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RESEARCH**

APPLICATION NUMBER:

204781Orig1s000

PHARMACOLOGY REVIEW(S)

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)**MEMORANDUM TO FILE****DIVISION OF MEDICAL IMAGING PRODUCTS****Pharmacology/Toxicology Review**

Date: March 08, 2012
Topic: Neonatal Rat Repeat-Dose Toxicity Study (Final Study Protocol Review)
Reviewer: Olayinka A. Dina, DVM, Ph.D
Through: Adebayo Lanionu, Ph.D. (Supervisory Pharmacologist)

Application Information

Title:	Dotarem – neonatal and juvenile (pre-post weaning) toxicity study by the intravenous route in the rat
Application number:	IND 65,041 / NDA 204-781
Supporting Document No./Serial No./Date/Type of Submission:	February 21, 2013 / Final Study Protocol / Submitted via e-mail (Submission to the IND pending)
Sponsor / Address:	Guerbet BP 57 400, F-95943 Roissy CDG Cedex, France (US Agent: Guerbet LLC 1185 W. 2 nd St., Bloomington, IN 47403-2160)
Sponsor's Letter Date:	February 22, 2013
Product:	DOTAREM [®] (Gadoterate meglumine) Injection
CDER stamp date:	N/A
Proposed Indication(s):	“Dotarem is a gadolinium-based contrast agent indicated for intravenous use with magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adult and pediatric patients (2 years of age and older) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity.”
Submission:	1. Final Study Plan ^{(b) (4)} 14315-D (3_13_00002_protocolIDGD33041.pdf) 2. Study plan amendment no.1 (3_13_00008_amendment.pdf)
Review Division:	Division of Medical Imaging (DMIP), HFD#: 160
Preclinical Reviewer:	Olayinka A. Dina, DVM, Ph.D.
Review Completion Date:	March 19, 2013
Supervisor/Team Leader:	Adebayo Lanionu, PhD
Division Director:	Rafel Dwaine Rieves, MD
Project Manager:	James W. Moore, Pharm.D, MA

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

Purpose of Memo: The purpose of this memorandum is to provide a Pharmacology/Toxicology evaluation of the study protocol # DGD-33-041 titled “Dotarem – neonatal and juvenile (pre-post weaning) toxicity study by the intravenous route in the rat”.

A. Background: Guerbet LLC sponsor) submitted the final protocol for a proposed repeat-dose toxicity study in neonatal and juvenile rats to provide nonclinical support for a proposed pediatric indication for Dotarem in children aged 0-2 years in NDA 204781. The proposed study will be conducted in compliance with the FDA Guidance for Industry for nonclinical safety evaluation of pediatric drug products (FDA, 2006); EMEA Guidelines on nonclinical testing in juvenile animals for pharmaceuticals for pediatric products (EMEA, 2008) and the ICH S3A (Guidance on Toxicokinetics: the assessment of systemic exposure in toxicity studies).

B. Protocol Review:

Aim of study: The proposed juvenile toxicity in the final protocol ^{(b) (4)} 14315-D was to determine the toxicity of Gadoterate meglumine when administered to neonate and juvenile rats following a single intravenous administration on PND10 or following repeated intravenous administrations every four days starting from PND10 up to PND30 (4 weeks) and evaluate the regression of any signs of toxicity during a 60-day treatment-free period. The study would also assess development up to sexual maturation.

Experimental Design:

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)**Methods**

Conducting laboratory and location:	(b) (4)
Study number:	(b) (4) 14315-D; Sponsor reference No. DGD-33-041
Doses:	0 (0.9% NaCl), 0.6, 1.25 and 2.5 mmol Gd/kg of Gadoterate meglumine
Frequency of dosing:	Single dose: One single administration on PND10 Repeated doses: Six doses on PND 10, 14, 18, 22, 26 and 30)
Route of administration:	Slow bolus intravenous injection via the jugular or tail vein: PND10 : jugular vein PND14-30 : tail vein
Dose volume:	5, 1.2, 2.5 or 5 mL/kg for control, low, intermediate and high doses, respectively
Formulation/Vehicle:	Dotarem (0.5 mmol/mL) / 0.9% NaCl (saline for injection)
Species/Strain:	Rat/Sprague-Dawley: (b) (4)
Age:	PND 10 (at initiation of treatment)
Weight:	TBD
Number/sex/dose:	Subgroups A-C: 30/sex/group
Satellite group:	Subgroup D: 9/sex (control group); 18/sex for each of low, intermediate and high dose groups
GLP compliance:	Yes (x), No ()
Quality Assurance:	Yes (x), No ()

Reviewer's Table. Revisions to the methods are shown in boldface.

