decreases in urine electrolyte concentrations were observed.

The repeat dose studies suggest that the safety margin for MultiHance in terms of adverse effects is low (0-0.6 times human dose). In general, MultiHance was better tolerated in mokeys than in rats or dogs. The target organs for toxicity seem to be mainly liver (necrosis, vacuolation, changes in liver enzymes) and kidney (vacuolation, increased weight). In addition, vacuolation was also seen in testes (with abnormal spermatogenic cells) and pancreas at higher dose levels. In most cases, the vacuolation was not reversible.

The sponsor included ECG evaluation in dog and monkey repeat dose studies. However, these recordings were not continuous but carried out 2-hrs post-dosing. In addition, various ECG parameters such as QT interval were not evaluated. Therefore, the ECG data was deemed inadequate, and does not replace an adequately designed safety pharmacology study.

28 Immunotoxicology:

28.1 Gd-BOPTA/Dimeg: Histamine release from rat peritoneal mast cells:

Study number: RF4511
Study located in Vol. 21, page 193
Certificate of analysis: Yes
Site: Bracco SpA, Milan, Italy
Dose: 125 & 250 mM

Report date: April 4, 1995
Batch # RG4/92
GLP: Yes

Experimental design: This *in vitro* study was conducted to evaluate the potential of 0.5 M MultiHance (125 & 250 mM) to induce histamine release and cause degranulation in rat peritoneal mast cells. Magnevist (125 & 250 mM) and hyperosmolal sucrose (304 & 608 mM) were tested as reference compounds. 0.9% saline and compound 48/80 (histamine-releasing agent) were used as negative and positive controls, respectively. Histamine was measured fluorometrically and mast cell degranulation was assed using light microscopy.

Results: The results obtained in this study are summarized in the table below:

Compound	% Histamine release	% Mast cell degranulation
Saline	1.2	3.8
Gadobenate dimeglumine: 125 mM 250 mM	 1.6 23.2	 3.5 29.0
Magnevist: 125 mM 250 mM	 3.9 32.0	 3.5 45.9
Hyperosmolal sucrose solution: 304 mM 608 mM	 1.3 33.0	 4.1 45.0

*$p<0.05$ versus negative control (saline)

Gadobenate dimeglumine did not cause significant histamine release or mast cell degranulation at 125 mM concentration. However, these parameters were significantly affected at 250 mM. Magnevist produced similar effects (increased histamine release and mast cell degranulation). Data obtained with the sucrose solution suggests that the observed effects (increased histamine and mast cell degranulation) may be due to the hyperosmolality of the solution. According to the sponsor, the concentration of gadobenate dimeglumine (125 mM) used in this study is about 250-times higher than the maximum clinical concentration obtained in blood.

28.2 GBOP antigenicity study in guinea pigs:

Study number: 936161 Report date: Aug 22, 1994
Study located in Vol. 21, page 212 Batch # K2Y018ZZA
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: Female Hartley guinea pigs (323-390 g)
Doses: 0.25 and 1 mmol/kg

Experimental design: Gadobenate dimeglumine was examined for its antigenic potential in guinea pigs using active systemic anaphylaxis (ASA) test and passive cutaneous anaphylaxis (PCA) test. Guinea pigs were treated with either gadobenate dimeglumine solution (0.25 and 1 mmol/kg), complete Freund's adjuvant (CFA) emulsion of 0.5 M gadobenate dimeglumine (0.125 mmol/animal), CFA emulsion of saline (negative control) or CFA emulsion of ovalbumin (OVA, 0.25 mg/animal, positive control). Six animals were used for each group, except for the OVA-treated group, which contained 3 animals. The gadobenate dimeglumine solutions were injected intraperitoneally from day 1 through day 5 and from day 15 through day 19 (sensitization phase, total 10 doses). The CFA emulsions were injected subcutaneously on day 1 and day 15. Blood samples from these animals, which were used for antibody examination in the PCA test, were collected on day 29. On day 31, the guinea pigs were subjected to the ASA test. In this test, each animal received intravenous injection of gadobenate dimeglumine (0.25 mmol/animal, challenge phase) and was examined for anaphylactic reactions (graded/scored into 10 classes) for 30 minutes post-injection.

PCA test was performed by provoking anaphylactic responses in passively sensitized guinea pigs with an injection of gadobenate dimeglumine (0.25 mmol/animal). For the PCA test, test sera from the animals in the ASA test were injected intradermally (0.1 ml/site) onto the back of receipient guinea pigs. Next day these guinea pigs received intravenous injection of 1 ml of the PCA test solution (consisting of 0.25 mmol of gadobenate dimeglumine, 1 mg OVA and 10 mg Evans blue). Thiry minutes later, animals were sacrificed and the skin on the back was removed and scored for the presence of a PCA reaction. The short and long diameters of the Evans blue colored spot at the site of intradermal injection were measured. The results were considered positive when the mean of the diameters exceeded 5 mm. The antibody titer was expressed as the reciprocal of the maximum positive dilution.

Results: The animals treated with gadobenate dimeglumine solution did not show an anaphylactic response in the ASA test. Severe ASA reactions were observed in all positive control group animals (convulsions, collapse and/or death). For the PCA test, sera obtained from animals were examined for antibodies to gadobenated dimeglumine and ovalbumin. The gadobenate dimeglumine solution was negative in the PCA test. The antibody titer in positive control group was $>10^3$. It was concluded that gadobenate dimeglumine did not show antigenic potential in the guinea pig ASA test and PCA test.

Reviewer's comments: Under the conditions of this study (ASA and PCA tests), gadobenate dimeglumine did not show antigenic potential. No anaphylactic reaction was seen in the ASA or PCA test.

28.3 *In vitro* complement activation with Gd-BOPTA/Dimeg:

Study number: RF2681
Study located in Vol. 21, page 103
Certificate of analysis: Yes
Site: Bracco SpA, Milan, Italy
Dose: 25 x 10^{-4} M to 4 x 10^{-2} M

Report date: Nov 11, 1991
Batch # RG9/89
GLP: Yes

Experimental design: The aim of this study was to assess any potential activation of the complement system (which is considered to be one of the causes for anaphylaxis) by 0.25 M gadobenate dimeglumine. The activation of the complement system by Gd-BOPTA/Dimeg was assessed *in vitro* spectrophotometrically measuring the hemolysis induced in the immuno-hemolytic system (sheep erythrocytes 5% suspension sensitized with their antibody hemolysin). The percent of complement activation (which is inversely proportional to hemolysis) was determined.

Results: Gd-BOPTA/Dimeg (0.25 M) in the range of 25 x 10^{-4} M to 4 x 10^{-2} M, induced a concentration-dependent 11 to 94% complement activation. The IC50 value for the complement activation was 15.2 mmol/L (1.5 x 10^{-2} M). According to the sponsor, this IC50 value (15.2 mmol/L) for gadobenate dimeglumine is about 15-times higher than the maximum plasma level in humans after intravenous administration of 0.2 mmol/kg of gadobenate dimeglumine.

Summary of Immunotoxicity Studies:

The antigenicity of gadobenate dimeglumine was investigated in guinea pigs for active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA). Under the conditions of this study (# 936161), 0.5 M gadobenate dimeglumine did not show antigenic potential. No anaphylactic reaction was seen in the ASA or PCA test.

An *in vitro* study (# RF4511) was conducted to evaluate the potential of 0.5 M MultiHance (125 and 250 mM) to induce histamine release and cause degranulation in rat peritoneal mast cells. MultiHance did not cause any significant histamine release or mast cell degranulation at 125 mM concentration. However, significant histamine release (23% versus 1% in saline control) and mast cell degranulation (29% versus 4% in saline control) was observed at 250 mM MultiHance concentration. Magnevist and hyperosmolal sucrose solution produced similar effects. Data obtained with the sucrose solution suggested that it is possible this effect is due to the hyperosmolality of MultiHance. According to the sponsor, the no effect MultiHance concentration (125 mM) for histamine release is about 250-times higher than the clinical concentartion obtained in blood.

The sponsor also conducted an *in vitro* study (# RF2681) to assess potential of 0.25 M MultiHance to activate complement system (which is considered to be one of the causes for anaphylaxis). MultiHance in the range of 25×10^{-4} M to 4×10^{-2} M, induced dose-dependent 11 to 94% complement activation. The IC50 value for this effect was 1.5×10^{-2} M (15.2 mmol/L). According to the sponsor, this IC50 value for MultiHance is about 15-times higher than the maximum plasma levels in human after intravenous administration of 0.2 mmol/kg of MultiHance.

28 Genetic toxicity studies:

MultiHance was evaluated for genetic mutation in bacteria (Ames test using *S. typhimurium and E. coli.*) and in mammalian cells (V79 hamster cells), chromosome mutation *in vitro* (human lymphocyte assay) and *in vivo* (mouse micronucleus assay), and DNA damage (unscheduled DNA synthesis in human cells).

(A) *In vitro* mutagenicity studies:

28.1 Study of the capacity of B19036/7 to induce gene mutations in strains of *Salmonella typhimurium:*

Study number: 880376 Report date: May 30, 1989
Study located in Vol. 28, page 01 Batch # RG7/88
Certificate of analysis: Yes GLP: Yes
Site:
Tester strains: TA1535, TA1537, TA1538, TA98 and TA100
Dose: 1, 10, 100, 1000 and 5000 µg/plate

Experimental Design: The aim of this study was to evaluate in the Ames test the potential mutagenic effect of MultiHance. *S. typhimurium* tester strains (TA1535, TA1537, TA1538, TA98 & TA100) were incubated for a period of 72 hr in the presence of MultiHance (1-5000 µ g/plate) with and without metabolic activation by rat liver S9 fraction. All tests were performed in triplicate. Appropriate positive (hydrazine sulfate, 9-aminoacridine HCl monohydrate, doxorubicine HCl and 2-aminofluorene) and negative (deionized sterile water) controls were used for each tester strain.

Results: MultiHance (0.5 M) at a concentration up to 5000 µg/plate did not cause any significant increase in the number of revertant colonies relative to the negative control (deionized sterile water) in any of the strains tested in either the presence or absence of metabolic activation.

Reviewer's Comments: Current guidelines (59FR 48734) recommend the inclusion of a tester strain capable of detecting T-A point mutations such as *S. typhimurium* strain TA102 or *E. coli* strains WP2 uvrA or WP2 uvrA (pKM101) in the *in vitro* bacterial mutagenicity assay. A strain capable of detecting T-A point mutations was not included in this study. However, the study is deemed acceptable, due to the lack of effects in the tester strains used.

28.2 Study of the capacity of B19036/7 to induce gene mutations in *E. coli* CM891 (Agar plate test):

Study number: 920876 Report date: May 18, 1993
Study located in Vol. 28, page 41 Batch # RG2/92
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Tester strains: CM891 (WP2, uvrA⁻, pKM101)
Dose: 50, 150, 500, 1500 and 5000 µg/plate

Experimental Design: E. coli tester strain CM891 was incubated for a period of 72 hr in the presence of MultiHance (50-5000 µg/plate) with and without metabolic activation by rat liver S9 fraction. All tests were performed in triplicate. Appropriate positive (methylmethane--sulphonate and 2-aminoanthracene) and negative (water for injection) controls were used for the study.

Results: MultiHance (0.5 M) at a concentration up to 5000 µg/plate did not cause any significant increase in the number of revertant colonies relative to the negative control (water for injection) in either the presence or absence of metabolic activation.

Reviewer's Comments: In this study, a tester strain (*E. coli* CM891) capable of detecting T-A point mutations was evaluated. Under the conditions of this study, MultiHance (up to 5000 µg/plate) did not show any significant mutagenic effect in CM891 tester strain.

28.3 Study of the capacity of B19036/7 to induce gene conversion in *Saccharomyces cerevisiae* D4:

Study number: 880377 Report date: Feb 23, 1989
Study located in Vol. 28, page 71 Batch # RG7/88
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Tester strains: *Saccharomyces cerevisiae* D4 strain
Dose: 125, 250, 500, 1000 and 5000 µg/plate

Experimental Design: This test was conducted to assess the gene conversion frequency induced in 'Ade2' and 'Trp5' loci on different chromosomes of the yeast strain *S. cerevisiae D4*. The tester strain was incubated for a period of 96 hr in the presence of MultiHance (125-5000 µg/plate) with and without metabolic activation by rat liver S9 fraction. All tests were

performed in triplicate. Appropriate positive (methylmethanesulfonate and cyclophosphamide) and negative (deionized sterile water) controls were used for the study.

Results: MultiHance (0.5 M) at a concentration up to 5000 µg/plate did not cause any significant increase in the gene convertant frequency in *S. cerevisiae* D4 strain relative to the negative control (deionized sterile water) in either the presence or absence of metabolic activation.

Reviewer's Comments: This tester strain (*S. cerevisiae* D4) is capable of detecting T-A point mutations. Under the conditions of this study, MultiHance (up to 5000 µg/plate) did not produce any mutagenic effect in *S. cerevisiae* D4 tester strain.

28.4Study of the capacity of B19036/7 to induce chromosome aberrations in human lymphocytes cultured *in vitro*:

Study number: 880379
Study located in Vol. 28, page 100
Certificate of analysis: Yes
Site: [redacted]
Dose: 1, 10, 100 and 1000 µg/ml

Report date: Nov 30, 1989
Batch # RG7/88
GLP: Yes

Experimental Design: This study was conducted to evaluate the clastogenic potential of MultiHance in cultured human lymhocytes *in vitro*, in the absence and presence of metabolic activation. Whole blood was obtained from a healthy human volunteer. Whole blood cultures were established and incubated at 37^{0}C for 48 hr. The cultures were centrifuged to obtain the cultured blood cells. The blood cells were exposed to MultiHance in the presence and absence of a rat liver S9 fraction for a period of 3 hr. The cells were washed and cultured for an additional 20 hrs. The frequency of chromosomal aberrations in the blood lymphocytes was determined from 100 metaphase spreads for each culture exposed to MultiHance. Mitomycin C in the absence of rat liver S9 and cyclophosphamide in the presence of S9 were used as positive controls. Water for injection was used as a negative control.

