CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-357

21-358

PHARMACOLOGY REVIEW

Supervisory Pharmacologist Memo

NDA: **21-357/21-358**
Drug: **MultiHance**
Sponsor: **Bracco**

MultiHance (Gadobenate dimeglumine) is an injectable ionic contrast MRI agent proposed for magnetic resonance imaging of the CNS to visualize lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues. The proposed dose is 0.1 mmol/kg (0.2 mL/kg) for — adults ——— Dr. Tushar Kokate reviewed the original NDA and concluded that the NDA is approvable subject to satisfactory completion of recommended preclinical Pharmacology/Toxicology studies prior to approval. The sponsor addressed all outstanding preclinical issues in this resubmission dated 10/14/2004, and reviewed by Dr. Ouyang.

Based on this latest review, Dr Ouayng's recommended that the NDA be approved, and that no further preclinical Pharmacology/Toxicology study is required. Please see Dr. Ouayng's review for details.

The sponsor conducted the following studies for this review cycle:

1. Cardiovascular safety pharmacology studies in Cynomolgus Monkey evaluating a battery of cardiovascular parameters.
2. *In vitro* electrophysiological studies evaluating effects on potassium channels.
3. Respiratory safety study in rats
4. An *in vivo* rat micronucleus assay.
5. Local tolerance study.

The cardiovascular safety study in Cyanomolgus Monkeys addressed the potential risk of QT prolongation with MultiHance use. MultiHance had no significant effect on arterial blood pressure and heart rate. Moreover, QT_c intervals (Bazett's or Fredericia's correction) were not significantly modified by MultiHance (3mmol/kg; dose multiple: 10 fold). The *in vitro* electrophysiological study showed that MultiHance, Mannitol (osmotic-matched control), and Omiscan (a marketed Gadolinium) all produced significant inhibition of HERG tail current. Dose response relationship was difficult to establish. Given the potential disruptive effect of high osmolarity on cell membrane integrity, the inhibitory effect on HERG current is perhaps not surprising. It is pertinent to note that no significant inhibition of HERG current occurred at anticipated maximal plasma concentration of Multihance. In a study, evaluating the effect of MultiHance on action potential parameters in dog isolated Purkinje fibers, MultiHance at up to 50 mM had no significant effects on action potential duration, maximum rate of depolarization, upstroke amplitude, and resting membrane potential. Taken together, the sponsor concluded and Dr. Ouyang concurred that no clear evidence of QT prolongation was seen with MultiHance use in these preclinical studies.

The respiratory safety pharmacology study in rats highlighted the impact of rate of IV administration and dose on adverse events occurrence (table) in a small animal model. IV administration of MultiHance at 4 mmol/kg resulted in immediate death of animals when administered at rate of 2 mL/min (1/5) or 6 mL/min (2/2). MultiHance at 2 mmol/kg caused breath ceasing when administered at 6 mL/min. No animal died or had breathing difficulties in either mannitol (osmolarity control) or omniscan (gadolinium class control) groups. Therefore, one may conclude that the effect of MultiHance on respiratory parameters in rats may be intrinsic

Effects of MultiHance and IV rate on respiration

	MultiHance [mmol/kg (dose multiples)]						Omniscan [mmol/kg (dose multiples)]		Mannitol	
	4 (6.5)		2 (3.2)		1 (1.6)		4 (6.5)			
	IV rate (ml/min)		IV rate (ml/min)		IV rate (ml/min)		IV rate (ml/min)		IV rate (ml/min)	
	6	2	6	2	6	2	6	2	6	2
Death	2/2	1/5	0	0	0	0	0	0	0	0
Breath ceasing	N/A	-	+ *	-	-	-	-	-	-	-
Transient increase in RR, TV, or MV (+)	N/A	+	+	+	+	+	+	+	+	+

* Breath ceasing for 23-49 seconds

to Multihance since it is not shared by either mannitol or omniscan. However, no death or breath ceasing was reported in the cardiovascular safety study in monkeys (3 mmol/kg (dose multiple: 10) at 6 mL/min. For the rat study, Dr. Ouyang concluded that the results clearly revealed the potential risk associated with administration of high doses of MultiHance especially when administered rapidly in a small animal. She opined that potential severe respiration risk associated with MultiHance administration in humans may be relative small in view of lack of respiratory problems in the monkey study and the fact that in clinical trials, potential respiration related observations such as dyspnea and hyperventilation occurred only in low incidence (<0.5%, regardless of causality). Nevertheless, she recommended that caution should be exercised when MultiHance is administered as a rapid bolus injection. I agree with her conclusions.

MultiHance showed no evidence of clastogenicity in an in vivo bone marrow micronucleus assay.

The local tolerance studies conducted using intramuscular, intravenous or perivenous routes of administration showed that intramuscular injection produced no local reaction (unlike outcome of a study submitted with the original NDA) where intramuscular administration produced local irritant effects characterized by hemorrhage, edema, cellular infiltration, degeneration and necrosis of muscle fibres. Both intravenous and perivenous routes caused mild to moderate but reversible local reactions including erythema, edema, hemorrhage, and mild inflammatory cell infiltration. Perivenous route produced a more severe reaction. In a study reviewed by Dr. Kokate during the first review cycle, he noted that perivenous administration produced moderate to severe irritant effects characterized by reddening, thickening, inflammatory cell infiltrates, eschar and large areas of necrosis. According to Dr. Kokate "these adverse local reactions were qualitatively more pronounced than Magnesvist® (a marketed gadolinium)." He opined that local reaction is likely after accidental extravasation of MultiHance during clinical administration and those effects maybe somewhat more adverse than Magnevist®.

Magnevist® package insert describes cases of phlebitis and thrombophlebitis (in rare cases potential to develop thrombotic syndromes, thrombosis with fascilitis and surgical intervention) associated with Magnevist® injection. The degree to which local extravasation inflammatory reactions contributed to these cases is not known. However, given similar extravasation reaction patterns shown by both MultiHance and Magnevist®, I recommend that in the absence of definitive clinical data to the contrary for MultiHance, that appropriate language be included in MuliHance proposed package insert describing results from preclinical local tolerance studies.

Overall Evaluation:

The sponsor has successfully addressed all outstanding preclinical Pharmacology/Toxicology issues. I concur with Dr. Ouyang's recommendations that the application be approved and that no further pre-clinical Pharmacology/Toxicology study is required prior to approval.

Adebayo Laniyonu, Ph.D.

Supervisory Pharmacologist

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/s/

Adebayo Laniyonu
4/6/04 11:55:26 AM
PHARMACOLOGIST

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

NDA number: 21-357, 21-358

Review number: 02

Sequence number/date/type of submission:

Designation	Letter Date	Stamp Date	Content
N 000 AZ	OCT-14-2003	OCT-14-2003	Resubmission
N 000 BP	FEB-17-2004	FEB-18-2004	Response to Pharm/Tox comments

Information to sponsor: Yes ( ) No (X)

Sponsor and/or agent: Bracco Diagnostics Inc.,
Princeton, NJ 08543

Manufacturer for drug substance: Altana Pharma AG,
78224 Singen, Germany

Reviewer name: Yanli Ouyang, MD, PhD

Division name: Medical Imaging and
Radiopharmaceutical Drug Products

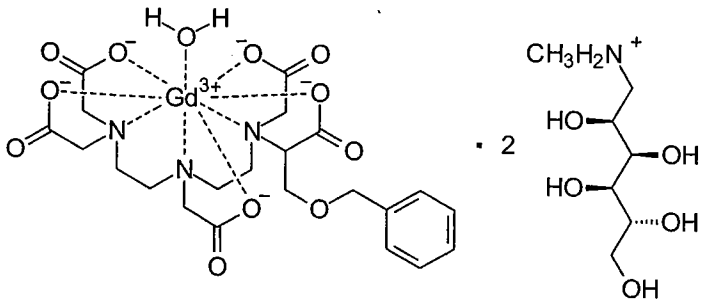
HFD #: 160

Review completion date: March 3, 2004

Drug:

Trade name: **MultiHance**
Generic name: Gadobenate dimeglumine
Code name: Gd-BOPTA/Dimeg, B19036/7, E7155
Chemical name: (4RS)-[4-carboxy-5,8,11-tris(carboxymethyl)--1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5-)} gadolinate(2-) dihydrogen compound with 1-deoxy-1-(methylamino)-D-glucitol (1:2)
CAS registry number: CAS 127000-20-8
Molecular formula/molecular weight: $C_{22}H_{28}GdN_3O_{11}.2C_7H_{17}NO_5$/1058.2

Structure:



Relevant INDs/NDAs/DMFs: IND 43,779

Drug class: **MRI Contrast Agent**

Indication: For intravenous use in magnetic resonance imaging (MRI) of the central nervous system (CNS) to visualize lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues.