Table 1: Subgroups, frequency of treatment, treatment-free period and time of sacrifice

Subgroup	No. of animals/sex/group	Treatment / Day(s) of treatment	Treatment-free period (days)	Day of sacrifice
A	15	Single dose on PND10	61 or 62	End of treatment-free period (PND71/72)
B	15	Repeated doses on PND10, 14, 18, 22, 26 & 30)	None	2 days after last treatment on PND31 or 32
C	15	Repeated dose on PND10, 14, 18, 22, 26 & 30) + TK sampling on PND30	61 or 62	End of treatment-free period (PND91/92)
D (Satellite)	18 (except in the control group with 9/sex/group)	Single dose on PND10 + TK sampling on PND10	None	24h after treatment (PND11) 6 rats/sex/group)

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

Reviewer's Table based on sponsor's data on page 4 of 37

Table 2: Study Design: Dose groups, Dose multiples and Number of animals

Dose groups (+ total no. of rats)	Dose level (mmol/kg)	Dose Volume (mL/kg)*	Human Dose multiples	Number of animals	
				Males	Females
Control (54)	0	5	0	15(A) + 15(B) +15(C) + 9(D)	15(A) + 15(B) +15(C) + 9(D)
Low (63)	0.6	1.2	0.97x	15(A) + 15(B) +15(C) + 18(D)	15(A) + 15(B) +15(C) + 18(D)
Intermediate (63)	1.25	2.5	2.03x	15(A) + 15(B) +15(C) + 18(D)	15(A) + 15(B) +15(C) + 18(D)
High (63)	2.5	5	4.05x	15(A) + 15(B) +15(C) + 18(D)	15(A) + 15(B) +15(C) + 18(D)

Reviewer's Table adapted from of Experimental Design (page 1 of 37). Rats in the control group will be administered saline (0.9% NaCl), * = All animals from a dose group will receive the same dose volume until PND18 (males and females considered together) based on the mean weight of each dose group. From PND22, all animals from a dose group and sex (males and females being considered separately) will receive the same volume based on the mean weight of each dose group and sex. Human dose multiples were based on body surface area and a human dose of 0.1mmol/kg or 3.7mmol/m², assuming a 60 kg adult.

Table 3: Summary of Protocols

Protocol	Method, frequency and/or objectives
Morbidity/mortality	2x/day; Dead or moribund animals will be necropsied. Satellite animals will be observed for mortality/morbidity but will not be necropsied
Clinical observations	Daily; Before treatment and once after each dosing to detect clinical reactions to treatment. A full clinical examination will be performed once weekly up to termination
Body weight	Subgroup A: Each pup will be weighed on PND10, 14 and 17 then weekly up to termination Subgroups B & C: Each pup will be weighed on PND10, 14, 18, 22, 26 and 30, then weekly up to termination for subgroup C Subgroup D (Satellite): Weighed only to calculate dose volumes. Weight is not reported
Food consumption	Recorded 2x/week starting after weaning up to the end of the treatment period then 1x/week up to termination for subgroups A & C pups
Growth measurements	Tibia lengths: Tibial length of all animals will be measured with calipers before dosing (PND 10) and then on the relevant weigh days up to termination
Ophthalmology	On subgroup A & C in groups 1 and 4 on a suitable day during the last week of treatment-free period. - Animals in study groups 2 and 3 may be examined (if necessary).