Results: Under the conditions of this experiment, MultiHance in concentration up to 1000 µg/ml did not cause any significant increase of cells with chromosome aberrations in cultured human lymphocytes either in the presence or absence of metabolic activation. Positive controls produced significant increases in the percentage of cells with chromosome aberration. It was concluded that MultiHance (up to 1000 µg/ml) does not induce chromosome aberrations in cultured human blood lymphocytes.

Reviewer's Comments: It is not clear why higher concentration of MultiHance (5000 µg/plate)

was not tested in this *in vitro* human lymphocyte assay. The sponsor has used 5000 µg concentration for all other battery of mutagenicity tests. Under the conditions of this study, MultiHance in concentration up to 1000 µg/plate did not produce any clastogenic effect in an *in vitro* human lymphocyte assay.

28.5 Study of the capacity of B19036/7 to induce unscheduled DNA synthesis in cultured HeLa cells:

Study number: 880378 Report date: Sept 6, 1989
Study located in Vol. 28, page 122 Batch # RG7/88
Certificate of analysis: Yes GLP: Yes
Site:
Dose: 10, 100, 1000 and 5000 µg/ml

Experimental Design: The assay for unscheduled DNA synthesis (repair synthesis) was conducted to evaluate potential of MultiHance to provoke repairable damage to the DNA molecule, in the absence and presence of metabolic activation. The solution for metabolic activation (S9 mix) was obtained from the liver of rats treated by intraperitoneal route with Aroclor 1254. Cultured HeLa cells were exposed to MultiHance for 24 hrs in the presence of labeled nucleotidic precursor (tritiated thymidine). For repair synthesis to be distinguished from semiconservative replication and the absolute & relative repair synthesis, the cells were treated with hydroxyurea after having been treated with the test substance. Radioactivity was measured by liquid scintillation spectrometer for tritium counting. Methylmethanesulfonate in the absence of rat liver S9 and cyclophosphamide in the presence of S9 were used as positive controls. Water for injection was used as a negative control.

Results: Under the conditions of this experiment, MultiHance (up to 5000 µg/ml), both in the presence and absence of metabolic activation, did not induce statistically significant increases in the incorporation of tritiated thymidine in presence of hydroxyurea in cultured human cells (HeLa). It was concluded that MultiHance (up to 5000 µg/ml) does not induce unscheduled DNA synthesis in cultured HeLa cells *in vitro*.

28.6 Study of the capacity of B19036/7 to induce gene mutation in V79 Chinese Hamster Lung cells:

Study number: 910466 Report date: Dec 20, 1991
Study located in Vol. 28, page 149 Batch # RG4/91

Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Dose: 10, 100, 1000 and 5000 µg/ml

Experimental Design: The gene mutation test in V79 chinese hamster lung cells allows for screening the frequency of resistants to 6-thioguanine due to deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT). V79 cells were exposed to the test article, then reseeded in complete medium and selective medium. After a period of expression in which segregation of the mutant allele, degradation and dilution of HGPRT within the mutant cells occurred, mutant phenotypes (mutant deficient of HGPRT) were detected as colony formation in the selective medium. Gadobenate dimeglumine (10, 100, 1000 & 5000 µg/ml) was incubated for 2 hrs. The solution for metabolic activation was obtained from the liver of rats treated by intraperitoneal route with Aroclor 1254. Ethylmethanosulphonate and N-nitrosodimethylamine were used as positive controls.

Results: Under the conditions of this experiment, MultiHance in concentration up to 5000 µg/ml did not induce any significant increase of gene mutation ($HGPRT^-$ frequency) in V79 chinese hamster lung cells either in the presence or absence of metabolic activation. Positive controls produced significant increases in the $HGPRT^-$ mutant frequency.

28.7 Micronucleus induction in bone marrow cells of rats treated by intraperitoneal route with B19036/7:

Study number: 880380 Report date: Sept 11, 1989
Study located in Vol. 28, page 182 Batch # RG2/89
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: CD (SD)BR rats (90-115 g)
Dose: 5 mmol/kg, intraperitoneal route

Experimental Design: This *in vivo* micronucleus test was conducted to evaluate potential of MultiHance to damage chromosomes by generating acenteric fragments. Gadobenate dimeglumine (5 mmol/kg) or mitomycin C (reference mutagen, 8 mg/kg) were administered by intraperitoneal route to rats (n=5/sex/time point). Animals were sacrificed at 18, 42 and 66 hrs after treatment. The femurs were removed and their bone marrow fixed and stained. For each animal, 2000 polychromatic erythrocytes were counted and scored for micronucleated cells (micronucleus frequency). For each animal, polychromatic to normochromatic erythrocyte ratio (P/N) was calculated on one slide by counting a total of 1000 polychromatic erythrocytes.

Results: Under the conditions of this experiment, MultiHance administered intraperitoneally at 5 mmol/kg dose did not cause a significant rise in the frequency of bone marrow micronuclei 18, 42 and 66 hr after treatment. P/N ratio of gadobenate dimeglumine treated animals was not significantly different from control. Positive control mitomycin C significantly increased the frequency of micronucleated cells. The sponsor concluded that gadobenate dimeglumine was negative in the *in vivo* rat micronucleus test at doses up to 5 mmol/kg.

Reviewer's Comments: In this study, MultiHance was administered by intraperitoneal and not intravenous (clinical route) administration. All other pre-clinical studies, including mutagenicity assays were carried out using intravenous route. It is not clear why only this particulat study was conducted after intraperitoneal administration. The sponsor should repeat this study using intravenous administration.

Summary of Genetic Toxicology Studies:

Following battery of genotoxicity tests were conducted for MultiHance (0.5 M): Ames test (S. *typhimurium* and E. *coli)*, human lymphocyte assay, unscheduled DNA synthesis in human cells (HeLa cells), *in vitro* mammalian cells assay (V79 Chinese hamster lung cells) and *in vivo* micronucleus assay (intraperitoneal injection) in rats. Under the conditions of these studies, MultiHance did not show any mutagenic effect in any of the above mutagenicity assays in the absence or presence of metabolic activation.

In vivo micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg), and not intravenous (clinical route) administration. The sponsor reported that the PK study after intraperitoneal administration in rats showed plasma Cmax value of [redacted] (15 minutes post-injection at 5 mmol/kg) and an $AUC_{0\text{-}240\ min}$ value of 7.4 mmol/L x hr. In humans, Cmax was [redacted] and AUC was 1.03 mmol/L x hr after 0.1 mmol/kg, iv dose. The sponsor concludes that this demonstrates effective transfer of gadobenate ion into the systemic circulation after intraperitoneal injection in rats. However, it should be noted that all other pre-clinical studies were carried out using intravenous administration. The sponsor needs to explain reasons for choosing intraperitoneal route only for this particular study (in vivo micronucleus assay) as opposed to intravenous route. Biodistribution studies after intravenous MultiHance administration in rats have shown some accumulation of free gadolinium (<1% of ID) in bone that was persistent. Biodistribution kinetics after intraperitoneal administration are not known. It is recommended that the sponsor repeat the *in vivo* micronucleus assay using intravenous administration of MultiHance at higher (and repeat?) dose levels.

All *in vitro* mutagenicity tests, except human lymphocyte assay, were carried out using 5000 mg/plate as the highest concentration of MultiHance. It is not clear why 1000mg/plate was chosen as the highest concentration for the human lymphocyte assay. MultiHance did not show any clastogenic effect in this assay at concentrations up to 100 mg/plate.

[redacted]

29 Reproductive and development toxicity studies:

Fertility and reproductive performance (Segment I) studies:
29.1 Gadobenate dimeglumine: Study of effects of fertility and early embryonic development in male CD rats by intravenous (bolus) injection:

Study number: BRO070-972236 Report date: March 31, 1998
Study located in Vol. 22, page 01 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site:
Species: CD (SD) rats (Wt.: 282-339 g)
Dose: 0.3, 1 and 2 mmol/kg/day for 13 weeks (0.3, 0.8 and 1.7 x clinical dose, mg/m^2)

Experimental design: This study was conducted to assess the effects of MultiHance on the general reproductive performance and fertility of male rats after intravenous administration. Four groups of male rats (22/group) received gadobenate dimeglumine at 0 (saline control), 0.3, 1 and 2 mmol/kg/day dose levels for 13 weeks. After 4-weeks of treatment, each male was paired (first pairing) with an untreated virgin female of the same strain. Each male was paired again after 10 weeks of treatment (second pairing) with another untreated female of the same strain. Once mating had occurred (daily vaginal smear checks, Day 0 of gestation), the females were separated from males. Pre-coital interval was recorded. All females were killed on day 14 of gestation for examination of uterine contents. Males were sacrificed after 13 weeks of treatment. Complete necropsy was performed. Following parameters were evaluated: clinical signs, body weight, food consumption, mating performance after pairing, litter size and survival, macroscopic examination of reproductive organs and weights (testes, epididymides, prostate gland and seminal vesicles), sperm count and motility. Mating performace and fertility index were determined (% mating, conception rate, fertility index). For each female, the reproductive tract was examined. This examination included number of corpora lutea in each overy, number of implantation and resorption (early & late) sites and number & distribution of fetuses in each uterine horn.

Results: There were no deaths during the study. Clinical signs associated with treatment were local inflammation at the injection site in animals dosed with 1 (12/22) and 2 (17/22) mmol/kg/day. The local reactions at injection sites included erythema, exfoliation, swelling, scab formation and bruising. This effect was not seen at 0.3 mmol/kg/day dose level. Body weight gain and food consumption for males & females were not significantly affected by the treatment. Mating performance after pairing in weeks 4 & 10 of treatment, and the commensurate litter size and survival to day 14 of pregnancy were not significantly affected. Macroscopic examination of males killed after 13 weeks of treatment revealed no significant findings associated with treatment. No remarkable treatment related effects were noted in males for the weights of the reproductive organs, sperm count and motility. At 0.3 mmol/kg dose

level, one male had no sperm visible in motility/count sample and the another one had low sperm motility. This was not considered a treatment related effect, since this effect was not seen in other 20 animals of the same group, and was not seen at higher doses. Teratology data (number of corpora lutea, implantations, live young and resorptions and the extent of pre- & post-implantation loss) suggested no remarkable paternal treatment related trends.

Reviewer's comments: Based on the data submitted, NOAEL for the general reproductive performance and fertility in male rats can be established at 2 mmol/kg/day. This dose is approximately 1.7 times the maximum clinical dose based on body surface area. The sponsor should have tested higher dose multiples for this sudy.

29.2 Gadobenate dimeglumine: Combined study of effects on fertility and embryo-fetal toxicity in female CD rats by intravenous (bolus) administration:

Study number: BRO071-970104 Report date: March 31, 1998
Study located in Vol. 22, page 158 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: CD (SD) female rats (200-237 g)
Dose: 0.3, 1 and 2 mmol/kg/day (0.3, 0.8 and 1.7 x clinical dose, mg/m^2)

Experimental design: This study was conducted to assess the effects of MultiHance on the general reproductive performance and fertility of female rats after intravenous administration. Four groups of female rats (22/group) received gadobenate dimeglumine at 0 (saline control), 0.3, 1 and 2 mmol/kg/day dose levels for 15 days before pairing. Treatment was continued throughout mating (Day 0 of gestation: day on which mating evidence was found) and up to Day 17 of gestation. All animals mated within five days of pairing. All female rats were killed on Day 20 of gestation for examination of their uterine contents and complete necropsy. During the treatment period, clinical signs, body weight gain and food consumption, estrous cycle and mating performance were evaluated. Reproductive tract examination included number of corpora lutea in each ovary, number of implantation and resorption (early & late) sites, and number & distribution of fetuses in each uterine horn. Weight and sex of individual fetuses, individual placental weights and external abnormalities were also recorded. Approximately half of each litter were allocated for visceral examination and remaining half for skeletal examination.

Results: One female rat that received 2 mmol/kg/day dose died during the study, this death was, however, not attributed to the treatment. Necropsy revealed a large and congested thymus, but the cause of death was not established. In view of isolated nature of this death, it was not attributed to the treatment. Body weight gain and and food consumption were not remarkably

affected before pairing or during gestation. Estrous cycle, mating performance (pre-coital interval and the numbers of animals mating and achieving pregnancy), litter size, fetal and placental weights, survival and development to day 20 of gestation were not significantly affected by the treatment. Teratology data (numbers of corpora lutea, implantations, live young and resorptions and extent of pre- & post-implantation loss) showed no remarkable trend that can be attributed to the treatment. The incidence of fetuses with skeletal anomalies was high in 2 mmol/kg/day dose group. The anomalies occurred at a low incidence or showed no doseage-relationship, hence association with the treatment was not considered.

Clinical signs associated with treatment were local inflammation at the injection site in animals dosed with 1 (2/22) and 2 (15/22) mmol/kg/day. The local raction at injection site was characterized by erythema, exfoliation, scab formation and bruising. This effect was not seen at 0.3 mmol/kg/day dose level.

Reviewer's comments: Based on the data submitted, NOAEL for the general reproductive performance and fertility in female rats can be established at 2 mmol/kg/day. This dose is approximately 1.7 times the maximum clinical dose based on body surface area. The sponsor should have tested higher dose multiples for this sudy, since maternal toxicity was not seen.

Teratology studies (Segment II):

29.3 Gadobenate dimeglumine: A dose range finding study by intravenous administration in the pregnant rabbit:

Study number: — 56-962671 Report date: July 2, 1997
Study located in Vol. 24, page 019 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: Female New Zealand white rabbits (3.1-4.1 kg)
Dose: 0.5, 1 and 3 mmol/kg/day (0.8, 1.7 and 5 times clinical dose, mg/m^2)

Experimental design: This was a dose range finding study for use in selection of doses for a main Segment II study. Time-mated female rabbits received saline or gadobenate dimeglumine (0.5, 1 and 3 mmol/kg/day) on Day 6 post-coitum through Day 18 post-coitum. The dams were observed for clinical signs, food consumption and body weight. On Day 29 of pregnancy, all surviving dams were sacrificed for post-mortem examination. The litter values were determined and fetuses examined for gross macroscopic changes.