Clinical formulation (0.5 M): Gd-BOPTA. water for injection: qs to —— The osmolality of the solution is 1970 mOsmol/kg at 37°C and 6.9 times that of plasma (285 mOsmol/kg). pH of the solution is 6.5-7.5.

MultiHance for injection is supplied as a sterile, non-pyrogenic solution and contains 529 mg of gadobenate dimeglumine per mL. The product will be supplied as a vial with a total volume of 5, 10, 15, or 20 mL.

The sponsor conducted required pharmacology and toxicology studies using current 0.5 M clinical formulation.

Route of administration: Intravenous (rapid infusion or bolus injection)

Clinical dose: Adults : 0.1 mmol/kg (0.2 mL/kg)

Maximum clinical dose = 0.1 mmol/kg

Disclaimer: The review is mainly based on the information contained in this NDA resubmission. They will be identified by statement if directly adopted from the NDA.

Studies reviewed within this submission:

Study # Vol # (page #)	Study Type	Species	Lot #	Dose, mmol/kg (Dose Multiples)	Review Page
886/026 21 (7)	Core battery of CVS	Cynomolgus monkeys	S2259	0.3, 1, 3 (1, 3.2, 10)	6
DGMH1002 22 (144)	HERG Tail Current	HEK 293 cells with HERG	S2259		9
DGMH1004 23 (1)	Action potential parameters	Beagle dog Purkinje fibers	S2259		12
DGMH1003 22 (1)	Respiratory parameters	Sprague-Dawley rats	S2259	1, 2, 4 (1.6, 3.2, 6.5)	15
KFF 009/024207 23 (180)	Micronucleus test	CD rats	S2259	1, 2, 4 (1.6, 3.2, 6.5)	20
KFF 011/023877 23 (111)	Intravenous local tolerance	New Zealand white rabbits	S2259		23
KFF 013/024149 23 (134)	Perivenous local tolerance	New Zealand white rabbits	S2259		25
KFF 012/024148 23 (157)	Intramuscular local tolerance	New Zealand white rabbits	S2259		27

Studies <u>not</u> reviewed within this submission: None

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: **Approval**

1.2 Recommendation for nonclinical studies: None

1.3 Recommendations on labeling: Pending

2. Summary of nonclinical findings

MultiHance is a gadolinium compound for intravenous use in magnetic resonance imaging (MRI) of the central nervous system (CNS) to visualize lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues. The proposed dose is 0.1 mmol/kg (0.2 mL/kg) for — adults. ———

2.1 Brief overview of nonclinical findings

The original NDA was received on April 27, 2001. The agency in an action letter dated May 24, 2002 informed the sponsor that the application was approvable for aforementioned CNS indication. Certain pre-clinical safety issues such as cardiovascular safety were not adequately addressed in the original application necessitating the conduct of new studies to address these issues. The findings from these new studies are briefly summarized below.

Cardiovascular safety pharmacology studies

In the original NDA submission, the following deficiencies were noted in the cardiovascular safety pharmacology studies:

1) Using low dose multiples (0.3 to 3.0 times the maximum human dose);
2) Single dose in most of the studies;
3) No continuous ECG recording and QT interval report;
4) Attributing adverse effects noted in safety pharmacology studies to hyperosmolality of MultiHance without adequately including a hyperosmotic control.

In response, the sponsor conducted three complementary studies to evaluate the CVS safety. These studies included:

1) Core battery of CVS studies in conscious Cynomolgus Monkey monitored by telemetry;
2) HERG tail current study in stably transfected HEK293 cells; and
3) Action potential parameter study in isolated dog Purkinje fibers.

These studies were conducted to mainly address the concern of potential risk of QT prolongation associated with MultiHance use. There is no evidence that MultiHance at up to 3 mmol/kg (dose multiple: 10 fold) induced QTc prolongation. In addition, MultiHance at up to 50 mM produced no significant effect on action potential parameters.

However, MultiHance induced a statistically significant inhibition of HERG tail current at 3, 10, and 50 mM (although not at 1 and 30 mM) when compared to the vehicle. No clear concentration-dependent relationship was noted. Of interest, this statistically significant inhibition elicited by MultiHance was no longer apparent when compared to their corresponding osmotic matched controls, indicating the hyperosmolality may contribute to this MultiHance-induced inhibition of HERG tail current. Furthermore, there was no significant HERG inhibition at 1 mM MultiHance, which is very close to calculated human maximal plasma concentration (1.35 mM at dose of 0.1 mmol/kg). Therefore, it is reasonable to predict that it is unlikely that MultiHance induces significant blockage of I_{kr} at intended clinical dose.

Taken together, no clear evidence of QT prolongation risk was associated with MultiHance use.

Respiratory safety pharmacology study

Respiratory safety study was conducted in anesthetized, spontaneously breathing rats upon FDA's request. IV administration of MultiHance at 4 mmol/kg resulted in immediate death of animals when MultiHance was administered at rate of 2 mL/min (1/5) or 6 mL/min (2/2). This dose is only 6.5-fold of intended clinical dose based on body surface area. MultiHance at 2 mmol/kg (dose multiple: 3.2) caused breath ceasing when administered at 6 mL/min, while transient increase in respiratory rate, tidal volume, and minute volume when administered at 2 mL/min. Only transient increase in respiratory parameters was noted at 1 mmol/kg MultiHance (dose multiple: 1.6), mannitol, and Omniscan (4 mmol/kg, dose multiple: 6.5) groups even at 6 mL/min. The results of this study clearly revealed the potential risk associated with administration of high doses of MultiHance especially when administered rapidly. However, no death or breath ceasing was reported in aforementioned monkey CVS safety study at doses up to 3 mmol/kg (dose multiple: 10) at 6 mL/min. Furthermore, in clinical trials, potential respiration related observations such as dyspnea and hyperventilation occurred only in low incidence (<0.5%, regardless of causality). Taken together, potential severe respiration risk associated with MultiHance administration in humans may be relative small even though MultiHance is recommended to be administered as a rapid IV infusion or bolus injection in PI. Nevertheless, caution should be exercised when the drug is rapidly administered to anesthetized patients.

Genetic toxicology study

In the original NDA submission, *in vivo* rat micronucleus assay was conducted using intraperitoneal rather than the intended intravenous route. In addition, the dose levels were considered to be inadequate.

In response to FDA's request, the sponsor conducted an *in vivo* bone marrow micronucleus assay. The results showed no evidence of clastogenicity when rats were exposed to up to 4 mmol/kg MultiHance (dose multiple: 6.5).

Local tolerance studies
In the original NDA submission, histological evaluation was not adequately performed.

In response to FDA's request, the sponsor conducted local tolerance studies using intravenous, perivenous, and intramuscular routes. Overall, MultiHance injection caused mild to moderate but reversible local reactions. The reactions included erythema, edema, hemorrhage, and inflammatory cell infiltration. The reactions were severer when MultiHance was injected by perivenous route than by intravenous route.

2.2 Pharmacologic activity: N/A

2.3 Nonclinical safety issues relevant to clinical use

Death and breath ceasing was observed in anesthetized rats at high doses of MultiHance (4 mmol/kg and 2 mmol/kg, respectively, dose multiple: 6.5 and 3.2, respectively) especially when the drug were administered rapidly (6 mL/min). Therefore, caution should be exercised when the drug is rapidly administered to anesthetized patients. MultiHance is recommended to be administered as a rapid IV infusion or bolus injection in PI.

Perivenous injection provoked severer local reactions than intravenous injection. Therefore, caution should be exercised to avoid local extravasation when intravenous administration of MultiHance.

Overall, the sponsor has adequately addressed pre-clinical issues in this resubmission, and therefore approval is recommended for MultiHance from pharmacology and toxicology perspective.

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

MultiHance (Gadobenate dimeglumine) is a gadolinium compound for intravenous use as a magnetic resonance imaging (MRI) contrast indicated for visualization of lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues. Gadobenate dimeglumine consists of an octadentate chelate of paramagnetic ion Gd^{3+}. The chelation of Gd^{3+} presumably results in substantially less toxicity than the free ion. A structural analog, Magnevist (gadopentetate dimeglumine injection), has been approved as a contrast enhancement agent with CNS indication.

MultiHance (0.5 M) has an osmolality of 1970 mOsmol/kg, which is 6.9 times that of plasma (285 mOsmol/kg), and is hypertonic under conditions of use. The osmolality of MultiHance is similar to Magnevist (1960 mOsmol/kg). The maximum clinical dose of MultiHance is 0.1 mmol/kg (0.2 mL/kg). It will be administered as a rapid intravenous infusion or bolus injection followed by saline flush of at least 5 mL. MultiHance was granted an approvable status in the original submission. The agency has requested additional preclinical safety pharmacology, genotoxicity, and local tolerance studies to further characterize the safety profile. This pharmacology/toxicology review summarizes and comments on these preclinical studies.

