urinary excretion.

Phase II: The plasma concentration of gadobenate ion at 4 hrs post-dosing (first time-point for plasma sampling) ranged from 0.151 mmol/L at 2 mmol/kg dose to 0.841 mmol/L at 6 mmol/kg (n=1 for each dose level of 2, 3, 4 and 6 mmol/kg). The cumulative urinary excretion ranged from 50 (at 2 mmol/kg) to 75% (at 4 mmol/kg) of ID for the maximum of 48 hrs of urine collected. For the same time period (up to 48 hrs), the fecal excretion was low with values ranging from 0.7% (at 6 mmol/kg) to 1.4% (at 2 mmol/kg). About 48-25% of ID recovery was not accounted.

Phase III: The mean Cmax (n=2) for plasma was 8.7 mmol/L (1-minute post-dosing, first time-

point) after 1 mmol/kg injection of gadobenate dimeglumine. The plasma level decreased rapidly with mean concentration of 0.19 mmol/L at 480 minutes post-dosing (last time-point). The cumulative urinary excretion up to 480 minutes (8 hrs) post-dosing was 55% (male) and 74% (female) of ID for two animals tested. Biliary excretion for the same time period (up to 8 hrs) was 2.7% and 11.6% of ID, respectively. Samples were not collected after 8 hrs.

Phase IV: The plasma concentration of gadobenate ion at 6 mmol/kg dose level was 32 mmol/L at 1 minute after injection. By 8 hrs (last time-point), it declined to 0.24 mmol/L.

Please note that the same animals (2 males and 2 females) were used for the entire study (Phase I to IV) with 24 or 48 hr washout period in between the dosing. So all above PK values should be treated with great caution. The recovery of the ID was incomplete (ranged from 50-80%).

Reviewer's comments: This study is deemed invalid for following reasons:

a) There was no systematic analysis of blood, urine, bile and feces samples at each dose level for each animal (see the experimental design for more details).
b) This study was mainly carried out as a preliminary maximum tolerated dose (MTD) study (described in the toxicology section). Four animals were used for the entire study evaluating PK parameters at total 8 dose levels. The number of animals used per dose were insufficient (1 or 2/dose), and furthermore, escalating dose levels (1 to 8 mmol/kg) were given to same animals just after 24 hr washout (48 hr washout in some cases). This makes difficult to evaluate various PK parameters at each dose separately without taking in to account the previous dose (cumulative effect). Each animal should have been tested only at a single dose level.
c) The time-points for sample collection were not uniform for each dose level, and the samples were not taken long enough (mostly 24 hrs, maximum 48-hrs in few cases) to account for the complete recovery of the injected doses.

26.7 Distribution into liver and excretion into urine and feces after a single intravenous administration of 153Gd-BOPTA/Dimeg in mice:

Study number: 36-030 Report date: Aug 24, 1998
Study located in Vol. 31, page 024 Batch # RG9/95
Certificate of analysis: No GLP: No
Site: ~~~~~
Species: Female C57BL/6N (SPF) mice (17.5-21.5 g)
Doses: 0.1 mmol/kg (0.04 x clinical dose, mg/m^2)

Experimental design: This study was conducted to determine distribution of radioactivity in

liver, urinary and fecal excretion in mice (n=3 for each time-point) after 0.1 mmol/kg intravenous administration. For liver radioactivity determination, mice were sacrificed at 15, 30 minutes, 1, 1.5, 2 and 4 hours post-dosing. For fecal and urinary excretion, samples were collected every 24 hours up to 168 hrs. The amount of 153Gd labeled gadobenate dimeglumine and free 153Gd were evaluated using TLC method (for urine and feces samples collected up to 24 hr post-dosing). Radioactivity was determined using a gamma counter.

Results: Plasma and liver radioactivity decreased rapidly with elimination half-life of 0.5 and 1.8 hrs, respectively. Plasma and liver concentrations of radioactivity at 15 minutes post-dosing were 335 µg equivalent/ml and 318 µg equivalent/ml, respectively. The distribution of radioactivity in liver was 14% of ID at 15 minutes after injection. At 4 hrs after injection (last time point), 1.5% of the initial concentration (at 15 minutes) remained in the liver. Most of the radioactivity was recovered within first 24 hours (95%). The urinary and fecal elimination of total radioactivity was 54% and 43%, respectively by 168 hrs. The TLC analysis of samples collected in first 24 hours did not detect free 153Gd in the urine or feces.

Reviewer's comments: This dose is too low (0.1 mmol/kg, 0.04 times clinical dose) to make any meaningful PK comparison with the clinical dose. The sponsor states that no free gadlinium was detected in urine and fecal samples. However, the analysis for free gadlinium was carried out only in the first sample collected at 24 hours after injection. In a previous study in dogs (#36-029), free gadolinium ( ) was detected in feces samples collected after 24 hours (48-168 hrs). Therefore, this conclusion (no free 153Gd in fecal matters) can not be established.

26.8Study of the distribution and elimination of 153Gd-B19036/7 in the rat:

Study number: ADME002 Report date: Jan 30, 1990
Study located in Vol. 31, page 076 Batch # RG9/88
Certificate of analysis: Yes GLP: No
Site:
Species: SD male rats (200-250 g)
Doses: 0.25 mmol/kg (0.2 x clinical dose, mg/m^2)

Experimental design: This biodistribution study was conducted in male rats (n=3 to 6 per time point) after single intravenous administration of 153Gd-gadobenate dimeglumine at 0.25 mmol/kg. Radioactivity was measured at 15 minutes, 1 and 7 hrs, and 1, 7 and 28 days after administration. Radioactivity was measured in following organs: heart, brain, lungs, liver, spleen, pancreas, kidneys, adrenals, stomach, testes, intestine, plasma, blood and carcass, including bone, white & brown fat, muscle and skin. Samples were counted for gamma radiation from 153Gd using a gamma spectrometer. The metabolic profile in urine and bile was determined by HPLC method.