Results: At highest dose tested (3 mmol/kg/day), one dam was found dead after 15 minutes post-dosing following 9 days of treatment (Day 14 of pregnancy). At this dose, marked loss of

body weight and reduction in food intake was noted. Loss of body weight and reduction in food intake was also seen at 0.5 and 1 mmol/kg dose levels, although effect was much less pronounced. At 3 mmol/kg dose, local inflammation at the injection site (swollen ears) was observed. At this dose, there was an increase of the incidence of in-utero deaths, which resulted in a decreased mean litter size and mean litter weight. At 1 mmol/kg, in-utero deaths were also increased, however, this was entirely due to a single litter with eight dead implants, of which six were late embryonic deaths. At 0.5 mmol/kg, no obivious effect on in-utero survival was noted. Based on these results, doses of 0.3, 0.9 and 2 mmol/kg were chosen for the main Segment II study in rabbits.

29.4 Gadobenate dimeglumine: Study for effects on embryo-fetal development by intravenous administration in the rabbit:

Study number: 58-963667 Report date: March 26, 1998
Study located in Vol. 24, page 057 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site:
Species: Female New Zealand white rabbits (2.8-4.3 kg)
Dose: 0.3, 0.9 and 2 mmol/kg/day (0.5, 1.5 and 3.3 times clinical dose, mg/m^2)

Experimental design: This study was conducted to assess toxicity of MultiHance in pregnant rabbits during the major period of organogenesis. Time-mated rabbits (20/group) received saline and gadobenate dimeglumine (0.3, 0.9 and 2 mmol/kg/day) via the ear vein from Day 6 to Day 18 post-coitum. The dams were observed for clinical signs, food consumption and body weight. On Day 29 of pregnancy, all dams were killed for macroscopic examination. The litter values determined and fetuses examined for visceral and skeletal changes.
Results: There were no deaths during the study. At 2 mmol/kg/day, notable loss of body weight and reduction in food intake occurred. In addition, six rabbits had local reaction (swollen ears) at injection site that prevented three of the animals from being dosed at the end of treatment period. At 0.9 mmol/kg dose, signs of inappetance, decreased fecal output, decreased body weight (statistically significant on Day 14 of pregnancy) and swollen ears were also noted, although incidences were lower than the high dose. No remarkable effects were noted at 0.3 mmol/kg dose level. There were no abortions during the study.

There were no drug-related significant differences in the incidence of early in-utero deaths or mean litter size, sex ratio and mean fetal weights. However, at 0.9 & 2 mmol/kg, slightly higher (statistically not significant) incidence of early in-utero deaths were noted. At 2 mmol/kg, retinal irregularities (small eye, microphthalmia) were observed in three fetuses from three separate litters. There was also increase in the incidence of additional and/or fused sternebral

centers and 20-thoracolumbar vertebrae. At 0.9 and 2 mmol/kg, there was an increase in the fetal incidence of cervical ribs and offset pelvic girdles.

Reviewer's comments: At 0.9 and 2 mmol/kg/day dose levels, maternal toxicity (decreased body weight, food consumption, local reaction) and fetotoxicity (retinal irregularities, additional/fused sternebral centers, cervical ribs, offset pelvic girdles) was observed. No obivious adverse effects were noted at 0.3 mmol/kg/day. Based on the data provided, NOAEL can be established at 0.3 mmol/kg/day (0.5 times clinical dose, mg/m^2) with respect to embryo-fetal and maternal toxicity in rabbits.

Peri- and Postnatal (Segment III) study:
29.5 Gadobenate dimeglumine: Study of effects on pre- and post-natal development in CD rats by intravenous (bolus) administration:

Study numbe —— 067-970081 Report date: March 31, 1998
Study located in Vol. 26, page 199 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site: ————————————
Species: CD (SD) rats (223-282 g)
Dose: 0.3, 1 and 2 mmol/kg/day (0.3, 0.8 and 1.7 x clinical dose, mg/m^2)

Experimental design: Mated female rats (22/group) received saline or gadobenate dimeglumine (0.3, 1 and 2 mmol/kg/day) from Day 6 of gestation to lactation Day 20. Maternal mortality, clinical signs, body weights & food consumption were recorded. During postnatal phase, parturition parameters and gestation lengths were also recorded. Offspring mortality, clinical signs, litter size, sex ratios and body weights were recorded. Offspring physical development, auditory and visual function, activity, learning ability, and neuromuscular observations were recorded. The F0 females were sacrificed and examined macroscopically after weaning of the F1 offspring. At approximately 5 weeks of age (after completion of behavioral and neuromuscular assessments), 20 F1 males and 20 F1 females from each group were assessed for physical and sexual maturation. These rats were mated and reproductive performance was assessed. Other F1 offsprings were sacrificed and examined macroscopically. F1 females selected for assessment of reproductive performance

were sacrificed on Day 14 after mating and uterine contents were examined. F1 males were sacrificed after the F1 females were sacrificed.

Results: No unscheduled deaths occurred during the study. Local inflammation (erythema, scab formation and necrosis) was seen at the injection site of some dams that received 1 or 2 mmol/kg/day doses. There were no drug-related effects on maternal body weight gains, food

consumption, gestation length, partutirtion or gestation index. No remarkable effects of maternal treatment were noted in F1 offsprings with respect to general condition, litter size, sex ratio, survival, body weight, physical development, auditory & visual function activity, learning ability or neuromuscular function. No remarkable effects were noted for F1 mating performance, fertility & litter size or survival to gestation Day 14 were noted.

Reviewer's comments: Based on the data submitted, gadobenate dimeglumine administered during organogenesis phase of the gestation and throughout the peri- & post-natal phase to female rats, had no significant effects at 0.3 mmol/kg/day. At 1 and 2 mmol/kg/day doses, local inflammation at the injection site was observed. There were no effects on the birth, survival, growth, development and fertility of the F1 generation at any dose levels. The NOAEL in rats with respect to peri- and post-natal toxicity can be established at 2 mmol/kg. This dose is approximately 1.7 times the maximum clinical dose based on body surface area. The sponsor should have tested higher dose levels as no maternal toxicity was seen.

Following reproductive toxicity study was conducted using 0.25 M MultiHance formulation. The clinical formulation for MultiHance is 0.5 M.

29.6 Fertility and reproductive toxicity study by intravenous route in male and female rats:

Study number: 900092 Report date: March 10, 1991
Study located in Vol. 23, page 01 Batch # B5/20
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: CD (SD)BR rats (200-240 g)
Dose: 0.4, 0.8 and 1.5 mmol/kg/day (0.3, 0.7 and 1.3 times clinical dose, mg/m^2)

Experimental design: This study was conducted to assess potential effects of MultiHance (0.25 M) on the reproductive performance of male & female rats when treated in the F0 generation. The study also evaluated subsequent morphological, physical and behavioral development and reproductive performance of the F1 generation. Rats received daily intravenous injections of gadobenate dimeglumine or saline via tail vein. The male rats (n=30/group) were treated for 60 days before mating. The female rats (n=30/group) for 14 days before mating, during pregnancy and during lactation. After mating, the dams were divided into two groups. One group was sacrificed on Day 20 of pregnancy and necropsied. Their fetuses examined for extrenal, skeletal and visceral malformations, anamolies and variants. The other group bore their pups (F1 generation) and reared them until Day 21 of lactation, before being sacrificed with their pups (except for one male and one female pup per litter). The sacrificed pups were examined macroscopically. The F1 pups that were not sacrificed were subjected to

behavioral tests during growing period. These F1 pups were mated at approximately 12 weeks of age and examined for effects on reproduction. The F1 females delievered and reared their pups (F2 generation) until weaning (Lactation Day 21). On this day, the F1 parents were sacrificed and examined. The pups were also sacrificed and necropsied.

Results: *F0 generation:* There were no drug-related effects reported on body weight gain and food intake or clinical signs. No remarkable effects were reported on mating and fertility indices. No remarkable macroscopic findings in females sacrificed on gestation Day 20. No drug-related embryotoxicity was reported. In the highest dose group (1.5 mmol/kg), one fetus had a thin tail and two fetuses had monolateral microphthalmia/amophthalmia. The skeletal anomalies and variants were similar in all experimental groups. No visceral anomalies were reported. There were no treatment-related effects on gestation and parturition in females.

F1 generation: No treatment-related behavioral changes were reported. No remarkable effects on fertility and mating behavior were noted. There was one pup with thin tail in 1.5 mmol/kg dose group. The probability of survival of the highest dose group pups was slightly lower than in control group, particularly in the first 4 days of life. No remarkable effects on mating and fertility indices were reported for the F1 generation.

F2 generation: No significant treatment-related effects were observed.

Reviewer's comments: Based on the above study, the NOAEL for this study can be set at 0.8 mmol/kg/day. This dose is 0.7 times the clinical dose. Gadobenate dimeglumine in this study was administered to F0 males & females through the entire reproductive cycle (pre-mating, mating, gestation and lactation periods). No remarkable effects were seen on mating and fertility in the F0 or F1 rats. A slightly lower probability of survival for the pups was noted in the high dose F1 group (1.5 mmol/kg/day). One fetus each had a thin tail in the F0 & F1 group. Also in the F1 group, two fetuses had monolateral micropthalmia and anopthalmia. All above effects were seen at 1.5 mmol/kg/day dose level. No significant effects were reported in the F2 generation. Adverse effects noted above (1.5 mmol/kg) were not seen in the other fertility and embryo-fetal development study in rat using 0.5 M formulation of MultiHance at 2 mmol/kg.

Summary of Reproductive Toxicology:

Fertility study in male rats: This study evaluated MultiHance (0.3-2 mmol/kg/day for 13 weeks) effects on fertility and general reproductive performance in male rats. The NOAEL for this study was established at 2 mmol/kg (1.7 times human dose). However, it should be noted that in repeat dose study in rats, MultiHance (1 & 3 mmol/kg/day for 28 days) produced vacuolation in testes and abnormal spermatogenic cells. This effect was not reversible after 28-days recovery period. This suggests that MultiHance may affect male fertility. The present fertility study in male rats should have been carried out at higher dose multiples for proper evaluation.

Fertility and embryo-fetal toxicity in female rats (Segment I): This study was carried out to assess the effects of MultiHance (0.3, 1 and 2 mmol/kg/day) on the general reproductive performance and fertility of female rats. Estrous cycle, mating performance, litter size, fetal & placental weights, survival and development to Day 20 of gestation were not significantly affected at any dose level. Teratology data (numbers of corpora lutea, implantations, live young and resorptions) showed no remarkable trend that can be attributed to the treatment. Based on these results, NOAEL for reproductive performance and fertility in female rats was established at 2 mmol/kg (1.7 times human dose). However, sponsor should have tested higher dose multiples, since maternal toxicity was not seen in this study. Segment I study in rabbits was not carried out.

Interestingly, a separate fertility and embryo-fetal development study (Segment I) was carried out using 0.25 M formulation of MultiHancestudy (0.4-1.5 mmol/kg/day) that showed some adverse effects in the F1 generation (noted below). Gadobenate dimeglumine in this study was administered to F0 males & females through the entire reproductive cycle (pre-mating, mating, gestation and lactation periods). No remarkable effects were seen on mating and fertility in the F0 or F1 rats. However, a slightly lower probability of survival for the pups was noted in the high dose F1 group. Also in the F1 group, two fetuses had monolateral micropthalmia and anopthalmia. All these effects were seen at 1.5 mmol/kg/day dose level. No significant effects were reported in the F2 generation. NOAEL for this study was established at 0.8 mmol/kg/day (0.7-times clinical dose).

Segment II studies: A segment II developmental study in rabbits was conducted after administration of 0.3, 0.9 and 2 mmol/kg/day doses of 0.5 M MultiHance to pregnant rabbits during the major period of organogenesis. At 0.9 and 2 mmol/kg dose levels, maternal toxicity (loss of bodyweight, decreased food consumption and local injection site reaction) and fetotoxicity (retinal irregularities, additional/fused sternebral centers, cervical ribs, offset pelvic girdles) was observed. The NOAEL for this study was established at 0.3 mmol/kg (0.5-times human dose) with respect to embryo-fetal toxicity and maternal toxicity in rabbits.

Above Segment II study suggests that MultiHance is teratogenic in rabbits. MultiHance should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus **(Category C)**.

Segment II reporoductive toxicity study in rats using 0.5 M formulation was not conducted. **It is recommended that the sponsor conduct a Segment II reproductive study in rats at dose levels where some maternal toxicity is observed.**

Segment III study: A segment III peri- and post-natal study in rats was conducted by administering 0.5 M MultiHance at doses of 0.3, 1 and 2 mmol/kg/day from gestation day 6 to

lactation day 21. The only maternal effect seen at 1 & 2 mmol/kg doses was local inflammation at injection sites. There were no effects on the birth, survival, growth, development and fertility of the F1 generation. The NOAEL with respect to peri- and post-natal toxicity was established at 2 mmol/kg (1.7 times clinical dose). Again, the dose levels were not high enough, since no maternal toxicity was seen in this study. The segment III study is generally not required for the drug intended as a single dose administration.

30 Special Toxicology Studies:

30.1 Local tolerance of 0.5 M Gd-BOPTA/Dimeg solution in the vessel wall of the marginal ear vein in rabbits:

Study number: RF2735 Report date: Dec 12, 1991
Study located in Vol. 21, page 120 Batch # RG2/91
Certificate of analysis: No GLP: No
Site: Bracco SpA, Milan, Italy
Species: Male Hy —white rabbits (2.3-2.6 kg)
Total volume injected: 0.5 ml (intravenous)

Experimental design: This study was carried out to assess the local tolerance of 0.5 M Gd-BOPTA/Dimeg in the vessel wall and surrounding tissues in normal and the congested (by applying pressure) vein. Magnevist, hyperosmolal saline (5.5% w/v, 1732 mosmol/kg) solution and 0.75% acetic acid were injected as reference articles. Sixteen male rabbits were injected intravenously (marginal ear vein) with the test article (0.5 ml). Impairment of the blood flow was induced in eight animals and other eight animals were studied with normal blood flow. Two animals per test article or each reference control were used for the study. Congestion of the vein was carried out by applying pressure proximal and distal (5-6 cm) to the injection site during a 30-sec injection period. Animals were observed at least once a day during the 7-day observation period. On day 8, animals were sacrificed and macroscopic & histopathological examination of the area around injection sites was conducted.