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

	<p>- A mydriatic agent (Tropicamide) will be instilled into the eyes prior to ocular examination.</p> <p>- Examination of adnexia, optic media and fundus: performed by indirect ophthalmoscopy. A slit lamp examination may be performed</p>
Clinical Pathology (hematology ¹ , coagulation ¹ and serum chemistry ¹) (Methods: Table 4 below):	<p><u>Hematology:</u> Erythrocytes (RBC), Hemoglobin (HB), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Reticulocyte count, Platelet count(PLAT), Total WBC and Differential WBC count</p> <p><u>Coagulation:</u> Prothrombin time, Activated partial prothrombin time (APTT) and fibrinogen level</p> <p><u>Serum chemistry:</u> Na⁺, K⁺, Cl⁻, Ca²⁺, Total Iron, Ferritin, Inorganic phosphate, Cu²⁺, Zn²⁺, Glucose, Urea, Creatinine, Total bilirubin, Total proteins, albumin, albumin/globulin (A/G) ratio, Total Cholesterol, Triglycerides, Alkaline phosphatase, Aspartate aminotransferase, and Alanine aminotransferase.</p>
Urine analysis ² (see Table 5 below)	<p>Volume, Specific gravity, appearance (color and turbidity); Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺, Inorganic phosphate, Iron, Cu²⁺, Zn²⁺; pH, Bilirubin, Urobilinogen, Proteins, Glucose Blood, Ketones, Nitrites, Leukocytes, urinary sediment (oxalate crystals, RBCs)</p>
Termination/ Gross pathology	<p>On Main group animals only (except if indicated otherwise). Animals will be killed by CO₂/Exanguination</p> <p><u>Tissue collection:</u> See organ processing Table (Table 6).</p> <p>In addition, the skin, bone, liver and kidneys will be sampled from the first 6 surviving animals/sex/group in each subgroup A, B, C and D for measurement of total Gadolinium. For subgroup D, animals will correspond to animals sampled at the +24 h time point). A sample of bone will be taken and kept deep frozen from subgroups A, B and C for possible further analysis</p> <p><u>Histopathology</u> (See below)</p>
Necropsy	<p><u>Subgroups A & C:</u> At the end of the treatment-free period - A = PND 71 or 72; C = PND 91 or 92</p> <p><u>Subgroup B:</u> At the end of the treatment-free period on PND 31 or 32</p> <p><u>Subgroup D:</u> 6 rats/sex/group (including animals sampled for blood at 24 h time point), will be sacrificed for organ sampling only. Animals sampled for blood at earlier time points will be discarded without necropsy. Animals will be weighed before necropsy unless if moribund or found dead.</p> <p><u>All animals in subgroups A, B or C found dead or killed moribund will be necropsied including examination of:</u> external surface, orifices, cranial cavity, external surface of the brain and cervical spinal cord; thoracic and abdominal cavities and organs, cervical tissues and organs, carcass and</p>

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

	<p>injection sites</p> <p>Sperm analysis: Subgroup C</p>
Organ weights	<p>See Table of Organs (Table 6). Organ weights will be expressed as absolute values and weight relative to body weight. Testes and epididymides will be weighed separately. The left testis and epididymides of subgroup C males will be reserved for histological examination.</p>
Histopathology	<ul style="list-style-type: none"> - On animals in subgroups A, B and C - SOP will be followed. - Fixatives: 10% neutral formalin (bone smears will be air dried; Harderian glands, testes, epididymides, eyes, optic nerves will be fixed in modified Davidson's fluid) - Histopathology will be performed for selected organs/tissues in animals found dead or killed moribund and animals in groups dose groups 1 (control) and 4 (high dose). - Liver, skin, kidneys and bone will be examined in all subgroup A, B and C animals in all dose groups - Any target organs identified in group 4 (high dose) animals will be examined in the low and intermediate dose groups at the end of the treatment period - Histopathology will be performed for all gross lesions from animals in all groups - Bone marrow smears will be prepared at scheduled necropsy for all animals including those killed moribund - Slides will be stained with H&E except bone marrow (May Grunwald Giemsa stain) In addition Von Kossa staining will be performed for organs with an asterisk in the organ processing Table (Table 6). The testes will be microscopically examined using H&E staining (tubular stages of the spermatogenic cycle may be examined with PAS staining) - Detailed qualitative examination of the testes, including tubular stages of the spermatogenic cycle, will be performed in all subgroup C males. Qualitative examination of the ovaries for developmental and other lesions will be performed in all subgroup C females.
Toxicokinetics (TK) ³ (see Table 7)	<p>C_{max}, T_{max}, AUC, Accumulation ratio and dose proportionality using non-compartmentalization methodology</p>
Pre-weaning development	<p>Physical development of all pups will be assessed by the intra-litter onset and duration of pinna unfolding, incisor eruption and eye opening. Functional tests proposed include surface righting reflex on PND 8, gripping reflex on PND 17 and Pupillary reflex and auditory reflex on PND 21.</p>
Sexual maturation	<p>Only subgroup C animals will be used for determination of sexual maturation. Females will be examined as from PND 28 to detect the day of vaginal opening. Body weight will be recorded on the day of occurrence. Vaginal smears will be examined daily in all females after the onset of vaginal patency (~PND 33) until the first cornified smear is</p>