3.2 PHARMACOLOGY:

3.2.1 Brief summary

3.2.2 Primary pharmacodynamics: N/A

3.2.3 Secondary pharmacodynamics: N/A

3.2.4 Safety pharmacology

Neurological effects: N/A

Cardiovascular effects:

FDA request:

The dose multiples used for safety pharmacology studies ranged from 0.3 to 3.0 times the maximum human dose, and were inadequate for establishing a clear safety profile for MultiHance. In most of the studies, only one dose was utilized for safety pharmacology evaluation. Evaluation at various dose levels in the same study is necessary for proper comparison and establishment of a dose-response curve. The identified toxicities of concern were EEG slowing, motor incoordination, convulsions, and death. Adverse effects noted in safety pharmacology studies were attributed to hyperosmolality.

However, most of these studies did not include a hyperosmotic control group to determine if these effects were due to hyperosmolality. In a few studies where a hyperosmotic control group was included, some of these effects (e.g., slowing of EEG and amplitude) could not be attributed to hyperosmolality as they were not seen with the active control. Additionally, although MultiHance permeability through a damaged blood brain barrier was low, the dose level tested was too low to assess the risk (i.e., 0.3 x MHD). Also, continuous ECG recording was not performed in these studies and effects on ECG parameters such as QT interval were not reported. Therefore, these studies were not sufficient to assess potential risk, determine labeling, or risk management approaches for drug effects.

To resolve these deficiencies, conduct a comprehensive safety pharmacology study in monkey because the pharmacokinetic profile is similar to humans. This study must be conducted at various dose levels (at least, three with MTD as the highest dose). The study must include a complete battery of cardiovascular (including continuous ECG monitoring, QT interval, etc.) and respiratory system parameters. A hyperosmotic control group (sucrose/mannitol solution), and at least an Omniscan control group (to provide a link to the clinical database) must be included for comparison.

Also, conduct *in vitro* electrophysiological studies evaluating effects of MultiHance on cardiac action potential or potassium channels.

In addition, in a letter dated on March 10, 2003, FDA recommended the sponsor "submit a study evaluating the effects of MultiHance on calcium channels" and "include a positive control in the revised CVS protocol".

Sponsor's response

Four additional studies were designed and conducted in compliance with the ICH guidelines ICH S7(A) and ICH S7(B):

1) Study 886/026: Core battery of CVS in the conscious Cynomolgus Monkey
2) Study DGMH1003: Respiratory parameters in anesthetized rats
3) Study DGMH1002: HERG Tail Current
4) Study DGMH1004: Action potential parameters in dog isolated Purkinje fibers

A hyperosmotic control group (mannitol solution isotonic to MultiHance), and an Omniscan (a marketed gadolinium compound, class control) were included for comparison.

These studies are reviewed separately below.

Study title: Effect on arterial blood pressure, heart rate and ECG, following intravenous administration of MultiHance in the conscious Cynomolgus Monkey monitored by telemetry

Key findings: There is no evidence that intravenous injection of MultiHance (dose multiples up to10) has associated with risk of QT prolongation.

Study no.: 886/026
Volume #, and page #: vol 21 and p7
Conducting laboratory and location: [redacted]

Date of study initiation: January 9, 2003
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #: S2259, **and % purity**: [redacted]

Method

Six Cynomolgus monkeys (3 males + 3 females, 30-33 months, 2.4 to 2.6 kg) were used for the study. Each animal was intravenously administered (6 mL/min) control (hyperosmotic mannitol solution, concentration: 1.55M, osmolality: 1963 mosmol/kg, 6 mL/kg), three doses of MultiHance (see Table 1), and reference control Omniscan (3 mmol/kg, concentration: 0.5M, osmolality: 780 mosmol/kg, 6 mL/kg) on Days 0, 3, 6, 9, and 13 according to the designed order.

Table 1. Summary of MultiHance Dosing*

Dose (mmol/kg)	Dose volume (mL/kg)	Dose Multiple to Adults (m^2 basis)**	Dose Multiple to Children (m^2 basis)	Dose Multiple to Human Cmax
0.3	0.6	1	1.4	3
1	2	3.2	4.8	10
3	6	10	14	NA

* Concentration: 0.5M, Osmolality: 1950 mosmol/kg
** Clinical dose: 0.1 mmol/kg for both adults and children

Heart rate (HR), systolic and diastolic BP, mean arterial BP (telemetry), and cardiac Lead II was recorded and the following ECG parameters were determined: RR, PR, QRS, and QT/QT_c intervals. Parameters were recorded predose (2 hr prior to dosing) and at 1, 2, 5, 10, 15, 20, 30, 45m, 1, 11/4, 11/2, 2, 3, 4, 8, and 24h post each dosing. ANOVA followed by a Dunnett's test (if needed) was performed to compare predose values (18 values obtained at 15, 10, and 5 min prior to the doing) to post-treatment values. MultiHance effects on cardiovascular parameters were evaluated against not only pre-dose values but also those of mannitol and Omniscan.

Result

Arterial blood pressure and Heart rate

Neither MultiHance nor Omniscan produced significant change in BP when compared to mannitol although both induced transient increase when compared to pretest levels (see Table 2).

Table 2. Effect of MultiHance on Arterial Blood Pressure

Groups	Arterial Blood Pressure (mmHg, Mean±SE, % change from pretest)					
	Pretest	5 min*	30 min	45 min	60 min	120 min
Mannitol	100±2	112±3, 11	106±2, 5	119±4, 19	118±3, 17	103±2, 2
MultiHance 0.3 mmol/kg	100±2	100±3, 0	94±3, -6	114±4, 15	111±4, 11	103±3, 3
MultiHance 1 mmol/kg	99±3	106±5, 8	97±3, -2	125±6, 26	114±4, 14	102±2, 3
MultiHance 3 mmol/kg	99±2	105±3, 6	104±4, 4	122±4, 22	114±4, 14	100±2, 1
Omniscan	97±2	97±3, 0	97±2, 0	111±4, 14	111±4, 14	98±4, 1

* Time post doing

Mannitol induced statistically significant increase in HR during 45-75 min post dosing and returned to the pretest levels by approximately 2 h post dosing (see Table 3). This increase might be related to the stress induced by animal's handling during the line wash-out, according to the sponsor. Both MultiHance and Omniscan at dose of 3 mmol/kg induced a statistically significant increase in HR (during 5 or 30 min post dosing) when compared with mannitol control. Because the magnitude of increase was small it was difficult to draw a clear-cut association with the drug administration.

Table 3. Effect of MultiHance on Heart Rate

Groups	Heart Rate (bpm, Mean±SE, % change from pretest)					
	Pretest	5 min[a]	30 min	45 min	60 min	120 min
Mannitol	174±5	190±7, 9	175±8, 1	246±4, 42	229±10, 32	190±10, 9
MultiHance 0.3 mmol/kg	174±4	184±4, 6	161±6, -7	236±5, 36	215±9, 24	187±8, 8
MultiHance 1 mmol/kg	177±7	191±7, 8	165±8, -7	243±4, 38	223±7, 26	191±5, 8
MultiHance 3 mmol/kg	180±10	203±8*,13	169±10, -6	236±4, 31	215±7, 19	190±5, 6
Omniscan	181±8	204±8*,13	196±6*, 9	240±4, 33	231±7, 28	192±8, 6

[a] Time post doing

* Statistically significant when compared with mannitol control

ECG parameters

No significant QTc (Bazett's and Fredericia's correction) prolongation was noted for all MultiHance groups and Omniscan group. Maximal increase in QTc intervals was 11 ms at 5 min post Omniscan injection (Bazett's correction) and 12 ms at 8h post MultiHance (1 mmol/kg) injection (Fredericia's correction). Slight decrease in QTc was noted in all groups at some time points, which, according to the sponsor, was considered to be related to decreased RR interval.

MultiHance at dose of 3 mmol/kg induced statistically significant changes in QRS when compared with mannitol, which the sponsor considered as non-biologically relevant events due to small magnitude of change (6% from pretest level after dosing).

In one animal, 10 premature ventricular contractions (PVCs) were noted during 21 to 23 hours (Day 6) after administration of MultiHance at 3 mmol/kg. In most occasions (8/10), only single PVC was noted (2 or 4 PVCs for other two occasions). PVCs were also observed before and after Omniscan treatment (on Day 9, approximately five PVCs per minute with an irregular rate) in the same animal but no PVCs were noted either before or after administration of mannitol (on Day 13). The sponsor stated that it is difficult to attribute these PVCs to the administration of high dose of MultiHance but this possibility cannot be excluded.

Conclusion

MultiHance had no significant effects on arterial blood pressure and heart rate when compared with mannitol and Omniscan. QTc intervals (Bazett's or Fredericia's correction) were not significantly modified by MultiHance up to the maximum tested dose. Occasionally dose-independent reductions were observed with Omniscan or MultiHance.

Taken together, the sponsor concluded that MultiHance at tested doses were considered to be devoid of any potential deleterious effect on the atrioventricular and intraventricular conduction or the ventricular repolarization.