Results: *Normal marginal ear vein:* Slight to moderate reddening in the area of the injection site was observed with Gd-BOPTA and Magnevist. This effect lasted (slight reddening) until day 5 of the observation period associated with thickening around the site from Day 2 to 4. Minimal reddening (lasting up to day 3) was seen with hyperosmolal saline solution. 0.75% acetic acid (positive control) caused moderate reddning and thickening of large areas that was persistent at the end of observation period (day 7). No such effects were seen with negative control (0.9% saline). Histological examination showed minimal edema and hemorrhage/ inflammatory cell infiltrate for all groups tested (including 0.9% saline).

Congested marginal ear vein: Slight to moderate reddening and thickening around the injection site was seen with Gd-BOPTA, Magnevist, hyperosmolal saline and acetic acid (effect with acetic acid lasting longer). Histological examination showed minimal congestion associated with edema, hemorrhage and inflammatory cell infiltrate for the test articles. Acetic acid treated animals showed histological signs of severe irritation.

The irritative reaction observed with both compounds (MultiHance & Magnevist) was greater than the hyperosmolal saline but less strong than acetic acid. The administration into congested vein also had a weak local irritant reaction that was comparable to hyperosmolal saline.

Reviewer's comments: Intravenous administration of MultiHance produced mildly irritant effects at local injection site when administered to the normal or congested vein. The effects were qualitatively similar to Magnevist. Hyperosmolal saline had lower irritant effect than the test compounds. Please note the histological examination was carried out eight days after administration. No intermediate evaluation (first 24 hours) was conducted. Under the conditions of this study, MultiHance is slightly irritating when injected into the marginal ear vein of the rabbit.

30.2 Local tolerance of 0.5 M Gd-BOPTA/Dimeg after paravasal injection in rabbits:

Study number: RF27321 Report date: Dec 12, 1991
Study located in Vol. 21, page 139 Batch # RG2/91
Certificate of analysis: No GLP: No
Site: Bracco SpA, Milan, Italy
Species: Male Hy —white rabbits (2.3-2.7 kg)
Total volume injected: 0.3 ml/animal (paravenous)

Experimental design: Male rabbits (n=8) received 0.5 M Gd-BOPTA/Dimeg, Magnevist, hyperosmolal saline (5.5%) and 0.75% acetic acid (2/test group) into the ear subcutis close to marginal vein. A same volume of physiological saline was injected into the contralateral ear, Animals were observed several times daily and sacrificed on Day 8 for histological examination of the area surrounding injection site.

Results: Clinical signs, gross pathology and histological findings revealed that gadobenate dimeglumine was moderately irritant after paravasal injection. Clinical signs of irritancy included reddening, local thickening and an eschar on the inner surface of the injected ear. These adverse local effects were persistent at the end of observation period (Day 8). Magnevist caused similar lesions, however, effects were qualitatively somewhat lower than MultiHance. Histological examination showed multifocal hemmorhage, moderate inflammatory cell infiltration, large areas with necrosis, focal acanthosis and eschar. Magnevist effects were limited to hemmorhage and cell infiltrate. No necrosis or eschar was observed with Magnevist. No such effects were seen with the hyperosmolal saline solution. Positive control acetic acid produced effects similar to MultiHance.

Reviewer's comments: Paravenous administration of MultiHance produced irritant effects at local injection site characterized by reddening, thickening, inflammatory cell infiltrates, eschar and large areas of necrosis. These adverse local reactions were more severe than Magnevist. Hyperosmolal saline solution did not produce such adverse effects. Local irritative reaction is likely after accidental extravasation of MultiHance during clinical administration and effects may be somewhat more adverse than Magnevist. It should be noted that the sponsor did not conduct systematic evaluation (histological examination within first 24 hours) of local adverse effects. If these local adverse effects are even more severe during first 24 hours can not be determined from this study.

30.3 Muscular irritation study of E7155 in rabbits:

Study number: 703915 Report date: Dec 12, 1996
Study located in Vol. 21, page 154 Batch # K56019AZB
Certificate of analysis: Yes GLP: Yes
Site: [redacted]
Species: Male Japanese white rabbits (2.1-2.6 kg)
Total volume injected: 1 ml/animal (intramuscular)

Experimental design: Six rabbits were administered MultiHance (1 ml/site) into the right vastus lateralis muscle, and saline into the left vastus lateralis muscle. The muscles were removed from three rabbits at 2 days post-injection and from the remaining three rabbits at 14 days post-administration. The muscles were subjected to macroscopic observation and histopathological examination. Additional animals (n=6/group) were also evaluated similar way using 0.425% acetic acid and 1.7% acetic acid solution.

Results: Macroscopic findings revealed hemorrhage with white and brown coloration at 2 days (3/3) and 14 days (1/3) post MultiHance administration. These effects were qualitatively somewhat less severe than acetic acid solution (0.425% or 1.7%). Histopathological findings indicated moderate hemorrhage, edema, cellular infiltration, degeneration & necrosis of muscle fibers at 2 days post MultiHance administration. Cellular infiltration, degeneration of muscle fibers, fibrosis, calcification of muscle fibers and foreign body giant cells were found at 14 days after administration. These adverse effects were qualitatively somewhat less severe than 0.425% acetic acid solution. It was concluded that MultiHance causes muscular irritancy (grade 2) after intramuscular administration.

Reviewer's comments: Intramuscular administration of MultiHance produced local irritant effects characterized by hemorrhage, edema, cellular infiltration, degneration & necrosis of muscle fibers, fibrosis and calcification of muscle fibers.

**30.4 Hemolytic potential of gadobenate dimeglumine 0.5 M on human blood (*in vitro*

study):

Study number: RF5435 Report date: Feb 7, 1996
Study located in Vol. 21, page 01 Batch # RG15D/95
Certificate of analysis: Yes GLP: Yes
Site: Bracco SpA, Milan, Italy

Experimental design: Gadobenate dimeglumine was added to the human whole blood in a ratio of 10:1 (v/v) in vitro. After incubation for 30 minutes, each sample was centrifuged for 15 min and resulting supernatant examined for hemolysis. Hemolysis was evaluated by determining the RBC count with an automatic cell counter.

Results: No significant change of the RBC count was observed when compared with 0.9% saline. Addition of distilled water (positive control) yielded a complete hemolysis.

30.5 Effect of Gd-BOPTA/Dimeg on human erythrocyte deformability: *in vitro* study:

Study number: RF4862 Report date: May 5, 1995
Study located in Vol. 21, page 13 Batch # RG15D/95
Certificate of analysis: Yes GLP: Yes
Site: Bracco SpA, Milan, Italy

Experimental design: The effect of gadobenate dimeglumine, Magnevist and hyperosmolal solution of mannitol (2000 mosmol/kg) on human erythrocyte deformability was evaluated in vitro. Gadobenate dimeglumine was tested at concentrations of 13.3, 20 and 30% (v/v), corresponding to 67, 100 and 150 mM, respectively.

Results: The table below shows the effect of various test agents on erythrocyte filterability and osmolarity measurements of the samples.

Compound	Strength (% v/v)	Filterability (µL/s)	Osmolarity (mosmol/kg)
Saline	--	94	304
Gadobenate dimeglumine	13.3	76	478
	20	47*	565
	30	9*	724
Magnevist	13.3	89	467
	20	53	552
	30	8*	702
D-mannitol	13.3	78	519
	20	27*	636

	30	2*	818

*p<0.01 versus negative control (saline)

The reduced deformability of human erythrocytes is due to osmolarity of the solution. According to the sponsor, no deformability should be expected in clinical settings as no-effect concentration found in this study is much higher than maximum plasma concentration reached at maximum clinical dose.

30.6 An *in vivo* test to evaluate the effects of B19036/7 (0.25 M) on blood cells and plasma factors of the coagulation after intravenous administration in rats:

Study number: RF1812 Report date: July 7, 1990
Study located in Vol. 21, page 47 Batch # RG9/89
Certificate of analysis: No GLP: No
Site: Bracco SpA, Milan, Italy

Experimental design: This study was conducted to determine potential in vivo effects of 0.25 M gadobenate dimeglumine on CD (SD)BR rat blood cells and plasma factors after intravenous injection (0.1 and 0.25 mmol/kg, n=5/sex/dose/time point). Blood was collected 7.5, 30 and 120 min post-injection. Saline group was used as control. The following parameters were determined: total and differential leukocyte count, platelet count, hemoglobin concentration, erythrocyte count, packed cell volume, prothrombin time, partial prothrombin time and fibrinogen.

Results: Under the conditions of this study, gadobenate dimeglumine (0.25 M) did not significantly alter the corpusculate and coagulative blood parameters in rats at doses up to 0.25 mmol/kg (0.2 times human dose based on body surface area).

30.7 An *in vitro* test to evaluate the effects of B19036/7 (0.25 M) on plasma factors of coagulation:

Study number: RF1776 Report date: July 4, 1990
Study located in Vol. 21, page 88 Batch # RG9/89
Certificate of analysis: No GLP: No
Site: Bracco SpA, Milan, Italy

Experimental design: Gadobenate dimeglumine (0.25 M) was incubated in the presence of rat plasma, at concentrations of 6.3, 12.5 and 25 mM for 2 minutes. Saline was used as control group. Following parameters were determined: prothrombin time (PT), partial prothrombin time (PTT) and fibrinogen.

Results: A dose-dependent increase in PT and PTT, with a concomitant decrease in fibrinogen was observed. The effects were biologically significant at 12.5 and 25 mM dose level. These changes were minimal (about 5%) at the lowest concentration (6.3 mM). According to the sponsor, this concentration corresponds to approximately three times the maximum plasma levels obtained after intravenous administration of 0.5 mmol/kg in rats (0.4 times clinical dose). The table below summarizes the data for the effects on coagulation parameters:

Compound	Concentration (mM)	PTT (sec)	PT (sec)	Fibrinogen ((sec)
Saline	0.9%	13.6	14.8	257
Gadobenate dimeglumine	6.3	14.6	15.4	255
	12.5	15.4	16.4	234
	25	17.7	18.9	214

Summary of Special Toxicology Studies:

The following special toxicology studies were conducted: 1) Local tolerance/irritation study in rabbits after intravenous, intramuscular and paravenous administration of gadobenate dimeglumine, 2) Hemolytic and erythrocyte deformability potential in human blood and 3) *in vivo & in vitro* test for effect on rat plasma coagulation factors.

Intravenous administration of MultiHance produced mild irritant (redness, edema, hemorrhage, cellular infiltrate) effects at local injection site when administered to the normal or congested (by applying pressure) vein. Paravenous administration of MultiHance produced moderate to severe irritant effects at local injection site characterized by reddening, thickening, inflammatory cell infiltrates, eschar and large areas of necrosis. These adverse local reactions were qualitatively more pronounced than Magnevist. Hyperosmolal saline solution did not produce such adverse effects. Intramuscular administration of MultiHance produced local irritant effects characterized by hemorrhage, edema, cellular infiltration, degneration & necrosis of muscle fibers, fibrosis and calcification of muscle fibers.

Local tolerance studies suggest that local irritative reaction is likely after accidental extravasation of MultiHance during clinical administration and effects may be somewhat more adverse than Magnevist. It should be noted that the sponsor did not conduct systematic evaluation (histological examination within first 24 hours) of local adverse effects. If these local adverse effects are even more severe during first 24 hours can not be determined from this study.

In vitro studies conducted using human blood suggested that gadobenate dimeglumine, at clinically relevant concentrations, may not produce significant effect on human erythrocyte deformability or hemolysis.

An *in vivo* study in rats conducted to evaluate MultiHance (0.25 mmol/kg) effect on blood cells and plasma coagulation factors did not show any significant effects on corpusculate or coagulative parameters. However, it should be noted that this study was conducted using 0.25 M formualtion and the maximum dose tested is only 0.2 times the human dose based on body surface area. An *in vitro* test was conducted to evaluate effects of MultiHance (0.25 M) on rat plasma factors of coagualtion. Effects on PTT, PT and fibrinogen were minimal (about 5%) at 6.3 mM dose level. According to the sponsor, this dose corresponds to about three times the maximum plasma levels obtained after intravenous administration of 0.5 mmol/kg in rats (0.4 times clinical dose). At higher doses (13 and 25 mM), somewhat significant increases in PT and PTT and concomitant decrease in fibrinogen was observed.

Overall Summary:

MultiHance (0.5 M gadobenate dimeglumine) is a MRI agent that is proposed for the imaging the central nervous system (CNS). Gadobenate dimeglumine consists of an octadentate chelate of paramagnetic ion gadolinium salified with meglumine. The chelation of gadolinium ion presumably results in substantially less toxicity than the free ion. Upon intravenous administration, the drug exits the vascular space rapidly and resides primarily in the extravascular space, but is also present within hepatocytes. MultiHance is excreted mainly through the renal and biliary routes.

. The proposed dose for imaging of the CNS in adults is 0.1 mmol/kg;

Thus, the **maximum clinical dose** for MultiHance that will be administered in a patient during a single examination will be **0.2 mmol/kg**. This maximum clinical dose of 0.2 mmol/kg was used for calculating human dose-multiples for pre-clinical studies in animals.

. MultiHance has an osmolarity (1970 mOsmol/kg, similar to Magnevist) that is 6.9 times that of plasma (285 mOsmol/kg), and is hypertonic under conditions of use. It is administered as a rapid intravenous infusion or bolus injection followed by 5-ml saline flush.

During the clinical development of MultiHance, the use of a 0.25 M formulation was tested in clinical studies, and several non-clinical safety and efficacy studies. The formulation for marketing is 0.5 M gadobenate dimeglumine. The sponsor conducted required major pharmacology and toxicology studies using 0.5 M clinical formulation. However, some of the pre-clinical studies, in particular, safety pharmacology, expanded acute dose toxicity, reproductive toxicity (Segment II) and local tolerance studies were not adequate to assess safety of MultiHance in humans (please see below). In general, for most of the studies low dose-multiples were used to adequately establish the toxicity profile of MultiHance. In addition, in most of the safety pharmacology studies only one dose was utilized for evaluation. Evaluation at various dose-levels in the same study is necessary for comparison and establishment of a dose-response curve.

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

Safety Pharmacology: These 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.