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	recorded. Vaginal smears will also be taken daily for 2 weeks prior to necropsy and used to determine the stage of estrus/female. Males will be examined as from PND 38 to detect the day of bano-preputial skinfold cleavage and body weight will be recorded on the day of occurrence.
Behavioral tests	Only subgroup C animals will be used for behavioral tests. The tests include the water maze test at 5 and 6 weeks of age and open field test at 7 weeks old.

Reviewer's Table constructed from sponsor's data. Revisions to the protocol are shown in boldface. All abbreviations are standard clinical pathology terms; ^{1,2,3}, 1 = see Table 4 for summary of blood collection; 2 = see Table 5 for summary of urine collection; 3 = see Table 7 for summary of blood collection for Toxicokinetics

Table 4: Blood Sampling (for Hematology, Coagulation and Serum Chemistry)

Blood collection:

Occasions	Animals*	Sampling conditions	Diet conditions	Analysis type	Anticoagulant	Tube identification	Blood volume
Subgroups A, B and C on the day of necropsy	6m+6f	Retro-orbital sinus under isoflurane	Fasted overnight (about 16h)	Coagulation	Trisodium citrate	CITR	0.9 mL
	Haematology			EDTA-K ₃	EDTA	0.5 mL	
	Chemistry			Without	SEC	2 mL	

*: The first 6 male and 6 female surviving pups per group will be sampled for coagulation and haematology and the following 6 male and 6 female surviving pups per group will be sampled for clinical chemistry.

Subgroup A: before necropsy (PND 71/72).

Subgroup B: before necropsy (PND 31/32).

Subgroup C: before necropsy (PND 91/92).

Samples will also be taken from moribund animals where possible.

Table 5: Urine Sampling (for Urinalysis)

Urine collection:

Occasions	Animals	Sampling conditions	Diet conditions	Specific request
Subgroups A, B and C on the day of necropsy	All	About 16h in metabolism cages	Deprived of food and water but gavage with approximately 20 mL/kg of filtered water	None

Pathology

(Organs will be collected at necropsy as shown in Table 6).

i) Subgroup A: Animals will be necropsied at the end of the treatment period (PND 31 or 32; see Table 1)

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ii) Subgroups A and C: Animals will be necropsied at the end of the treatment-free period on PND 71/72 (subgroup A) and PND 91/92 (subgroup C).

iii) Subgroup D: six animals/sex/dose group (including animals sampled for blood at 24 h time point (see section 5.1 and Table 7) will be sacrificed at 24 h after treatment for organ sampling only. Animals sampled for blood at earlier time points will be discarded without necropsy (see Table 7).