Reviewer's comment

The study was principally conducted in compliance with the protocol approved by FDA. The reviewer concurred with the sponsor's conclusion. MultiHance had no significant effects on ECG parameters including QTc intervals. However, the sponsor did not include a positive control in this study according to FDA's recommendation in a letter dated March 10, 2003. Furthermore, the sponsor did not provide the information regarding the sensitivity of the testing system. The reviewer has requested this information. However, the sponsor did not provide the requested information and rebutted that the sensitivity of the testing system was demonstrated in the literature. The reviewer agrees that the sensitivity of ECG is well established in general. Based on the fact that the study was conducted under GLP in a contract laboratory and the sensitivity

of the test system is well established in general, it is reasonable to consider the test system used by the sponsor has sufficient sensitivity to reveal drug-induced QT prolongation if existed. In addition, previous studies in rats and pigs showed that MultiHance at up to 1 mmol/kg [1.6 (rat) and 3.6 (pig)-times human dose] had no significant effects on ECG.

Premature ventricular contractions were noted in one monkey (1/6) during 21 to 23 h after administration of MultiHance at 3 mmol/kg, before and after Omniscan treatment but neither before nor after administration of mannitol. Considering short $t_{1/2}$ of MultiHance (Terminal elimination half lives in human subjects ranged from 1.17 ± 0.26 hours to 2.02±0.60 hours at doses up to 0.4 mmol/kg, in beagle dogs 2.92 hours when IV 1 mmol/kg) and low incidence, the association of this PVCs with MultiHance could not be established though cannot be excluded either. Furthermore, according to the medical reviewer, PVCs have not been observed in clinical trials.

Both mannitol and MultiHance induced a slight and transient increase in BP which was probably due to the high osmolality. Transient increase in HR was also noted in all groups and the association with drug treatment cannot be established.

Study title: Effect of MultiHance and Omniscan on HERG Tail Current recorded from stably transfected HEK293 cells

Key findings: Intravenous injection of MultiHance produced no significant drug related effect on HERG Tail Current when compared to osmolality control mannitol.

Study no.: DGMH1002
Volume #, and page #: Vol. 22, page 144
Conducting laboratory and location: [redacted]

Date of study initiation: July 8, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #: S2259, **and % purity**: [redacted]

Method

Potassium currents in HEK 293 cells stably transfected with HERG were recorded using a whole cell patch-clamp method. MultiHance, Omiscan, and mannitol were tested at various concentrations (see Table 4). E-4031, a selective inhibitor of HERG current, (100 nM, exposure time: 10-15 min) was used in 2 of the vehicle treated cells to confirm the sensitivity of testing system. ANOVA followed by a Dunnett's test (if needed) was used to compare the difference among the groups

Result

Mannitol produced statistically significant reduction of HERG current amplitude (see Table 4) when compared to the vehicle. Although there was a general tendency for reduction in tail current values with increasing osmolality, changes induced by mannitol were not concentration-dependent.

Table 4. Effects of MultiHance on HERG Tail Current

Compound	Concentration (mM)	Osmolality (mosmol/L)	HERG tail current (% control)
Control			100
Vehicle		292	85.7 ± 2.7
Mannitol	3	297	78.7 ± 1.1
	10	304	69.9 ± 1.1**
	30	322	58.9 ± 2.3**
	40	329	74.9 ± 5.5
	90	399	61.0 ± 3.4**
	100	409	39.8 ± 0.7**
	135	453	47.8 ± 3.2**
MultiHance	1	295-302	82.6 ± 2.1
	3	302-303	59.6 ± 6.0*
	10	313-327	39.2 ± 8.2**
	30	382-389	74.7 ± 5.5
	50	430-434	57.1 ± 6.6**
Omniscan	0.1	291-295	78.3 ± 4.5
	10	297-300	77.0 ± 6.6
	30	341	66.5 ± 4.7*
	50	343-352	63.9 ± 4.6*
E-4031	0.0001	N/A	2.8 ± 1.6

* $p<0.05$, ** $p<0.01$ compared to the vehicle.

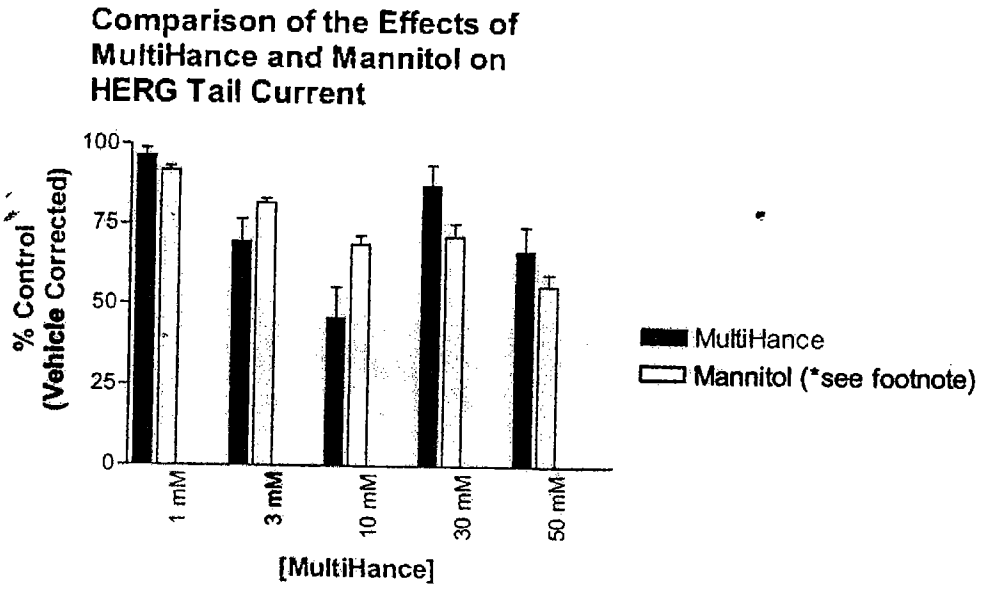
MultiHance produced a statistically significant inhibition of HERG tail current at 3, 10, and 50 mM when compared to the vehicle. However, no clear concentration response relationship could be established for this inhibition. Furthermore, there were no statistically significant differences between the effects of MultiHance and mannitol at the same osmotic load (see figure below, adopted from the submission).

Omniscan produced a significant inhibition of HERG tail current at 30 and 50 mM in a concentration-dependent manner. At lower concentrations tested, no statistically significant reduction of HERG tail current was noted.

The positive control (E-4031), as expected, markedly decreased the HERG tail current, indicating the sensitivity of testing system.

Considerable variability in the residual tail current measured from cells exposed to the same treatment was noted and this variability prevented function fits and derived the potency values (IC_{50}), according to the sponsor.

Comparison of the Effects of MultiHance and Mannitol on HERG Tail Current



*** Test concentrations of MultiHance were compared to Mannitol solutions with approximately equivalent osmolarity. Test concentrations of 1, 3, 10, 30 and 50 mM MultiHance were compared to 3, 10, 30, 90 and 135 mM Mannitol solutions, respectively.**

Conclusion

Both MultiHance and Omniscan elicited inhibition of HERG tail current. However, this effect could not be distinguished from the effects of increased osmolality as demonstrated by osmolality matched mannitol controls.

Reviewer's comment

The testing system appears sensitive enough to detect the blockage of potassium channel (I_{kr}) as evidenced by the almost dismissed HERG tail current by the positive control E-4031. It appears that HERG tail current is sensitive to osmolality. Increase in osmolality tends to lead to reduction of HERG tail current but no clear concentration response relationship was demonstrated. MultiHance elicited reduction in HERG tail current.

However, no clear concentration response relationship could be established. Omniscan inhibited HERG tail current in a concentration-dependent manner. This inhibitory effect on HERG tail current elicited by MultiHance and Omniscan can not be distinguished from the effects of increased osmolality as demonstrated by osmolality matched mannitol controls. The results suggested that hyperosmolality might contribute to reduction of HERG tail current elicited by MultiHance.

According to clinical pharmacology review, when male healthy volunteers were IV administered MultiHance at 0.1 mmol/kg, Vc (L/kg) was 0.074. Therefore, calculated maximal plasma concentration (C_0) in humans is 1.35 mM (C_0=dose/Vc, 2.68 mM at dose of 0.4 mmol/kg). No significant HERG inhibition was observed at this concentration. In general, HERG assay is a sensitive *in vitro* screening assay. If IC_{50} value of a drug in HERG assay is greater than 10 μM, the drug is considered as with weak evidence of risk to block I_{kr}. Taken together, it is reasonable to predict that it is unlikely that MultiHance induces significant blockage of I_{kr} at intended clinical dose.

Study title: MultiHance and Omniscan: Effect on action potential parameters in dog isolated Purkinje fibers

Key findings: Intravenous injection of MultiHance produced no effect on action potential parameters.