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 included 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 the patients with damaged BBB). In these studies, MultiHance caused hypoactivity, impaired motor coordination, convulsions and death. The NOAELs for various CNS parameters 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 (0.1-3 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 rat 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 in a conscious animal is totally stopped 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 evaluated in this study. The sponsor also studied effects of MultiHance on blood brain barrier permeability in BBB damaged animals. At 0.3 mmol/kg dose level (0.3-times clinical dose), MultiHance did not show any significant permeability (<0.2% ID) through the damaged BBB. However, higher dose levels should have been tested for any meaningful comparison.

Cardiovascular, renal and respiratory effects: These studies were conducted in rats, rabbits and pigs at 1 mmol/kg dose level. MultiHance at this dose produced transient but significant changes in various CVS (increased cardiac output and stroke volume, decreased total peripheral resistance, increased renal blood flow etc.) and respiratory (increase in intratracheal pressure and pulmonary blood flow) parameters. These transient effects were attributed to hyperosmolarity of the solution. However, the sponsor did not include a positive control (hyperosmotic mannitol solution) in these studies to support such conclusion.

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 (pre-dosing, during and after dosing). Also, sponsor did not report evaluation of any findings on various ECG parameters such as QT interval.

MultiHance effects on urine output and electrolyte excretion (sodium, potassium and chloride) were evaluated in rats. At the maximum dose level tested (1 mmol/kg), no significant effects were noted on these parameters. This dose is only 0.8-times the clinical dose based on body surface area.

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. The table below summarizes various adverse effects of MultiHance on CNS, CVS, renal and respiratory system.

Physiological system	Adverse effects
CNS*	Hypoactivity, impaired motor coordination, EEG: slowing of signal & transient flattening, convulsions and death
CVS and respiratory**	Transient effects: increased cardiac output & stroke volume, decreased total peripheral resistance, increased renal blood flow, increase in intratracheal pressure & pulmonary blood flow, reduction in papillary & atrial contractile force (*in vitro* studies), transient cardiac depressant activity (*in vitro* study)
Renal system	No effects on urine output and electrolyte concentration at 1 mmol/kg dose tested

*NOAELS: 0.3-2 mmol/kg, EEG effect seen at 4 mmol/kg; **Transient effects seen at 1 mmol/kg, only dose tested.

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. Hyperosmotic control group (sucrose/mannitol solution) and Magnevist should be included for

comparison purpose.

The sponsor should also conduct a study to evaluate effects of MultiHance on blood brain barrier (BBB) permeability in BBB damaged animals at clinically equivalent and higher dose levels.

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.**

Single dose acute toxicology Studies: Acute dose studies were conducted to determine LD_{50} in mice, rats and dogs after intravenous and intracerebral administration. The table below summarizes the LD_{50} data for these studies.

Species	Route of administration	LD_{50} (mmol/kg)	Highest non-lethal dose	Safety margin for lethality* (based on mg/m^2)	NOAEL dose level** (based on mg/m^2)
Mice	iv (1 ml/min) iv (0.2 ml/min) intracerebral	5.7 7.9 0.4	Not established*** 7.1 0.3	Not established 3 0.2	Not established Not established Not established
Rat	iv (6 ml/min) iv (1 ml/min) Neonate (iv,1 ml/min) Intracisternal	6.6 9.2 9 0.3	4.5 7.5 6.3 0.2	3.8 6.3 5.3 0.2	Not established Not established Not established Not established
Dogs	iv	6 (100% lethal dose)	2	5	Not established

*Based on body surface area, safety margin was calculated as a ratio of highest non-lethal dose noted above to the maximal clinical dose of 0.2 mmol/kg, **Dose level that did not show any adverse effects in animals, ***The lowest dose tested (5 mmol/kg) produced lethality in some animals.

As seen in the table above, there is a very low safety margin in terms of non-lethal dose and the highest clinical dose of 0.2 mmol/kg. According to the sponsor, these low safety margins may be due to high osmotic load relative to species blood volume (30-50 times higher osmotic load compared to the highest dose in human in terms of total blood volume of human and animals). It may be possible that the lethal effects are exaggerated due to hyperosmolarity of the solution in animals (in terms of total blood volume). However, this conclusion can not be confirmed in

the absence of positive control group (such as hyperosmotic mannitol solution) in above studies. Also NOAEL could not be established in any of the above studies as various adverse effects were noted at the lowest dose tested. Some of the severe adverse effects observed after acute administration of MultiHance were convulsions, dyspnea, shallow breathing/gasping, marked increase in liver enzymes and kidney vacuolation. **The sponsor did not conduct any systematic expanded acute dose toxicity study.**

A study was conducted in rats to evaluate the occurrence of renal tubular vacuolation after acute administration. Vacuolation in kidney was observed at doses 1 mmol/kg and above. The NOAEL for this study was established at 0.5 mmol/kg, which is 0.4 times the clinical dose based on body surface area. This study did not evaluate extent of reversibility of this effect (vacuolation) after 7/14 days recovery period.

It is recommended that the sponsor conduct a systematic expanded acute dose study in at least one species to adequately establish the toxicity profile for MultiHance. The study should be conducted in bigger animals to avoid the high osmotic load problem seen in small animals. This study should be carried out at various dose levels (at least three) and hyperosmotic mannitol/sucrose solution should be included as a comparative control. Various toxicity parameters should be evaluated 72-hours post-dosing, and also after 7/14-days recovery period. This recommendation is based on the following deficiencies/concerns noted in the acute dose studies:

(a) LD_{50} studies can not be substituted for the expanded single dose studies. Expanded acute dose toxicity study evaluating all necessary parameters (hematology, clinical chemistry, urinalysis, complete histopathology etc.) is needed.
(b) NOAEL was not established in any of the species for the dose levels tested.
(c) Lethality and adverse effects seen were attributed to high osmotic load in animals. However, in the absence of positive control group (hyperosmotic mannitol/sucrose solution), there is no evidence that all of the adverse effects are solely due to hyperosmolarity, and not due to drug itself.
(d) In repeat dose studies (please see below), NOAEL was established at dose multiples that were below the equivalent clinical dose of 0.2 mmol/kg (0.4-0.6 times the human dose based on body surface area as well as AUC data provided by the sponsor). In humans, MultiHance will be administered as a single dose contrast agent. Therefore, it is important to evaluate toxicity potential of MultiHance in animals (and likely target organs) after single dose administration at dose-multiples that are equivalent and higher than the intended clinical dose.

Repeat dose studies: Preliminary dose-escalation studies were conducted to determine the maximum tolerated dose (MTD) levels in various species. Based on these studies dose selection was made for the expanded repeat dose toxicity studies. These preliminary studies indicated

that the MTD levels for rats, dogs and cynomolgus monkeys were 4, 2 and 6 mmol/kg, respectively. All these MTDs were associated with significant toxicities (ranging from dyspnea to liver and kidney toxicity), and should not be considered as equivalent to NOAELs.

Expanded repeat dose toxicity studies were carried out in rats, dogs and cynomolgus monkeys. The table below summarizes various adverse effects seen and NOAELs for the repeat dose studies:

Species and dose levels	Adverse effects	NOAEL
Rats (0.3, 1 & 3 mmol/kg for 28 days)	1 & 3 mmol/kg: Vacuolation in kidney, bladder and testes (with abnormal spematogenic cells), pale corpus mucosa and mineralization in stomach, increased kidney weights. Additionally, at 3 mmol/kg dose hepatocyte necrosis was observed. Above effects not reversed after a 4-week recovery period. 0.3 mmol/kg: Vacuolation in kidneys and changes in plasma/urinary electrolyte concentrations. Dose-dependent increase in the incidence & degree of lesions at injection sites at all dose levels.	Not established (<0.3 mmol/kg)
Dogs (0.25, 1 & 2 mmol/kg for 28 days)	1 & 2 mmol/kg: Transient swelling/redness of facial area, significant loss of bodyweight & food consumption, kidney vacuolation (reversed after 4-week recovery period), increase in kidney & liver weights, changes in liver enzymes and decreased urinary electrolyte levels. Vacuolation & lesions in liver (not reversed by 28 days at 2 mmol/kg dose). Additionally, bone marrow hypocellularity at 2 mmol/kg dose. 0.25 mmol/kg: Although not significant, decrease in urine phosphorus levels.	0.25 mmol/kg (0.6-times human dose)
Cynomolgus monkey (0.25, 1 & 3 mmol/kg for 14 days)	1 & 3 mmol/kg: Reduction in bodyweight & food consumption, increase in kidney weight & vacuolation, decreased plasma zinc levels. Additionally, at 3 mmol/kg: increased liver weights, vacuolation in pancreas. Urinary electrolyte levels were not determined.	0.25 mmol/kg (0.4-times human dose)

In repeat dose study in monkeys, although statistically not significant, decreases in plasma zinc (33%) and phosphrous (21%) levels were reported at the highest dose (3 mmol/kg) tested. This may indicate possible transmetallation of the gadolinium, and possible evidence for the release of free gadolinium from the MultiHance complex. In addition, the bone marrow hypocellularity seen in dogs may indicate that free gadolinium was released from MultiHance and deposited in the bone marrow. Furthermore, PK studies in rats and dogs (see below) showed free gadolinium (6%ID) in feces and 2-3%ID accumualtion in bone (in rat, biodistribution in dog was not studied). Therefore, stability of MultiHance complex (gadobenate) is of concern.

The repeat dose studies suggest that the safety margin for MultiHance in terms of adverse

effects is very low (0-0.6 times human dose). In general, MultiHance was better tolerated in monkeys than in rats or dogs. The target organs for toxicity seem to be mainly liver (necrosis, vacuolation, changes in liver enzymes, increased liver weight) and kidney (vacuolation, increased weight). In addition, vacuolation was also seen in testes (with abnormal spermatogenic cells) and pancreas at higher dose levels. In most cases, the vacuolation was not reversible.

Pharmacokinetic studies: The PK studies were conducted in rats, rabbits, dogs and monkeys. A study was also conducted in the TR$^-$ rats, a mutant strain of rat that has defective ATP-dependent bile canalicular membrane organic anion transporter (cMOAT), to examine mechanisms of bile transport of gadobenate dimeglumine.

After intravenous administration, the plasma kinetics showed a bi-exponential profile in all species tested. MultiHance distributed rapidly from the plasma compartment to the extracellular space, and tissue levels increased rapidly in parallel with the decrease in plasma levels. The table below summarizes PK parameters in rats and dogs as compared to humans:

Parameters	Rat	Dog	Human
Cmax (mmol/L)			
Half-life (min): α-phase β-phase	 8-12 30	 25 90-175	 6-36 70-122
Vol. of distribution (L/kg)	0.2	0.3	0.17-0.28
AUC (mmol x hr/L)	1.3 (1 mmol/kg dose)	2.9 (1 mmol/kg dose)	0.9 (0.1 mmol/kg dose)
Clearance (ml/hr/kg)	720	360	90-270
Elimination: Kidney Feces	 50-65% 35-50%	 60% 40%	 80-96% 1-4%

*Maximum clinical dose is 0.2 mmol/kg for which PK parameters were not provided. It is assumed that at 0.2 mmol/kg, AUC in humans will be 1.9 mmol x hr/L (AUC: 0.9 at 0.1 mmol/kg dose x 2). In terms of clinically equivalent dose based on AUC: Dogs: 1 mmol/kg dose is 1.5 times human dose and rats: 1 mmol/kg dose is 0.7 times clinical dose. These dose multiples are similar to the one calculated by using body surface area for pre-clinical studies.

Gadobenate dimeglumine was excreted rapidly via the urine and feces in rats, rabbits and dogs. Unlike in humans, biliary elimination is a major excretion route for these species. The elimination half-life was shorter in rats than in rabbits and dogs. It should be noted that

significant amount (~6% ID) was detected in feces of rats and dogs (but not in rabbits) as a free gadolinium ion, indicating it may dissociate from the gadolinium complex/chelate. Also in two separate distribution studies in rats (see below), approximately 2.7% ID was detected in bones (free gadolinium ion). The sponsor has attributed this accumulation in bone to radiactive impurities, which according to the sponsor are not present in the clinical formulation. However, no data was provided to support this contention.

Biliary excretion and enterohepatic circulation: In a separate study in rats, MultiHance (0.1-0.5 mmol/kg, 0.1-0.4 times clinical dose) was almost equally eliminated through urine and bile (50% ID, each). However, at low dose level, biliary elimination was somewhat higher (~55%) than at high dose (~47%). This may indicate that the biliary excretion is somewhat saturable. The biliary excretion was accompanied by a dose-dependent increase in biliary flow (2-3 times basal value). The enterohepatic circulation studies in rats and rabbits indicated that enterohepatic recirculation of gadobenate dimeglumine is low (~5%).

Biliary transport mechanism: A study was conducted to evaluate transport mechanism of gadobenate dimeglumine (0.25 mmol/kg, 0.2 times clinical dose) across the bile canalicular membrane using mutant TR^- rats, which have defective hepatic excretory function associated with mutation of the ATP-dependent organic anion transporter cMOAT. According to the sponsor, this animal model resembles the hepatic dysfunction that occurs in human Dubin-Johnson syndrome. The biliary excretion of gadobenate ion in TR^- rats was greatly reduced (~3%) relative to that occurs in normal rats (~50%). According to the sponsor, these results suggest that transport of gadobenate ion from the cytoplasm of hepatocytes to bile occurs via cMOAT transporter, which is also involved with the canalicular transport of bilurubin and bromosulfophthalein. It was concluded that in spite of hepatic dysfunction, gadobenate ion was almost totally eliminated from the body of the TR^- rats by urinary elimination (~90%), which compensates for the absence of the hepatic transport system. However, this study would have been more confirmative if positive control group (bromosulfophthalein) was included in the study.

Distribution studies in rats and rabbits: Studies in rats and rabbits indicated that gadobenate dimeglumine distributed mainly to the organs of elimination (kidneys, intestine, liver and urinary bladder). In rats, significant levels were also found in muscle (8% ID), skin (11% ID) and bone (2.7% ID). After 28 days, 1% ID remained in the body and this radioactivity was mostly in bone (0.7% ID). Therefore, stability of gadolinium chelate is of concern (for comparison: Magnevist retention in bone after 28 days is about 0.05% ID). The total elimination in rats after 3 days was 98% (54% in urine and 44% in feces).