Table 6: Organ weight, Tissue Preservation and Microscopic Examination in all animals

Organs	Organ weight	Preservation	Tissue preparation	Microscopic examination
Macroscopic lesions		X	X	X
Adrenal glands	X	X	X	X
Aorta*		X	X	X
Bone (femur) and articulation		X	X	X
Bone (sternum) with bone marrow		X	X	X
Bone marrow smears		X	X	
Brain*	X	X	X	X
Bronchi (mainstem)		X	X	X
Cecum		X	X	X
Cervix		X	X	X
Colon		X	X	X
Duodenum		X	X	X
Epididymides* (both right and left) ^a	X	X	X	X
Esophagus		X	X	X
Eyes		X	X	X
Harderian glands		X	X	X
Heart*	X	X	X	X
Ileum		X	X	X
Injection site(s)		X	X	X
Jejunum		X	X	X
Kidneys*	X	X	X	X
Liver*	X	X	X	X
Lungs*		X	X	X
Lymph nodes (mandibular)		X	X (left only)	X (left only)
Lymph node (mesenteric)		X	X	X
Mammary gland*		X	X	X
Nasal cavity and Zymbal's glands		X		
Optic nerves		X	X	X
Ovaries*	X	X	X	X

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Oviducts (Fallopian tubes)		X		
Pancreas		X	X	X
Parathyroid glands		X	X	X
Pituitary gland	X	X	X	X
Prostate	X	X	X	X
Rectum		X		
Salivary glands (mandibular, parotid, sublingual)		X	X (left only)	X (left only)
Sciatic nerve (left only)		X	X	X
Seminal vesicles		X	X	X
Skeletal muscle		X	X	X
Skin*		X	X	X
Spinal cord (cervical, thoracic, lumbar)		X	X	X
Spleen*	X	X	X	X
Stomach*		X	X	X
Testes* ^a	X	X	X	X
Thymus*	X	X	X	X
Thyroid glands	X	X	X	X
Tongue		X		
Trachea		X	X	X
Urinary bladder*		X	X	X
Uterus*	X	X	X	X
Vagina		X	X	X

Reviewer's Table adapted from Sponsor's Table (page 30 of 37).

HistopathologyGeneral histopathological methods

- Histopathology will be performed according to SOPs for animals in subgroups A, B and C (see Table 3).
- Histopathological examinations will be performed on all selected organs and tissues for all animals dead or killed moribund and for all animals in groups 1 (control) and group 4 (high dose) sacrificed at the end of the treatment period.
- The liver, skin, bone and kidneys will be examined in all dose groups.
- Any target organs identified in group 4 will be examined from group 2 and 3 animals killed at the end of the treatment period.
- Histopathological examination will be performed for all gross lesions from animals in all groups.

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

- Bone marrow smears will be prepared for all animals including those killed moribund.

- Slide staining (see Table 3 for details)

Sperm analysis (count and motility) in Subgroup C animals

A detailed qualitative examination of the testes, including tubular stages of the spermatogenic cycle, will be performed in all subgroup C males. Cell- or stage-specificity of testicular findings will be recorded by PAS staining.

A detailed examination of the ovaries for developmental and other lesions will be performed in all subgroup C females

Toxicokinetics (TK)**a) Blood sampling (for Gadoterate meglumine measurement)**

i) Animals: Toxicokinetics will be performed on subgroup C and D animals (Table 7). After blood sampling on PND 30/31, Subgroup C animals will be kept for a 60-day treatment-free period.

ii) Test item bioanalysis in plasma: The test item in plasma will be assayed using a validated LC-MSMS method.

iii) TK evaluation: TK parameters (C_{max}, T_{max}, AUC and Accumulation ratio, and dose proportionality) will be determined by non-compartmentalization PK methods.

Table 7: Blood sampling time-points and sampling day(s) for control and treated groups

Subgroup	Dose group	Sampling time (post-dose)	No. of rats/dose group	Sampling Day
D	Group 1 (control)	1h	3 males + 3 females	PND 10
	Groups 2, 3 & 4	5 min		
		0.5h		
		2h		
		6h		
		24h		
		Total = 18/sex	PND 11	
C	Group 1 (control)	1h	15 males + 15 females	PND 30
	Groups 2, 3 & 4	5 min & 6h	3 males + 3 females	PND 30
		0.5h & 24h	3 males + 3 females	PND 30/31
		2h	3 males + 3 females	PND 30
				Total = 24/sex