Study no.: DGMH1004
Volume #, and page #: Vol. 23, page 1
Conducting laboratory and location:

Date of study initiation: July 10, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MultiHance, lot #: S2259, purity:

Method

Action potential parameters in dog (beagle dog, weight 11.9-17.9 kg, 237-369 days) isolated Purkinje fibers electrically stimulated at 1 and 0.5 Hz were recorded using a microelectrode technique. The parameters analyzed included action potential duration at 60% and 90% repolarization (APD_{60} and APD_{90}), maximum rate of depolarization (MRD), upstroke amplitude (UA), and resting membrane potential (RMP). MRD at a pacing frequency of 3 Hz was also included to assess any effects of test compounds on sodium channels at the highest concentrations.

MultiHance, Omniscan and mannitol were tested at various concentrations (see Table 5). dl-Sotalol hydrochloride (50 μM) was used as a positive control.

The mean of 10 sequential waveforms prior to the markers were analyzed for determination of baseline values. In most of the experiments, each solution was applied

for approximately 30 min and tested in 4 fibers. Statistical analyses were done using unpaired, 2-tailed, Student's *t*-test (for homogeneity data) or Mann-Whitney *U*-test (for heterogeneity data).

Result

There were no significant differences in pretreatment action potential parameters among 5 treatment groups paced at either 1 or 0.5 Hz, indicating that the samples were drawn from comparable populations.

The vehicle had no effects on APD, MRD, UA, or RMP paced at stimulation frequencies of 1 and 0.5 Hz. Mannitol induced a statistically significant increase in APD_{60} and APD_{90} when compared to the vehicle (see Table 5). Furthermore, this increase appears concentration-dependent.

Table 5. Effects of MultiHance on isolated dog Purkinje fibers [a]

Compounds	Conc. (mM)	Osmolality (mosmol/L)	1 Hz	
			APD_{60} (ms)	APD_{90} (ms)
Vehicle	0[b]	≈300	*244.6±10.1*	*293.8±10.5*
Mannitol	0[b]		*261.4±16.4*	*321.8±19.1*
	4.2		6.2±2.1	5.1±2.1
	42		20.8±3.4##	16.1±3.7#
	140	440	34.8±3.0##	24.3±3.6#
MultiHance	0[b]		*245.2±7.9*	*294.3±10.7*
	1.5		0.30±1.0	-0.2±1.2
	15		3.5±2.0	1.1±2.3
	50	440	7.2±3.5	2.2±4.2

# $p<0.05$, ## $p<0.01$ compared to the vehicle (physiological salt solution)
a: expressed as change from baseline (%) except for 0[b]
0[b]: baseline expressed as mean±SE (n=4)

MultiHance at 1.5, 15, and 50 mM had no effects on MRD, UA, or RMP while slightly increased APD_{60} and APD_{90} paced at stimulation frequency of 1 Hz. However, the increase was less marked than those caused by the osmolality matched mannitol group at all concentrations tested (7% increase in APD_{60} at 50 mM MultiHance vs. 35% at osmolality matched mannitol). At 0.5 Hz, MultiHance at 1.5 and 15 mM had no effects on MRD, UA, or RMP while MultiHance at 50 mM slightly increased RMP (4 mV vs. baseline) and decreased UA (-2 mV vs. baseline). The changes were statistically significant when compared to mannitol but was considered as normal variation by the sponsor based on the small magnitude of change and occurring at 0.5 Hz only. Interestingly, when paced at stimulation frequency of 0.5 Hz, MultiHance failed to induce further increase in APD (comparable change at either 1 or 0.5 Hz).

A slight and comparable decrease in MRD was observed in all groups when the fibers were paced at stimulation frequency of 3 Hz, indicating MultiHance was unlikely to have an effect on cardiac sodium channels.

Omniscan at 1.5, 15, and 50 mM had no effects on APD, MRD, RMP, and UA at both 1 and 0.5 Hz when compared to the corresponding osmolality matched mannitol groups except for a decrease in RMP at 1 Hz when exposure to 15 mM Omniscan. This decrease was considered as normal variation by the sponsor because of the small magnitude of change (-0.4 mV vs. baseline), no concentration response, and occurring at 1 Hz only.

However, when compared to baselines, Omniscan increased APD. At 1 Hz, Omniscan at 1.5, 15, and 50 mM induced an increase in APD_{60} by 3.7%, 8.1%, and 17.5%, respectively.

The positive control dl-Sotalol hydrochloride (50 μM), when administered to the vehicle and mannitol treated fibers, induced significant increases in APD_{60} and APD_{90} as expected in a reverse rate-dependent manner (at 1 Hz, increased APD_{60} and APD_{90} by 58% and 55%, respectively, in 140 mM mannitol; at 0.5 Hz, increased APD_{60} and APD_{90} by 65% and 61%, respectively). dl-Sotalol hydrochloride had no effect on MRD, UA, or RMP.

Conclusion

MultiHance up to 50 mM had no significant effects on APD, RMP, UA, or MRD on dog isolated Purkinje fibers when compared to the vehicle control or the baseline values. When compared to the corresponding osmolality matched mannitol groups, changes induced by MultiHance were less marked than those by mannitol.

Omniscan induced a concentration-dependent increase in APD when compared to the vehicle control or the baseline values. However, this change was not significant different when compared to the osmolality matched mannitol groups.

The positive control, dl-Sotalol hydrochloride, induced expected increases in APD.

Taken together, according to the sponsor, these results indicated that MultiHance *per se* is not expected to have direct effects on QT interval and cardiac contractility if no change in osmolality occurs.

Reviewer's comment

MultiHance at up to 50 mM had no significant effects on APD, RMP, UA, or MRD on dog isolated Purkinje fibers as evidenced by the fact that no significant changes from baselines were observed when treated the fibers with MultiHance at up to 50 mM. Interestingly, mannitol induced more increase in APD than MultiHance even at same osmolality. The sponsor didn't provide any explanation for this observation.

Omniscan increased APD in a concentration dependent manner when compared to baselines. However, this increase is no longer apparent when compared to the corresponding osmolality matched mannitol groups.

It appeared that MultiHance, mannitol, or Omniscan prolonged APD in different magnitude even at similar osmolality (mannitol ≈ Omniscan > MultiHance ≈ vehicle), suggesting other factors (chemicals? other unknowns?) rather than osmolality alone involved in modulation of APD by those products.

Pulmonary effects:

Study title: Effect of MultiHance on respiratory parameters in anesthetized rats

Key findings: Intravenous injection of MultiHance produced a dose- and injection rate-dependent change on respiratory parameters.

Study no.: DGMH1003
Volume #, and page #: vol 22 and p1
Conducting laboratory and location:

Date of study initiation: September 25, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #: S2259, **and % purity**:

Method

The study was conducted in anesthetized, spontaneously breathing, male rats (Sprague-Dawley, 246-390 g, 5/group). Each animal was intravenously administered (2 mL/min) control (hyperosmotic mannitol solution, concentration: 1.55 M, osmolality: 1963 mosmol/kg, 8 mL/kg), three doses of MultiHance (see Table 6) and reference control Omniscan (concentration: 0.5 M, 4 mmol/kg, 8 mL/kg).

Table 6. Summary of MultiHance Dosing*

Dose (mmol/kg)	Dose volume (mL/kg)	Dose Multiple to Adults (m^2 basis)**	Dose Multiple to Children (m^2 basis)	Dose Multiple to Human Cmax
1	2	1.6	2.4	3
2	4	3.2	4.8	NA
4 (MTD)	8	6.5	9.6	NA

* Concentration: 0.5M, Osmolality: 1950 mosmol/kg
** Clinical dose: 0.1 mmol/kg for both adults and children

The following respiratory parameters (data were averaged over approximately 60 s and expressed as mean ± SE) were recorded: respiratory rate, tidal volume, and minute volume. The data were extracted at 15 (t-15) min predose and at 0 (t0, at the start of

administration of each dose), 1, 5, 10, 15, 30, 45, and 60 min after the end of each dose administration. A comparison was made at each time point using ANOVA.

Result

IV administration of Mannitol control, 1 and 2 mmol/kg MultiHance, and Omniscan caused a slight, transient increase in respiratory rate, tidal volume and minute volume (see Table 7). All parameters returned to baseline levels by 5 min post dosing. No statistically significant difference was noted among the groups.

At 4 mmol/kg one animal died within 1 min post dosing and was related to the drug administration, according to the sponsor. The remaining 4 animals showed an increase in respiration rate and minute volume following dosing and have a trend to return to baseline level by 15 min post dosing although slightly elevated levels was observed up to 60 min (last time point observed), indicating prolonged effects of high dose MultiHance on respiratory parameters.