As compared to rat biodistribution study, the total radioactivity distribution in rabbit was relatively poor in various tissues (dose: 1 mmol/kg, 1.7 times clinical dose). The majority of the

drug remained in carcass (60%). The highest tissue levels were detected in organs of elimination (liver, kidney and GI tract). Low activity was detected in bone (0.01% ID). The cumulative drug elimination after 7 days was mainly through urine (65%) and feces (25%).

Binding to plasma proteins and effect on hepatic enzymes: In an *in vitro* study gadobenate ion did not show appreciable binding to rat and rabbit plasma proteins and to human serum albumin (MultiHance dose: 0.2-10 mM). In a separate study, gadobenate dimeglumine in doses up to 0.5 mmol/kg (0.4 times clinical dose) administered twice a day for 7 days did not cause any significant effect on hepatic drug metabolizing enzymes.

PK studies in pregnant rats: In pregnant rats, the distribution of 153Gd labeled gadobenate dimeglumine (0.5 mmol/kg) was similar to that in normal rats, and was not influenced by the stage of pregnancy (7^{th}, 14^{th} and 20^{th} days of gestation). Radioactivity distributed into the maternal tissues, crossed the placental barrier, and reached the fetus. The accumulation in placenta was about 0.5% ID. Placental levels decreased to <0.02% after 2 hours post-injection. The diffusion to fetus was low (0.03% ID). However, some radioactivity detection was noted in fetal tissues, in particular in fetal liver (0.01% ID). The fetal liver activity decreased to comparable level after 24 hours (0.005% ID). Similar results were obtained with Magnevist.

Transfer to neonates via milk: Transfer of gadobenate dimeglumine (0.5 mmol/kg, 0.4 times clinical dose) and Magnevist (0.5 mmol/kg) *via* milk of lactating rats to neonates was about 0.5-1% ID. More than 75% of the transferred amount (radioactivity) was found in the GI tract of the neonate and no detectable residual activity was observed in neonatal liver.

Overall, PK studies in pregnant and lactating rats suggest that MultiHance is transferred (although low %) to neonates through the milk of the lactating rats, and diffusion of it to fetus occurs in pregnant rats.

Reproductive toxicity studies: *Fertility study in male rats:* This study evaluated MultiHance (0.3-2 mmol/kg/day for 13 weeks) effects on fertility and general reproductive performance in male rats. The NOAEL for this study was established at 2 mmol/kg (1.7 times human dose). However, it should be noted that in repeat dose study in rats, MultiHance (1 & 3 mmol/kg/day for 28 days) 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. This may be reflected in the label.

Fertility and embryo-fetal toxicity in female rats (Segment I): This study was carried out to assess the effects of MultiHance (0.3, 1 and 2 mmol/kg/day) on the general reproductive performance and fertility of female rats. Estrous cycle, mating performance, litter size, fetal & placental weights, survival and development to Day 20 of gestation were not significantly affected at any dose level. Teratology data (numbers of corpora lutea, implantations, live young

and resorptions) showed no remarkable trend that can be attributed to the treatment. Based on these results, NOAEL for reproductive performance and fertility in female rats was established at 2 mmol/kg (1.7 times human dose). However, sponsor should have tested higher dose multiples, since maternal toxicity was not seen in this study. Segment I study in rabbits was not carried out.

Interestingly, a separate fertility and embryo-fetal development study (Segment I) was carried out using 0.25 M formulation of MultiHancestudy (0.4-1.5 mmol/kg/day) that showed some adverse effects in the F1 generation (noted below). Gadobenate dimeglumine in this study was administered to F0 males & females through the entire reproductive cycle (pre-mating, mating, gestation and lactation periods). No remarkable effects were seen on mating and fertility in the F0 or F1 rats. However, a lower probability of survival for the pups was noted in the high dose F1 group. Also in the F1 group, two fetuses had monolateral micropthalmia and anopthalmia. All these effects were seen at 1.5 mmol/kg/day dose level. No significant effects were reported in the F2 generation. NOAEL for this study was established at 0.8 mmol/kg/day (0.7-times clinical dose).

Segment II study in rabbits: A segment II developmental study in rabbits was conducted after administration of 0.3, 0.9 and 2 mmol/kg/day doses of 0.5 M MultiHance to pregnant rabbits during the major period of organogenesis. At 0.9 and 2 mmol/kg dose levels, maternal toxicity (loss of bodyweight, decreased food consumption and local injection site reaction) and fetotoxicity (retinal irregularities, additional/fused sternebral centers, cervical ribs, offset pelvic girdles) was observed. The NOAEL for this study was established at 0.3 mmol/kg (0.5-times human dose) with respect to embryo-fetal toxicity and maternal toxicity in rabbits.

Above Segment II study suggests that MultiHance is teratogenic in rabbits. MultiHance should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus **(Category C)**.

Segment II study in rats using 0.5 M MultHance was not conducted. **It is recommended that the sponsor conduct a Segment II reproductive toxicity study in rats at dose levels where some maternal toxicity is observed.**

A segment III peri- and post-natal study in rats was conducted by administering 0.5 M MultiHance at doses of 0.3, 1 and 2 mmol/kg/day from gestation day 6 to lactation day 21. The only maternal effect seen at 1 & 2 mmol/kg doses was local inflammation at injection sites. There were no effects on the birth, survival, growth, development and fertility of the F1 generation. The NOAEL with respect to peri- and post-natal toxicity was established at 2 mmol/kg (1.7 times clinical dose). Again, the dose levels were not high enough, since no maternal toxicity was seen in this study.

Immunotoxicity studies: MultiHance was not antigenic in the guinea pig active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) tests. No anaphylactic reactions were seen in the ASA or PCA test.

In an *in vitro* study, MultiHance did not cause any significant histamine release or mast cell degranulation at 125 mM concentration. However, significant histamine release and mast cell degranulation was observed at 250 mM MultiHance concentration. Magnevist and hyperosmolar sucrose solution produced similar effects. This effect may be due to the hyperosmolality of MultiHance. According to the sponsor, the no effect MultiHance concentration (125 mM) for histamine release is about 250 times higher than the clinical concentration obtained in blood. In a separate *in vitro* study, 0.25 M MultiHance in the range of 25×10^{-4} M to 4×10^{-2} M, induced dose-dependent 11-94% complement activation. The IC_{50} value for this effect was 1.5×10^{-2} M (or 15.2 mmol/L). According to the sponsor, this IC_{50} value is about 15-times higher than the maximum plasma levels in human after intravenous administration of 0.2 mmol/kg of MultiHance.

Genetic toxicology studies: MultiHance did not produce any mutagenic effect in the following battery of genotoxicity tests (in the absence & presence of metabolic activation): a) Ames test (S. *typhimurium* and E. *coli).* b) Human lymphocyte assay. c) Unscheduled DNA synthesis in human cells (HeLa cells). d) *In vitro* mammalian cells assay (V79 Chinese hamster lung cells). e) *In vivo* micronucleus assay (intraperitoneal injection) in rats.

In vivo micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than intravenous administration. According to the ICH guidelines, this study should be carried out using intravenous administration. Also dose level used in this study was not high enough. The sponsor needs to explain reasons for choosing intraperitoneal route only for this particular study (*in vivo* micronucleus assay). Biodistribution studies after intravenous MultiHance administration in rats have shown some accumulation of free gadolinium (<1% of ID) in bone that was persistent. **It is recommended that the sponsor repeat the *in vivo* micronucleus assay using intravenous administration of MultiHance at higher dose levels.**

Local tolerance/irritation study in rabbits: Intravenous administration of MultiHance produced mild irritant (redness, edema, hemorrhage, cellular infiltrate) effects at local injection site when administered to the normal or congested (by applying pressure) vein. Paravenous administration produced moderate to severe irritant effects characterized by reddening, thickening, inflammatory cell infiltrates, eschar and large areas of necrosis. These adverse local reactions were qualitatively more pronounced than Magnevist. Hyperosmolal saline solution did not produce such adverse effects. Intramuscular administration of MultiHance produced local irritant effects characterized by hemorrhage, edema, cellular infiltration, degeneration & necrosis of muscle fibers, fibrosis and calcification of muscle fibers.

Local tolerance studies suggest that local reaction is likely after accidental extravasation of MultiHance during clinical administration and effects may be somewhat more adverse than Magnevist. It should be noted that the sponsor did not conduct systematic evaluation of local adverse effects. Histological evaluation was carried out 8-days post-dosing. Additional 24-hr time point should have been included for the histological evaluation. If these local adverse effects are even more severe during first 24 hours can not be determined from this study. Clinically, Magnevist is known to cause phlebitis, thrombophlebitis and faciitis in some patients (and amputation of arm in some cases). **In view of clinical importance of these studies, it is recommended that sponsor conduct a systematic local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation after 24 hours and at later time points, until the local adverse effects are resolved.**

Effect on blood cells and plasma coagulation parameters: *In vitro* studies conducted using human blood suggested that MultiHance, at clinically relevant concentrations, might not produce significant effect on human erythrocyte deformability or hemolysis. An *in vivo* study in rats conducted to evaluate MultiHance (0.25 mmol/kg) effect on blood cells and plasma coagulation factors did not show any significant effects on corpusculate or coagulative parameters. However, this study was conducted using 0.25 M formualtion and the maximum dose tested is only 0.2 times the human dose based on body surface area. A separate *in vitro* test was conducted to evaluate effects of MultiHance (0.25 M) on rat plasma factors of coagulation. Effects on PTT, PT and fibrinogen were minimal (about 5%) at 6.3 mM dose. According to the sponsor, this dose corresponds to about three times the maximum plasma levels obtained after intravenous administration of 0.5 mmol/kg in rats (0.4-times clinical dose). At higher doses (13 and 25 mM), moderate but somewhat significant increases in PT and PTT and concomitant decrease in fibrinogen were observed.

Likely target organs: In terms of adverse effects, following are the target organs for MultiHance toxicity:

Brain (convulsions, hypoactivity, slowing of EEG), liver (necrosis, vacuolation, histiocytosis, changes in liver enzyme levels, increased liver weights), kidney (vacuolation, transient polyuria, increased kidney weights, changes in urinary electrolyte levels), pancreas (vacuolation) and testes (vacuolation and abnormal spermatogenic cells). In addition, EKG effects were not properly evaluated (QT interval).

The safety margin in terms of lethality (LD_{50} studies) ranged from 2 to 8 (maximum non-lethal dose/clinical dose of 0.2 mmol/kg, based on body surface area). This effect may be exaggerated due to high osmolality of the solution in animals compared to humans. However, no positive control group (hyperosmotic mannitol/sucrose solution) was included in these studies to confirm it.

Local tolerance studies suggest that local reaction is likely after accidental extravasation of MultiHance during clinical administration

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Recommendations:

Internal Comments:

The NDA application for MultiHance contained required major pharmacology and toxicology sections. However, some of the pre-clinical studies, in particular, safety pharmacology, expanded acute dose toxicity, reproductive toxicity (Segment II) and local tolerance studies were not adequate to assess safety of MultiHance in humans (please see below). In addition, most of the animal studies were carried out using low dose-multiples to adequately establish the toxicity profile of MultiHance. Following are the major issues that need to be addressed by the Sponsor:

1) *Safety pharmacology studies:* Major deficiencies are summarized below:

a) 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.
b) 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.
c) 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.
d) 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 a larger 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 using hyperosmotic control group (sucrose/mannitol solution) and Magnevist for comparison purpose.

2) *Electrophysiological studies:* In humans, gadolinium type contrast agents can cause prolongation of QT interval. 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 effects on QT interval.

3) *Expanded single dose studies:* It is recommended that the sponsor conduct a systematic expanded acute dose study in at least one species to adequately establish the toxicity profile for MultiHance. The study should be conducted in bigger animals to avoid the high osmotic load problem seen in small animals. This study should be carried out at various dose levels (at least three) and hyperosmotic mannitol/sucrose solution should be included as a comparative control. Various toxicity parameters should be evaluated 72-hours post-dosing, and also after 7/14-days recovery period. This recommendation is based on the following deficiencies/concerns noted in the acute dose studies:

a) No expanded acute dose toxicity studies evaluating all necessary parameters included in the ICH guidelines for single dose toxicity studies (such as hematology, clinical chemistry, urinalysis, complete histopathology etc.) were submitted. LD_{50} studies can not be substituted for the expanded single dose studies.
b) Low safety margins in terms of lethality after acute dose administration.
c) Proper NOAEL was not established in any of the species for the dose levels tested. No dose-response relationship was established (in terms of toxicity), since only one dose was utilized for most of the studies.
d) Sponsor attributes the lethality and adverse effects to high osmotic load in animals. However, in the absence of positive control group (hyperosmotic mannitol solution), there is no evidence that all of the adverse effects are solely due to hyperosmolarity, and not due to drug itself.

4) *Reproductive toxicity studies:* Segment II reproductive toxicity study in rats was inadequate. It is recommended that the sponsor conduct a Segment II reproductive toxicity study in rats at dose levels where some maternal toxicity is observed.

5) *Mutagenicity studies: In vivo* micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than intravenous administration. According to the ICH guidelines, this study should be carried out using intravenous administration. Also dose levels used in this study were not high enough. The sponsor needs to explain reasons for choosing intraperitoneal route only for this particular study. It is recommended that the sponsor repeat the *in vivo* micronucleus assay using intravenous route at various doses with higher dose multiples.

6) *Local tolerance studies:* These studies suggest that local reaction is likely after accidental extravasation of MultiHance during clinical administration, and effects may be somewhat more adverse than Magnevist. The sponsor did not conduct systematic evaluation of local adverse effects. Histological evaluation was carried out 8-days post-dosing. If these local reactions are even more severe during first 24 hours can not be determined from this study. Clinically, Magnevist is known to cause phlebitis, thrombophlebitis and faciitis in some patients (and amputation of arm). In view of clinical importance of these studies, it is recommended that sponsor conduct a local tolerance study (intravenous, paravenous & intramuscular

administration) with histological evaluation at 24 hours and at a later time point, until the local adverase effects are resolved.

In view of above major deficiencies (also see external comments) compounded by narrow safety margins obtained in pre-clinical studies (please see below), MultiHance is deemed not approvable from the pharmacology and toxicology perspective.

Likely target organs: In terms of adverse effects, following are the target organs for MultiHance toxicity:

Brain (convulsions, hypoactivity, slowing & transient flattening (?) of EEG), liver (necrosis, vacuolation, changes in liver enzyme levels, histiocytosis, increased liver weights), kidney (vacuolation, increased kidney weights, changes in urinary electrolyte levels, polyuria), pancreas (vacuolation) and testes (vacuolation and abnormal spermatogenic cells). In addition, EKG effects were not properly evaluated (QT interval).

The safety margin for adverse effects was 0.3-3 times clinical dose. The safety margin in terms of lethality ranged from 2 to 8 (maximum non-lethal dose/clinical dose of 0.2 mmol/kg, based on body surface area). This effect may be exaggerated due to high osmolality of the solution in animals compared to humans. However, no positive control group (hyperosmotic mannitol/sucrose solution) was included in these studies to confirm it.

Local tolerance studies suggest that local reaction is likely after accidental extravasation of MultiHance during clinical administration

[redacted]

Pharmacology and Toxicology External Comments:

The NDA application for MultiHance contained all the major pharmacology and toxicology sections. However, following pre-clinical issues need to be addressed to fulfill the PharmTox requirements for the NDA:

1) *Safety pharmacology studies:* We recommend a comprehensive safety pharmacology study in bigger species to address various deficiencies noted below. 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 using hyperosmotic control group (sucrose/mannitol solution) and Magnevist for comparison purpose.

a) 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.
b) 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.
c) Most of the adverse effects were attributed to hyperosmolarity of the solution but studies did not include hyperosmotic control group (mannitol/sucrose solution) to attribute these effects to hyperosmolarity.
d) 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.

2) *Effect on Blood brain barrier permeability:* We recommend a safety pharmacology study to evaluate effects of MultiHance on blood brain barrier (BBB) permeability in BBB damaged animal model at clinically equivalent and higher dose levels.

3) *Electrophysiological studies:* In humans, gadolinium class of contrast agents can cause prolongation of QT interval resulting in cardiac arrhythmia. We recommend that the sponsor conduct an *in vitro* electrophysiological study evaluating effects on cardiac action potential or potassium channels for MultiHance.

4) *Effect on EEG:* MultiHance (4 mmol/kg) caused transient flattening (for 2 min) of EEG in conscious rats. How is it possible to have brain electrical activity completely stopped in a conscious animal even though it is a transient effect? Please explain.

5) *Expanded single dose studies:* We recommend a systematic expanded acute dose study in at least one species to adequately establish the toxicity profile for MultiHance. The study should

be conducted in bigger animals to avoid the high osmotic load problem seen in small animals. 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 a comparative controls. Various toxicity parameters should be evaluated 72-hours post-dosing, and also after 7/14-days recovery period. This recommendation is based on the following deficiencies/ concerns noted in the acute dose studies:

a) No comprehensive expanded acute dose toxicity studies evaluating all necessary parameters (such as hematology, clinical chemistry, urinalysis, complete histopathology etc.) were conducted. LD_{50} studies can not be substituted for the expanded single dose studies.
b) Low safety margins in terms of lethality after acute dose administration.
c) The lethality and adverse effects were attributed to high osmotic load in animals. However, in the absence of positive control group (hyperosmotic mannitol solution), there is no evidence that all of the adverse effects are solely due to hyperosmolarity, and not due to drug itself.
d) MultiHance is clinically intended as a single dose administration agent. Therefore, it is important to establish adequate safety profile of MultiHance after single dose administration.

6) *Reproductive toxicity studies:* Segment II study in rats was inadequate. We recommend a Segment II reproductive toxicity study in rats using doses of MultiHance (0.5 M) where some maternal toxicity is observed.

7) *Male fertility studies:* No adverse effects were reported on male fertility at doses up to 2 mmol/kg. However, in repeat dose toxicology study in rats, MultiHance (3 mmol/kg/day) produced irreversible effects on spermatogenic cells. This suggests that effects on male fertility are likely. Additionally, at 3 mmol/kg, there were other toxicities such as effects on the stomach, kidneys and liver. For proper assessment of reproductive risk, the male fertility study should have been carried out at higher dose levels where some maternal toxicity is observed. We recommend a segment I reproductive toxicity study in order to evaluate the effects of 0.5 M MultiHance on fertility parameters in males. This study should be conducted at doses that produce maternal toxicity.

8) *Mutagenicity studies: In vivo* micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than intravenous administration. According to the ICH guidelines, this study should be carried out using intravenous administration. Also dose levels used in this study were not high enough. Please explain reasons for choosing intraperitoneal route specifically for this particular study. We recommend an *in vivo* micronucleus assay using intravenous route and various dose levels of MultiHance (with higher dose multiples).

9) *Local tolerance studies:* These studies suggest that local reaction is likely after accidental

extravasation of MultiHance. Histological evaluation was carried out 8-days post-dosing. If these local adverse effects are even more severe during first 24 hours can not be determined from the present study. Additional 24-hr time point should have been included for the histological evaluation. In humans, hyperosmotic gadolinium contrast agents can cause phlebitis, thrombophlebitis and faciitis in some cases. In view of the clinical relevance of these studies, we recommend a local tolerance study (intravenous, paravenous & intramuscular administration) with histological evaluation after 24 hours and at later time points, until the local adverse effects are resolved.

10) *PK studies:* Stability of gadolinium chelate/complex is of concern. Free gadolinium ion was detected in feces of rats and dogs (~6%). Also in biodistribution study, significant retention of radioactivity was observed in bone (~3% ID). This retention was attributed to the impurities in the formualtion that according to your conclusion are not present in the clinical formulation. Please provide data supporting this conclusion.

11) *Effects on coagulation parameters (in vivo):* No effects on coagulation parameters were reported *in vivo*. However, this study was conducted at a dose of 0.25 M MultiHance that was equivalent to 0.2-times the clinical dose, based on body surface area conversion (0.25 mmol/kg). In an *in vitro* study, at higher doses, there were significant increases in PT & PTT and concomitant decreases in fibrinogen. This indicates that MultiHance has the 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.

12) *Vacuolation in pancreas:* In a repeat dose study in monkey, MultiHance (3 mmol/kg) caused vacuolation of islet cells in the pancreas. Does this affect pancreas function? Please comment.

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/s/

Tushar Kokate
2/8/02 03:19:51 PM
PHARMACOLOGIST
MultiHance: Primary Pharmacology & Toxicology Review

Nakissa Sadrieh
2/8/02 03:40:07 PM
PHARMACOLOGIST

Supervisory Pharmacologist Memo

NDA: 21-357
Drug: MultiHance
Sponsor: Bracco

MultiHance (Gadobeneate dimeglumine) is an injectable ionic, linear, gadolinium contrast agent intended for use in MRI. The indication includes the diagnostic assessment of lesions with abnormal vascularity and lesions causing abnormality in the blood brain barrier in adults. The maximal clinical dose to be used is 0.2 mmol/kg. The osmolarity of 0.5 M MultiHance is approximately 7 times that of plasma.

There are currently four gadolinium contrast agents approved and on the market in the United States. Structurally, MultiHance shares certain characteristics of some the approved agents. Specifically, the sponsor has used Magnevist, another gadolinium agent that is currently on the market, as a comparator in many of the preclinical studies. Magnevist is also an ionic, linear, high viscosity, hyperosmolar gadolinium contrast agent. The purpose for comparing MultiHance with Magnevist is due to their similarities, and to highlight that the effects of MultiHance are not worse than those of the approved drug, Magnevist. Although this review is not the appropriate place to talk about Magnevist, it should be pointed out that the latter drug, being used by the sponsor as a comparator for safety, is associated with significant adverse events in postmarketing, such as phlebitis, thrombophlebitis, fasciitis and partial amputation of the upper limbs. Data on the postmarketting experience with Magnevist was recently (October 10, 2001) presented by Dr. Roger Li at a scientific rounds hosted by HFD-160.

The pharmacology and toxicology review of this NDA was done by Dr. Tushar Kokate. This supervisory memo is not intended to duplicate the review of Dr. Kokate. Rather, I will be pointing out the major deficiencies in this application, for the purpose of emphasis. It should be pointed out that the pharmacology/toxicology section of the NDA for MultiHance contained over 150 studies. Therefore, many of the studies which were conducted with the non-marketed formulations of MultiHance were not reviewed

The deficiencies will be listed according to sections of the application, as follows.

1. Safety Pharmacology:

Safety pharmacology studies were conducted to characterize the CNS and cardiovascular safety profile of MultiHance.

For the CNS studies, effects such as a decrease in motor activity, prolongation of phenobarbital anesthesia and flattening of an already

reduced EEG were seen following IV administration . These effects were seen in mice and rats at doses that were below the clinical dose, based on body surface area. MultiHance did not disrupt the blood brain barrier, nor did significant amounts cross an already disrupted blood brain barrier. Following intracisternal and intraventricular administration, the effects in the animals were more pronounced. Decreased activity, decreased motor coordination, convulsions and death were reported. These effects were seen starting from 0.06 mmol/kg, with death and convulsions and death reported at 0.1 and 0.3 mmol/kg.

For the CVS studies, the major findings included increases in cardiac output and stroke volume and transient increases in renal and pulmonary blood flow. These effects were seen in anesthetized rabbits at 1 mmol/kg. At this dose, no effects on blood pressure and heart rate were reported, however, at doses above 4 mmol/kg, there were decreases in heart rate and left ventricular end diastolic pressure, and at 8 mmol/kg the anesthetized micropigs died from hypotension. In a pig model with myocardial ischemia, there were dose-dependent transient decreases in heart rate, blood pressure, left ventricular pressure and systemic vascular resistance at 2-3 mmol/kg, with a concomitant increase in cardiac output and stroke volume. The NOAEL for these above effects was 1 mmol/kg, which is about 4 times the clinical dose, based on body surface area conversion. In vitro, there were decreases in contractile force of rat papillary muscle and decreased amplitude and frequency of atrial contractions.

Many of the safety pharmacology included Magnevist as a comparator, and it was reported that the effects were similar for both drugs. Many of the effects were attributed to hyperosmolarity of MultiHance, however, in the studies that included mannitol as a hyperosmolar control, the effects seen with mannitol were less pronounced, indicating that the hyperosmolarity did not account for the all the effects seen.

These safety pharmacology studies are however considered deficient for the following reasons:

1. In most of the studies, only one dose of MultiHance was used (1 mmol/kg).
2. The doses used were too low to provide and adequate margin of safety.
3. No dose response curve could be established.
4. ECG monitoring was not adequate: no continuous monitoring during dosing and no QT interval evaluation.

The sponsor will be requested to conduct an adequate safety pharmacology to assess the CNS effects of MultiHance, using several doses, in order to establish a dose response curve and to provide an adequate margin of safety, based on conversion to body surface area. The sponsor will also be asked to conduct an in

vitro study to assess effects on cardiac electrophysiology; specifically effects on potassium channels and action potential duration should be evaluated.

2. Toxicology

Acute toxicology:

Several single-dose IV LD50 studies were conducted in rats and mice. These studies are no longer accepted as acute dose toxicity studies, however, since they were included in the submission, they were reviewed. In general, these studies were not acceptable, since a) they did not provide a NOAEL, b) a low safety margin was reported for lethality (2-6 times the human dose), c) the sponsor did not include a control group using a hyperosmolar drug, yet the sponsor reasoned that the effects were due to hyperosmolarity, and d) there was an indication that the dose response curve would be very steep, with the effect noted in animals being prostration, dyspnea, convulsions and lung congestion in most of the animals.

Similar studies were conducted in mice and rats in order to determine the intracisternal LD50. The results indicated that the effect of intracisternal MultiHance were more pronounced at lower doses, as compared to IV administration.

The NOAEL for proximal renal tubule vacuolization in rats was 0.5 mmol/kg, which is 0.5 times the clinical dose based on body surface area.

In rabbits, the MTD was reported to be 4 mmol/kg (5x the human dose), in dogs, the MTD was reported to be 2 mmol/kg (5x the human dose) and in cynomolgus monkeys, the MTD was reported to be 6 mmol/kg (15x the human dose). All these MTDs were associated with significant toxicities (ranging from dyspnea to liver and kidney toxicity), and should not be considered as equivalent to a NOAELs.

Repeat dose toxicology

In a repeat dose study in rats dosed with MultiHance for 28 days at 0.3, 1 and 3 mmol/kg/day, there were effects noted in several organs. Specifically, at all dose levels, lesions in the veins of all treated animals were reported, including scabbing/ulceration, thrombi, perivascular inflammation and fibrosis and vein obliteration), vacuolization of the cortical tubular epithelial cells of the kidneys with accompanying increased kidney weight. At 1 and 3 mmol/kg/day, there were plasma and urinary electrolyte imbalances, increased water consumption, mineralization of the corpus region of the stomach, vacuolization of the epithelial cells of the bladder. These effects were not recovered in the high dose group after 4 weeks recovery. In the high dose group, there was hepatocyte necrosis with inflammatory cells in the liver, degeneration of spermatogenic cells and epithelial

degranulation of interlobular ducts of salivary glands. There was no NOAEL established for this study. The dose multiples used range from 0.3 to 2.5 times the human dose based on body surface area conversion. Based on some of the effects seen, such as liver necrosis, it is clear that MultiHance is not an extracellular fluid or blood pool imaging agent. MultiHance has the capacity to enter cells and cause significant toxicity. This is different from some of the other gadolinium contrast agent which are limited to the extracellular compartment. The effects on the kidneys indicate that in addition to histopathological changes, there are functional changes in the kidneys. These lead to alterations in plasma electrolyte balance, which could in fact affect cardiac function. Possible consequences of electrolyte imbalance were not discussed by the sponsor.

In a 4-week repeat dose study in dogs dosed with 0.25, 0.5, 1 and 2 mmol/kg/day MultiHance, similar findings as in the rat repeat-dose study were noted. However, in addition to the effects in the liver and kidneys (histopathological changes as well as increased liver enzymes and decreased electrolytes in the urine), bone marrow hypocellularity was observed. In the rat study this was not seen, since histopathology of bone smears was not conducted. This may indicate that free gadolinium was released from MultiHance and deposited in the bone marrow. The NOAEL for the repeat dose study in dogs was 0.25 mmol/kg (0.6 times the clinical dose).