Table 7. Effects of MultiHance on Respiratory Parameters

Group	RP*	Baseline**	1 min (%)***	5 min	10 min	15 min
Mannitol	RR	68±5	74±6 (8)	69±5	-****	-
	TV	1.44±0.08	1.58±0.11 (10)	1.45±0.12	-	-
	MV	97±5	116±7 (19)	99±7	-	-
MultiHance 1 mmol/kg	RR	74±4	79±5 (7)	73±5	-	-
	TV	1.55±0.11	1.78±0.09 (16)	1.55±0.10	-	-
	MV	113±4	140±9 (24)	111±8	-	-
MultiHance 2 mmol/kg	RR	69±4	80±6 (15)	70±6	-	-
	TV	1.38±0.09	1.70±0.09 (26)	1.28±0.08	-	-
	MV	94±7	137±16 (48)	90±12	-	-
MultiHance 4 mmol/kg	RR	76±4	78±7	89±8 (13)	87±6	81±4
	TV	1.40±0.08	1.77±0.10 (19)	1.45±0.09	1.50±0.04	1.45±0.03
	MV	107±11	138±18 (17)	128±10	129±9	119±8
Omniscan	RR	69±5	78±6 (14)	70±6	-	-
	TV	1.28±0.04	1.54±0.05 (20)	1.35±0.05	-	-
	MV	89±8	122±12 (38)	95±11	-	-

* RP: respiratory parameters, RR: respiratory rate (breaths/min), TV: tidal volume (ml), MV: minute volume (ml/min).
** average values of T-15 and t0
*** Time of post dosing (percentage of increase from baseline)
**** Data not shown

When MultiHance was intravenously administrated at 6 ml/min, rats at 2 mmol/kg group (2/group) ceased breathing for 23 or 49 seconds immediately following dosing, while both rats at 4 mmol/kg group died shortly (within 5 min) following the administration. No death and breath ceasing were noted for rats in mannitol, Omniscan, and 1 mmol/kg

MultiHance groups. A slight, transient increase (only at 1 min post injection) in respiratory rate, tidal volume, and minute volume was observed when 1 mmol/kg MultiHance was IV at 6 ml/min. The sponsor amended the administration rate to 2 ml/min for the final study.

Conclusion

IV administration of 1 and 2 mmol/kg MultiHance had no notable effects on respiration rate, tidal volume or minute volume when compared to the mannitol solution. At 4 mmol/kg one animal died and the remaining 4 animals showed an increase in respiration rate and minute volume 5-10 min following dosing. These slight, transient changes were not statistically different from those induced by either mannitol or Omniscan.

Reviewer's comment

IV administration of MultiHance at 4 mmol/kg resulted in immediate death of animals when MultiHance was administered at rate of 2 mL/min (1/5) or 6 mL/min (2/2). Four mmol/kg MultiHance was considered as MTD. This dose is only 6.5-fold of intended clinical dose for adults and 9.5-fold for children based on body surface area. MultiHance at 2 mmol/kg (dose multiple was 3.2 for adults and 4.8 for children) caused breath ceasing when administered at 6 mL/min, while transient increase in RR, TV, and MV when administered at 2 mL/min. MultiHance at 1 mmol/kg (dose multiple was 1.6 for adults and 2.4 for children), however, caused only slight, transient increase (only at 1 min post injection) in RR, TV, and MV and no significant difference was observed with different injection rate. The above results indicate that MultiHance may induce the severe adverse respiratory effects in dose and injection rate dependent manners. Furthermore, no death and breath ceasing were noted in mannitol and Omniscan (4 mmol/kg) groups even at 6 mL/min. Taken together, the results clearly indicate that the respiratory risk is associated with administration of MultiHance especially when administered rapidly and at high doses.

However, no death and breath ceasing were reported in monkey CVS safety study at doses up to 3 mmol/kg (dose multiple: 10) at 6 mL/min. Potential respiration related observations such as dyspnea and hyperventilation in clinical trials occurred only in low incidence (≤0.5%, regardless of causality). Taken together, potential severe respiration risk associated with MultiHance administration in humans may be relative small even MultiHance is recommended to be administered as a rapid IV infusion or bolus injection. Nevertheless, caution should be exercised when the drug is administered rapidly to anesthetized patients.

Renal effects: N/A

Gastrointestinal effects: N/A

Abuse liability: N/A

Other: N/A

Summary of Safety Pharmacology Studies

CVS and respiration safety studies were conducted upon FDA's request and are summarized below.

Cardiovascular safety

Three complementary studies were conducted to evaluate the CVS safety in compliance with FDA approved protocol or the ICH guidelines ICH S7(A) and ICH S7(B). These studies included: 1). core battery of CVS studies in conscious Cynomolgus Monkey monitored by telemetry; 2) HERG tail current study in stably transfected HEK293 cells; and 3). action potential parameter study in isolated dog Purkinje fibers. The studies were conducted to mainly address the concern of potential QT prolongation risk associated with MultiHance use. The studies were adequately designed and conducted under GLP. MultiHance at up to 3 mmol/kg (dose multiple: 10 fold) produced no QTc prolongation. In addition, MultiHance at up to 50 mM produced no significant effect on action potential parameters.

However, MultiHance induced a statistically significant inhibition of HERG tail current at 3, 10, and 50 mM (although not at 1 and 30 mM) when compared to the vehicle. No clear concentration-dependent relationship was noted. Of interest, this statistically significant inhibition elicited by MultiHance was no longer apparent when compared to their corresponding osmotic matched controls, indicating the hyperosmolality may contribute to this MultiHance-induced inhibition of HERG tail current. Furthermore, there was no significant HERG inhibition at 1 mM MultiHance, which is very close to calculated human maximal plasma concentration (1.35 mM at dose of 0.1 mmol/kg). Therefore, it is reasonable to predict that it is unlikely that MultiHance induces significant blockage of I_{kr} at intended clinical dose.

Omniscan (3 mmol/kg), a reference drug in this class, had no effect on QTc. Omniscan inhibited HERG tail current and increased action potential duration when compared to the vehicle but elicited no significant effects when compared to corresponding osmolality control mannitol.

In a letter dated March 10, 2003, FDA recommended the sponsor "submit a study evaluating the effects of MultiHance on calcium channels". The sponsor did not address this recommendation in current resubmission. However, based on the negative findings from *in vivo* ECG study and action potential parameter study, it could reasonably be assumed that it is unlikely that MultiHance elicits biologically significant effects on calcium channels.

Taken together, no clear evidence of QT prolongation risk was associated with MultiHance use.

Respiratory safety

Respiratory safety study was conducted in anesthetized, spontaneously breathing rats. IV administration of MultiHance at 4 mmol/kg resulted in immediate death of animals when MultiHance was administered at rate of 2 ml/min (1/5 death) or 6 ml/min (2/2 death). This dose is only 6.5-fold of intended clinical dose for adults and 9.5-fold for children based on body surface area. MultiHance at 2 mmol/kg (dose multiple was 3.2 for adults and 4.8 for children) caused breath ceasing when administered at 6 ml/min, while transient increase in RR, TV, and MV when administered at 2 ml/min. Only transient increase in respiratory parameters was noted at 1 mmol/kg MultiHance (dose multiple: 1.6), mannitol, and Omniscan (4 mmol/kg, dose multiple: 6.5) groups even at 6 mL/min. The results clearly indicate that the respiratory risk is associated with administration of MultiHance especially when administered rapidly and at high doses.

However, no death or breath ceasing were reported in aforementioned monkey CVS safety study at doses up to 3 mmol/kg (dose multiple: 10) at 6 mL/min. Furthermore, in clinical trials, potential respiration related observations such as dyspnea and hyperventilation occurred only in low incidence (<0.5%, regardless of causality). Taken together, potential severe respiration risk associated with MultiHance administration in humans may be relative small even though MultiHance is recommended to be administered as a rapid IV infusion or bolus injection in PI. Nevertheless, caution should be exercised when the drug is rapidly administered to anesthetized patients.

3.2.5 Pharmacodynamic drug interactions: N/A

3.3 PHARMACOKINETICS/TOXICOKINETICS: N/A

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology: N/A

Genetic toxicology:

In vivo bone marrow micronucleus assay in rats was conducted in compliance with FDA recommended administration route and maximal dose. The study was deemed valid by this reviewer. The results showed no evidence of clastogenicity when rats were exposed to up to 4 mmol/kg (dose multiple: 6.5 for adults and 9.6 for children). Furthermore, MultiHance was negative in *in vivo* rat micronucleus assay at a dose of 5 mmol/kg via ip route in a previous study.

Carcinogenicity: N/A

Reproductive toxicology: N/A

Special toxicology: N/A

Local tolerance:

The local tolerance studies using intravenous, perivenous, and intramuscular routes were conducted with some deviations with the protocols approved by FDA. The main deviation is injecting 0.2 mL instead of proposed 0.5 mL in perivenous local tolerance study, which could have had significant impact on the outcome of the study. Overall, MultiHance injection caused mild to moderate but reversible local reactions including erythema, edema, hemorrhage, and inflammatory cell infiltration. The reactions were more severe when injected by perivenous route than by intravenous route (please note injection volume was 0.2 mL in perivenous study while 0.5 mL in intravenous study). Therefore, caution should be exercised to avoid local extravasation when intravenous administration of MultiHance. No significant difference between injection rates (0.1 mL/min vs. 3 mL/min) could be discerned when administered by intravenous route.

3.4.2 Single-dose toxicity: N/A

3.4.3 Repeat-dose toxicity: N/A

3.4.4. Genetic toxicology

FDA request

In vivo micronucleus assay in rats was carried out using intraperitoneal (5 mmol/kg) rather than the intended intravenous route. Also, the dose level used in this study was inadequate.

In order to resolve this deficiency, an *in vivo* micronucleus assay using the intravenous administration route and higher dose levels of MultiHance (MTD) must be conducted.

Sponsor's response

In vivo micronucleus assay in rats was conducted according to FDA recommendation.

This study is briefly reviewed below.

Study title: MultiHance rat micronucleus test

Key findings: MultiHance is negative in the *in vivo* bone marrow micronucleus test in rats.

Study no.: KFF 009/024207

Volume #, and page #: vol 23 and p180
Conducting laboratory and location: [redacted]

Date of study initiation: July 17, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MultiHance, lot #: S2259, purity: [redacted]

Method

Strains/species/cell line: Sprague-Dawley CD/rat (7/male/negative control and MultiHance groups/time point, 5/male/positive control group/time point)

Doses used in definitive study: 0 (0.9% sodium chloride), 1, 2, and 4 mmol/kg MultiHance (see Table 8), IV

Table 8. Summary of MultiHance Dosing*

Dose (mmol/kg)	Dose volume (mL/kg)	Dose Multiple to Adults (m^2 basis)**	Dose Multiple to Children (m^2 basis)	Dose Multiple to Human Cmax
1	2	1.6	2.4	3
2	4	3.2	4.8	NA
4 (MTD)	8	6.5	9.6	16

* Concentration: 0.5M, Osmolality: 1950 mosmol/kg
** Clinical dose: 0.1 mmol/kg for both adults and children

Basis of dose selection: Four mmol/kg was considered as the maximum tolerated dose according to the previous toxicity test and therefore was selected as a maximal dose for the test. During micronucleus test, animals treated with 2 and 4 mmol/kg MultiHance showed clinical signs including underactivity, abnormal gait, and irregular respiration.

Negative controls: 0.9% sodium chloride (8 mL/kg)

Positive controls: Cyclophosphamide (20 mg/kg, orally by gastric intubation, 10 mL/kg)

Incubation and sampling times: Bone marrow was harvested 24h after rats received a single IV dose of MultiHance for all groups and 48h for 0.9% sodium chloride and 4 mmol/kg MultiHance groups.

Result

Study validity:
The study was valid for the following reasons: 1) the species and number of animals/group were acceptable; No substantial differences in toxicity were observed between the sexes in preliminary toxicity test, therefore using male rats only was adequate; 2) tissue sampling and analysis was acceptable; 3) the vehicle control group

had 1 micronucleated PCEs (polychromatic erythrocytes) in 2000 PCEs (the historical data: mean 0.79); and 4) positive controls exhibited significantly higher micronucleated PCEs than the vehicle control group (2.45% vs. 0.05%, $p<0.01$).

Study outcome:
The results revealed no statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the portion of immature erythrocytes compared to vehicle control values. MultiHance did not show any evidence of chromosome damage or bone marrow cell toxicity.

Reviewer's comments: The sponsor conducted an *in vivo* micronucleus assay using the intravenous administration route at MTD of MultiHance in compliance with FDA's recommendation. The results had shown no evidence of clastogenicity when exposure the rats to up to 4 mmol/kg (MTD, dose multiple: 6.5 for adults and 9.6 for children). Furthermore, MultiHance was negative in *in vivo* rat micronucleus assay at a dose of 5 mmol/kg via ip route in previous study.

3.4.5. Carcinogenicity: N/A

3.4.6. Reproductive and developmental toxicology: N/A

3.4.7 Local tolerance

FDA request:

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

To resolve this deficiency, conduct a local tolerance study (intravenous, paravenous, and intramuscular administration) with histological evaluation at earlier time points (e.g., 24 hours) and at later time points, until the local adverse effects are resolved. Also, MultiHance is proposed for direct bolus or infusion. The study must include the rates of infusion on tolerance.

Sponsor's response

Three local tolerance studies (intravenous, intramuscular, and perivenous administration) were conducted in rabbits in compliance with the guideline CPMP/SWP/2145/00. All studies included histological evaluations at an earlier time point (24h) and at a later time point (day 22). For the IV route two infusion rates (0.1 mL/min and 3.0 mL/min) were evaluated.

The studies are separately reviewed below.

A.
Study title: MultiHance Intravenous local tolerance study in the rabbits

Key findings: Intravenous injection of MultiHance produced mild, reversible local reactions.

Study no.: KFF 011/023877
Volume #, and page #: vol 23 and p111
Conducting laboratory and location:

Date of study initiation: August 6, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MultiHance, lot #: S2259, purity:

Method

The study design was shown at Table 9. MultiHance was administered into the lateral ear vein of the right ear of male New Zealand white rabbits (2.54 to 3.19 kg) while contralateral ear was similarly administered with sterile physiological saline and served as controls. The dermal reactions surrounding the injection site were assessed qualitatively for signs of erythema, edema, and eschar formation. The dermal reactions were quantified on a scale of 0 to 4, with 0 being no local reaction and 4 being severe erythema or edema. The animals were observed for the local reactions at following time points: 1-hour post-dosing and Days 2, 3, 4, 5, 6, 7, 8, 15, and 22 post-dosing. Animals were sacrificed on Days 2 and 22 post-dosing and the injection site and surrounding tissues were examined macroscopically and microscopically.

Table 9. Experimental Design

Group	# of Male New Zealand rabbits	Rate of injection (mL/min)	Volume of injection (mL, 0.5 mmol/mL)	Termination
1	4	0.1	0.5	Day 2*
2	4	3	0.5	Day 2
3	4	0.1	0.5	Day 22*
4	4	3	0.5	Day 22

Day 2: 24 hours post-administration, Day 22: 21 days post-administration

Result

As shown at Table 10, a higher incidence of mild erythema was observed on Day 2 at the injection sites of MultiHance groups than at controls. The erythema was no longer observable by Day 5. Bruising was evident at both test and control injection sites in numerous animals and was resolved by Day 5. Bruising was considered as procedure–

related rather than drug related effects. No dermal reactions except for bruising were noted at 1 hour post injection. No other local reactions were reported. No clear association of erythema with injection rates could be established.

Table 10. Summary of Local Reactions

Group*	Erythema on Day 2		
	Grade** 0	Grade 1	Grade 2
	# of Rabbits	# of Rabbits	# of Rabbits
1	3	1	0
1-C	4	0	0
2	1	3	0
2-C	3	1	0
3	1	2***	1****
3-C	4	0	0
4	2	2	0
4-C	4	0	0

* See Table 9 for the design; C:control
** 0: no erythema, 1:very slight erythema, 2: well-defined erythema
*** Erythema persisted in 1 rabbit until Day 4
**** Erythema persisted until Day 4 but grade decreased to 1 since Day 3

No macroscopic abnormalities were observed on both Days 2 and 22. Minimal or slight perivascular odema and/or hemorrhage at and/or around injection sites were noted at both test and control animals in histopathology examination on Day 2. Only minimal perivascular hemorrhage was noted at 1 test site on Day 22. These microscopic findings were not considered to be related to administration of MultiHance, according to the sponsor. No significant difference between two injection rates could be discerned.

Conclusion

Intravenous injection of MultiHance elicited some non-persistent mild dermal reactions. There were no other effects of treatment and the dermal reactions were considered to be an adverse effect at the site of injection.

Reviewer's comment

The study was principally conducted in compliance with the protocol approved by FDA. Additional observation time-points (6 and 9 hours) were recommended by FDA but weren't included in the study, which may not significantly compromise the study outcome and interpretation. Intravenous injection of MultiHance produced only mild reversible local reactions in this study. The local tolerance study previously conducted by the sponsor showed MultiHance produced slight to moderate reddening and slight reddening lasted until Day 5 and minimal edema, hemorrhage, and inflammatory cell infiltration were noted on Day 8 for all groups tested (Please refer to the previous review for details regarding the previous study). Although the local reaction rates in MultiHance

groups was not higher than placebo group, injection site reactions characterized as anesthesia, blisters, burning sensation, erythema, and local pain have been reported in postmarketing experience outside the U.S. No significant difference between two injection rates could be discerned.

B.
Study title: **MultiHance perivenous local tolerance study in the rabbits**

Key findings: Perivenous injection of MultiHance produced mild to moderate reversible local reactions.