In a 14-day repeat dose study in cynomolgus monkeys dosed with 0.25, 1 and 3 mmol/kg/day (0.4, 1.7 and 5 times the clinical dose), findings similar to those in rats and dogs were reported in the liver and kidneys. But the severity of the effects in the liver was not as great as in rats and dogs, since no effects on liver enzymes were reported. However, there were also effects such as vacuolization of the islet cells of the pancreas. The study in the monkeys did not contain a recovery group as in the rats and dogs, and urinalysis did not include electrolyte levels. Interestingly, zinc levels were reported to be decreased in the plasma in the mid and high dose groups. This could indicate possible transmetallation of the gadolinium. Again, this could be evidence for the release of free gadolinium from the MutiHance complex, into the plasma and tissues. The NOAEL in monkeys was set at 0.25 mmol/kg/day (0.4 times the clinical dose).

Therefore, repeat-dose treatment with MutiHance in rats, dogs and monkeys revealed similar toxicities to the liver and kidneys, with possibly lower sensitivity in the monkeys. There were however certain organs that were affected in some species and not others. Some of the interesting findings included the decreased bone marrow cellularity in dogs and the decreased zinc levels in monkeys. Both of these effects indicate possible transmetalation of the gadolinium and possible release of free gadolinium in the plasma and tissues. This remains a safety concern. Another interesting finding is the effect on the liver, where liver necrosis was noted, accompanied by liver enzyme increases. This indicates that MultiHance does not remain in the extracellular compartment, like some of the other gadolinium contrast agent. The sponsor did not include in the toxicology

studies, a control group using Magnevist or any other approved gadolinium contrast agent. Such a control group would have shown if the effects noted were due to MultiHance, hyperosmolarity or gadolinium contrast agents in general. In the absence of such data, all the effects reported have to be considered a being due to MultiHance.

The repeat dose toxicology studies were nevertheless considered adequate. However, the acute toxicology studies were considered deficient, since there were no expanded acute studies, rather the sponsor submitted MTD and LD50 studies. These studies showed significant lethality with a low safety margin for lethality in mice, rats and dogs, but not in monkeys. No NOAELs were established and no sense of a dose response curve was provided. These types of studies are no longer acceptable as acute toxicology studies. As elaborated in Dr. Kokate's review, it is recommended that the sponsor conduct an adequate expanded acute toxicology study, using appropriate doses that cover a wide range and provide a NOAEL, adequate safety margins and a dose response curve. In the proposed expanded acute study, the sponsor is a advised to use a hyperosmolar control as well as Magnevist and possibly other approved gadolinium contrast agents.

3. PK studies

Several PK studies were conducted in rats, mice, rabbits, dogs, and monkeys. The dose multiples tested in most of the studies were well below the maximal clinical dose, based on body surface area conversion. The reported t ½ for elimination of the drug were:

Rats: 15-20 minutes
Dogs: 90-175 minutes
Mice: 30 minutes

In rats, biodistribution studies showed that the main organs where radioactivity from 153-Gd-gadobenate Dimeglumine accumulated were the GI, liver, kidney and bladder. In rats, there was accumulation of radioactivity in the femoral bone, which remained constant from 8-28 days. Interestingly, there was hypocellularity reported upon histopathological evaluation of dog bone marrow, however, such histopathological evaluation of the bone marrow was not done in rats. Therefore it is unclear if the radioactivity detected in the rat bone marrow was from free gadolinium or parent drug, and whether it could have lead to toxicity such as hypocellularity. It is possible that free gadolinium accumulation in the bone marrow would result in hypocellularity.

In rats, excretion in the feces was significant, ranging from 40-50% of the injected dose. Of the amount excreted in the feces of rats, it was reported that 16% was free gadolinium. Similar results were found in dogs. This accounts for

a significant amount of free gadolinium, which is considered to be a toxic metal. In general, over 90% of the drug is eliminated within the first 24 hours after administration.

In a single dose biodistribution study in rats, using a very low dose (0.2 times the maximal clinical dose based on surface area) of 153-Gd-gadobenate dimeglumine, after 28 days of dosing, 1% if the injected dose remained in the body, of which 0.7% was retained in the bone. It is likely that the radioactivity remaining in the bone was due to free gadolinium, and not the parent drug. However, this was not confirmed by the sponsor.

In general, the elimination of MultiHance is slower than the elimination of Magnevist which is excreted only in the urine, and within 6 hours, only 1% of the injected dose is remaining in the body.

In general, the elimination of MultiHance was similar in all the species studied. Therefore, elimination in the urine and feces were more or less equivalent across species. This data was in agreement with the biliary excretion data reported by the sponsor. In rats, 50% of the drug was eliminated in urine and 50% in the bile, with only 5% undergoing enterohepatic recirculation. Also, data in rats with defective hepatic excretory function showed that in the absence of biliary excretion, the kidneys would compensate and eliminate all the drug. No effect on liver cytochrome P450 enzymes were reported.

The sponsor also studied the biodistribution of MultiHance in pregnant rats. There was accumulation of radioactivity in the placenta that accounted for 0.35% of the injected dose, however only 0.02% of that was reported to pass to the fetus. However, it should be pointed out that these studies were performed at doses of the drug that were below the recommended human dose (0.5 mmol/kg). The sponsor therefore concluded that the placental diffusion to the fetus was very low. In a milk transfer study, the sponsor found that 0.5% of the injected dose of the drug was in the neonates, of which 75% was in the GI. This indicates that the drug can get excreted in milk. However the quantification of this milk excretion is not possible based on the data provided. Additionally, the doses used were too low, as compared to the recommended human dose.

4. Genetic Toxicology:

Genotoxicity studies included mutation assays in Salmonella typhimurim strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100, in E. coli CM891, in Saccharomyces cerevisiae. A chromosome aberration assay in cultured human lymphocytes, an unscheduled DNA synthesis assay in HeLa cells and a gene mutation assay in V79 Chinese Hamster Lung Cells were also conducted. All these assay were negative for genotoxicity. An in vivo rat micronucleus assay was conducted, using an intraperitoneal injection rather than an IV injection.

The results of the test were negative, however the route of administration is not considered the appropriate route of exposure. In this regard, the rat micronucleus test is not considered adequate and may need to be repeated.

5. Reproduction toxicology:

Reproduction toxicology studies were conducted in rats and rabbits. In general, the studies were done with doses of MultiHance that were no high enough, since in most studies, maternal toxicity was not seen and therefore potential toxic effects could not be assessed. In rats, the highest dose used in the segment 1, 2 and 3 studies was 2 mmol/kg, which corresponds to 1.7 times the maximal clinical dose. At that dose, no effects on fertility were reported, and there were no effects on embryo-fetal development or post-natal development. However, in toxicology studies, MultiHance was tested at 3 mmol/kg, and at that dose, there were irreversible effects on spermatogenic cells, which would no doubt have lead to effects on fertility. Additionally, at 3 mmol/kg, there were other toxicities such as effects on the stomach, kidneys and liver. No such effects were reported in the dams at 2 mmol/kg. In fact, one of the deficiencies of the reproduction toxicology studies is that maternal toxicity was not reached. This precludes proper assessment of reproductive risk and does not provide an adequate design for the reproduction toxicology studies. Interestingly, in a fertility and embryo-fetal toxicity study in rats using a not-to-be marketed strength of MultiHance (0.25 M instead of 0.5 M), doses up to 1.5 mmol/kg were used (1.3 times the maximal clinical dose). At that dose, 2 F1 fetuses had monolateral microphthalmia and a lower probability of survival compared to controls. These effects were not seen in the other fertility and embryo-fetal development rat studies using a 0.5 M strength of MultiHance at 2 mmol/kg.

In rabbits, a segment 2 study was conducted using doses of MultiHance up to 2 mmol/kg (3.3 times the maximal clinical dose). The maternal and fetal NOAEL was reported to be 0.3 mmol/kg (0.3 times the maximal clinical dose, based on body surface area). Maternal toxicity was characterized by decreased body weight, food consumption and a local reaction to the injection at the 0.9 and 2 mmol/kg doses. The fetal toxicity was characterized by retinal irregularities, additional or fused sternebral centers, cervical ribs and offset pelvic girdles. These effects were also seen at the 0.9 and 2 mmol/kg doses.

So in summary, the rat reproduction toxicity are not considered adequate and the rabbit study showed signs of fetal toxicity. The drug also has potential for fertility effects in males, based on the repeat dose toxicology studies. These findings will be reflected in the label. Regarding the rat studies, it is recommended that a segment 2 study be repeated, using doses of MultiHance that are significantly higher than those used in the present study. The purpose would be to obtain maternal toxicity and provide an adequate margin of safety which would be reported in the label. Additionally, a segment 1 study is also recommended in order to evaluate the effects of MultiHance on fertility

parameters in males. This study would have to be conducted at doses that are higher than those used in the present fertility study, and even those used in the repeat dose toxicology study (3 mmol/kg). In the repeat dose toxicology study in rats, irreversible effects on the spermatogenic cells were reported.

6. Immunotoxicology studies:

Three immunotoxicology studies were conducted. Two of the studies were in vitro and one was in vivo. In vivo, guinea pigs were tested for active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA). Gadobenate dimeglumine was found to be negative in both the ASA and PCA tests, indicative that the drug had a low likelihood to induce an antigenic response.

In vitro, gadobenate dimeglumine was tested for its potential to induce mast cell degranulation and histamine release. It was found that at 250 mM and not 125 mM, gadobenate dimeglumine induced both mast cell degranulation and histamine release. The results were similar to those obtained with Magnevist and a hyperosmolar solution of sucrose. Therefore, it is likely that the effect was caused by the hyperosmolarity of the drug. The 125 mM concentration of gadobenate dimeglumine was reported to be equivalent to 250 times the clinical plasma concentration of the drug, therefore, the effect is not likely to occur in the clinical setting. The sponsor also studied the in vitro effect of gadobenate dimeglumine to cause complement activation, using sensitized sheep red blood cells. There was a concentration effect reported, with an IC50 for the activation of complement that was equivalent to 15 times the maximal level expected to be reached in the clinical setting, using a 0.2 mmol/kg dose of MultiHance.

MultiHance is therefore not expected to induce an antigenic response. Additionally, even though results showed that MultiHance can induce mast cell degranulation and histamine release, as well as complement activation, these effects are not likely to occur at concentrations of MultiHance that are achieved systemically with clinical use of the drug.

7. Special Toxicology studies:

Several special toxicology studies were conducted to determine the local tolerance, hemolytic and erythrocyte deformability potential and the effects on coagulation parameters of MultiHance.

IV injection of MultiHance produced mild irritant effects, whereas paravenous and intramuscular administration produced moderate to severe irritant effects in rabbits, characterized by cell infiltrates and necrosis. These effects were more severe than those observed with Magnevist. In light of the concern regarding the potential for Magnevist to cause phlebitis and thrombophebitis, there is reason to be concerned about these irritant effects of MultiHance, especially since the sponsor reports that these effects are more severe than with Magnevist.

MultiHance did not have any hemolytic potential with human blood, and the effects on red blood cell deformability reported were due to the hyperosmolarity of the solution, as shown with a hyperosmolar control.

Regarding effects on coagulation parameters, there were no findings in an in vivo study in rats. However, the study was conducted at a dose of MultiHance that was equivalent to 0.2 times the clinical doe, based on body surface area conversion (0.25 mmol/kg). Similarly, in an in vitro study where PTT, PT and fibrinogen were assessed in rats, there were no significant effects at a dose that corresponded to 0.4 times the maximal clinical dose, or 3 times the maximal plasma levels achieved in clinical studies. However, at both 1 and 2 times the maximal clinical dose (or 6 and 12 times the equivalent plasma concentration), there were significant increases in PT, PTT with a concomitant decrease in fibrinogen. This indicates that MultiHnace has the potential to affect coagulation parameters, at higher doses.

In conclusion, MultiHance has a significant irritation potential, and based on the in vitro results, there may be some concerns regarding effect on coagulation parameters. Since the in vivo study in rats was not adequate to assess this effect, one cannot conclude about possible effects on coagulation based on in vivo effects. It might be prudent to ask to the sponsor to conduct an in vivo study, using high doses of MutiHance, in order to determine the effects of the drug on coagulation parameters and bleeding time.

Overall evaluation:

The application for MultiHance contains the major sections usually submitted in NDAs. However, there are major deficiencies which are listed above and in much more detail in Dr. Kokate's review. With regards to pharmacology and toxicology, the drug is considered **not to be approvable** at this time. There are several studies that the sponsor will be requested to conduct. These studies are listed in Dr. Kokate's review, under "Recommendations".

The following are the studies that I believe should be considered as necessary prior to the approval of MultiHance:

1. A safety pharmacology to assess the CNS toxicity of MultiHance in an animal model with a disrupted BBB, using several doses, in order to establish a dose response curve and to provide an adequate margin of safety, based on conversion to body surface area.
2. An in vitro study to assess the effects of MultiHance on cardiac electrophysiology; specifically effects on potassium channels and action potential duration. An in vivo cardiovascular is also recommended to assess possible effects on QT prolongation following continuous ECG monitoring.

3. An expanded acute toxicology study, using appropriate doses of MultiHance that cover a wide range and provide a NOAEL, adequate safety margins and a dose response curve. In the proposed expanded acute study, the sponsor is a advised to use a hyperosmolar control as well as Magnevist and possibly other approved gadolinium contrast agents.
4. A mouse micronucleus study using an IV injection of MultiHance, instead of an IP injection of the drug, as was used in the present micronucleus study.
5. A segment I fertility study in rats, using at least one dose of MultiHance that is higher than 3 mmol/kg (and including a dose equal to 3 mmol/kg, as in the repeat dose toxicology study).
6. A segment II embryo-fetal development study in rats using doses of MultiHance where maternal toxicity is seen.
7. A study assessing effects on coagulation parameters and bleeding time in an in vivo animal model.

Additional comments regarding the PK studies, the effects on the EEG and the bone marrow histological evaluation of rat bone marrow, and listed in Dr. Kokate's review, should also be conveyed to the sponsor.

/S/

Nakissa Sadrieh, Ph.D.
Supervisory Pharmacologist

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/s/

Nakissa Sadrieh
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