Study no.: KFF 013/024149
Volume #, and page #: vol 23 and p134
Conducting laboratory and location:

Date of study initiation: August, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MultiHance, lot #: S2259, purity:

Method
Two groups (3 rabbits/sex/group) of New Zealand White rabbits (2.7 to 3.31 kg) were injected 0.2 mL (0.5 mmol/mL) of MultiHance near the lateral vein of the ear. Other parts of protocol were same as above described for IV study.

Result
Slight erythema at injection sites was noted in all rabbits (6) administered MultiHance from Day 2 (see Table 11) and erythema progressed to well-defined in two animals by

Table 11. Summary of local reactions

Groups	Erythema*			Odema**	Hemorrhage**	Inflammation infiltration**
	Day 2	Day 3	Day 4	Day 2	Day 2	Day 2
	Grade (# of Rabbits)	Grade (# of Rabbits)	Grade (# of Rabbits)	Grade (# of Rabbits)	Grade (# of Rabbits)	Grade (# of Rabbits)
Treatment-M (3)	1 (3)	1 (1), 2(1)	1 (1), 2(1)	2 (3)	1 (1)	1 (3)
Treatment-F (3)	1 (3)	1 (1), 2(1)	1 (1)	3 (3)	1 (1), 2 (1), 3(1)	2 (1), 3(2)
Control-M (3)	0 (3)	0 (3)	0 (3)	--	--	1 (1)
Control-F (3)	0 (3)	1 (1)	0 (3)	3 (1)	--	1 (2), 2 (1)

* 0: no erythema, 1:very slight erythema, 2: well-defined erythema; Very slight erythema persisted in 3 males until Day 6, and 1 female rabbit until Day 8.
** Grades for odema, hemorrhage, and inflammation infiltration: --: finding not present, 1: minimal, 2: slight, and 3: moderate.

Day 3. The erythema in all animals resolved by Day 9. Slight erythema was noted in control site in one rabbit and on Day 3 only. Clearly, higher incidence of mild erythema was observed at the injection sites of MultiHance than at controls (6/6 vs. 1/6). Bruising around most test and control injection sites was noted in most animals during the first week of injection and resolved by the end of the first week in most cases. Bruising was considered as procedure–related rather than drug related effects by the sponsor. No dermal reactions except for bruising were noted at 1 hour post injection.

No macroscopic abnormalities were observed on Days 2 and 22 except that areas of scabbing were noted on the ears of one rabbit sacrificed on Day 2 and considered as procedure–related rather than drug related effects by the sponsor. Subcutaneous odema, hemorrhage, and inflammation infiltration were noted in histopathology examination at most MultiHance injection sites on Day 2 while such lesions occurred in fewer animals and in lesser degree in controls (see Table 11). Overall, it appears that females have severer local reactions than males. No subcutaneous odema, hemorrhage, and inflammation infiltration were observed in rabbits sacrificed on Day 22.

Conclusion

Perivenous injection of MultiHance elicited reversible, slight to well-defined erythema at the sites of injection and this reaction was related to the increased incidence of subcutaneous odema and hemorrhage.

Reviewer's comment

The study was not conducted in compliance with the protocol approved by FDA. Only 0.2 mL instead of proposed 0.5 mL was used for injection. This deviation might have significant impact on outcome of the study. Additional observation time-points (6 and 9 hours) were recommended by FDA but weren't included in the study.

Perivenous injection of MultiHance produced mild to moderate, reversible local reactions in this study. However, severer reactions including eschar and necrosis were noted even on Day 8 post MultiHance dosing in previous study [the reactions in MultiHance group was severer than in Magnevist group and similar to 0.75% acetic acid (positive control) group]. The injection volume was 0.3 mL in this study.

Taken together, perivenous injection of MultiHance produced severer local reactions than intravenous injection. Therefore, caution should be exercised to avoid local extravasation when intravenous administration of MultiHance.

C.
Study title: **MultiHance intramuscular local tolerance study in the rabbits**

Key findings: Intramuscular injection of MultiHance produced no local reactions.

Study no.: KFF 012/024148
Volume #, and page #: vol 23 and p157
Conducting laboratory and location: [redacted]

Date of study initiation: August 27, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MultiHance, lot #: S2259, purity. [redacted]

Method

Two groups (3 rabbits/sex/group) of New Zealand white rabbits were injected 0.5 mL (0.5 mmol/mL) of MultiHance into the paravertebral muscle to the right of the spine. Other parts of protocol were same as above described for IV study.

Result

Well-defined erythema was observed on Day 2 at the control site of 1 rabbit and was considered as background irritation by the sponsor. Bruising around the test injection site was noted at in 1 animal and resolved by Day 5. No other dermal reactions were noted.

No macroscopic abnormalities except for dark areas within the musculature/subcutis at both test (4 sites) and control (2 sites) injection sites were observed on Days 2 and 22. Dark areas were considered as administration procedure-related rather than treatment-related by the sponsor.

Minimal dermal inflammation was noted in both test and control injection sites in all animals on Day 2 and was still present in 2 animals on Day 22 in histopathology examination. Minimal or slight dermal, subcutaneous, and muscular hemorrhage, muscular necrosis, and myositis were noted at both test and control injection sites in 1 or 2 animals on Day 2 only. These microscopic findings were not considered to be related to administration of MultiHance, according to the sponsor.

Conclusion

Intramuscular injection of MultiHance did not elicit any treatment related local reactions, according to the sponsor.

Reviewer's comment

The study was principally conducted in compliance with the protocol approved by FDA. Additional observation time-points (6 and 9 hours) were recommended by FDA but weren't included in the study. The study was conducted under GLP, however, blunder such as "an intramuscular injection of MultiHance into the lateral vein of rabbit" was noted in the conclusion of the study report. However, the reviewer concurred with the sponsor's conclusion: intramuscular injection of MultiHance did not elicit drug-related local reactions.

3.4.8 Special toxicology studies: N/A

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The sponsor has adequately addressed pre-clinical issues in this resubmission, and therefore approval is recommended for MultiHance from pharmacology and toxicology perspective (see executive summary for details).

Unresolved toxicology issues (if any): None

Recommendations: Approval

Suggested labeling: Pending

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Information to sponsor: No

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

/s/

Yanli Ouyang
4/7/04 12:12:20 PM
PHARMACOLOGIST

Adebayo Laniyonu
4/7/04 12:19:41 PM
PHARMACOLOGIST

Acting Pharmacology/Toxicology Team Leader Memo

NDA: **21-358**
Drug: **MultiHance**
Sponsor: **Bracco**

MultiHance (Gadobenate dimeglumine) is an injectable ionic contrast MRI agent proposed [redacted] CNS imaging of lesions with abnormal vascularity, and lesions causing abnormality in the blood brain barrier in adults [redacted] Dr. Tushar Kokate reviewed the original NDA. Based on the identification of several deficiencies in the preclinical pharmacology/toxicology section, he recommended that the NDA not be approved until successful completion of studies identified as deficient and clarification of other issues raised by the review of the NDA. Dr. Kokate's preliminary comments on the NDA were faxed to the sponsor on 03/13/02 the sponsor responded on 4/10/02 and Dr Kokate has reviewed the sponsor's response.

Based on this latest review, Dr Kokate's recommendation is that the NDA is approvable subject to satisfactory completion of recommended preclinical Pharmacology/Toxicology studies prior to approval. This memo is not intended to duplicate his review, rather, it is to highlight the issues that Dr. Kokate believes have been resolved successfully by the sponsor, and issues that need resolved prior to approval. Please see Dr. Kokate's review for details.

Studies that are still required prior to approval:

1. Safety pharmacology studies in a large animal species evaluating a battery of cardiovascular and respiratory parameters.

2. *In vitro* electrophysiological studies evaluating effects on cardiac action potential or potassium channels.

3. An *in vivo* micronucleus assay.

4. Local tolerance study.

I concur with Dr. Kokate's recommendations.

/S/ 5/1/02

Adebayo Laniyonu, Ph.D.

Acting Team Leader

PHARMACOLOGY/TOXICOLOGY COVER SHEET

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

Proposed use: (a) For adults

Dose: 0.1 mmol/kg,

Maximum clinical dose: 0.2 mmol/kg.

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

Executive Summary

I Recommendations:

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

(B) Recommendation for Nonclinical Studies:

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

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

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

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

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

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

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

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

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

III Administrative:

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/ 4/29/02 . See attached memo BBK

Non-Concurrence - _____
(see memo attached)

C. cc: list:

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION:

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

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

I. GENERAL PHARMACOLOGY/TOXICOLOGY COMMENTS:

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

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

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

Cmax was chosen based on the following observations:

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

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

II. SAFETY PHARMACOLOGY:

(A) General comment:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(B) In vitro electrophysiology study:

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

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

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

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

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

III. PHARMACOKINETICS/TOXICOKINETICS:

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

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

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

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

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

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

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

IV. GENERAL TOXICOLOGY:

(A) Acute toxicity studies:

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

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

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

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

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

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