Reviewer's Table based on sponsor's data. In subgroup D, blood samples (125 µL/rat) will be collected after decapitation under pentobarbital anesthesia. In subgroup C, blood samples (500

Neonatal Rat Repeated-Dose Toxicity (Final Study Protocol Review)

µL/rat) will be collected via the retro-orbital sinus under isoflurane anesthesia. In both subgroups, rats will not be fasted before blood collection. Samples will contain Lithium-Heparin as anticoagulant and will be centrifuged within 1 h of sample collection.

b) Organ sampling (for total Gadolinium measurements)

i) Subgroups A, B and C: At necropsy, the first 6 surviving animals/sex/group in subgroups A, B and C will be selected and sacrificed for determination of total Gadolinium (Gd) in skin, bone, liver and kidneys

ii) Subgroup D: After blood sampling at the 24 h time-point, 6 animals/sex/group will be sacrificed for measurement of Gd in the skin, bone, liver and kidneys. Subgroup D (satellite) animals sampled for blood at previous time points will be killed and discarded without necropsy after blood sampling.

Sponsor's proposed study dates for study DGD-33-041/(b) (4) 14315-D:

- Current study status: Ongoing
- Projected completion: September, 2013
- Final study report to FDA: December, 2013

(Source: Guerbet Pediatric Plan Deferral Request – Email of 2/25/2013 Re: PERC Documents)

Reviewer's comments

The aim of the proposed study is acceptable and reflects FDA recommendation to include i) a single dose toxicity and ii) a 60-day treatment-free period. The choice of species (rat), age (starting age PND10), route of administration (intravenous) and doses of the test article are acceptable. All FDA recommendations to the sponsor have been included in the final protocol.

Recommendation

The final study protocol is acceptable.

COMMENT TO SPONSOR

The proposed single dose and repeat dose toxicity study in neonate and juvenile rats titled, "Dotarem – neonatal and juvenile (pre-post weaning) toxicity study by the intravenous route in the rat" and described in your revised study protocol (b) (4) -14315-D for study number DGD-33-041 is acceptable.

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/s/

OLAYINKA A DINA
03/19/2013

ADEBAYO A LANIYONU
03/19/2013

Tertiary Pharmacology Review

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

NDA: 204781

Submission date: 9/20/2012

Drug: gadoterate meglumine

Sponsor: Guerbet LLC

Indication: magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adult and pediatric patients (2 years of age and older) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity

Reviewing Division: Division of Medical Imaging Products

Background Comments:

The pharmacology/toxicology reviewer and team leader in the Division of Medical Imaging Products reviewed the nonclinical information for gadoterate meglumine and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above in adults and children ages 2 and older. The reviewer noted that juvenile animal toxicity studies to support use in ages less than 2 years were not provided. It also appears that exposure to pups in the pre/postnatal study in rats was likely to have been very low given the negligible excretion in milk, low oral absorption, short half-life and because the pups were not dosed directly. The division has routinely recommended that juvenile animal studies be conducted to support the use of all gadolinium-based contrast agents in pediatric subjects. This recommendation is, in part, based on the concern of potential effects of gadolinium-based agents on the developing kidney and a lack of sufficient human data in children under 2 years of age.

Carcinogenicity studies have not been conducted with gadoterate meglumine. This is acceptable because the product is an imaging agent that is used acutely.

Conclusions:

I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. Calling gadoterate meglumine a gadolinium-based contrast agent for its Established Pharmacologic Class is consistent with other drugs in the class. I concur with the labeling changes suggested in the pharm/tox review.

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/s/

PAUL C BROWN
03/15/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Pharmacology/Toxicology Review 2

Purpose: To include the review of studies inadvertently omitted in the finalized Pharmacology / Toxicology Review of Gadoterate meglumine (DOTAREM) archived in DARRTS on 02/21/2013 under NDA 204781.

Table 1: Application Information

Application number:	NDA 204-781
Supporting document/s:	SD-1 / eCTD sequence No. 0000
Review No.	002
Applicant's letter date:	September 20, 2012
CDER stamp date:	September 20, 2012
Product:	Gadoterate meglumine Injection
Indication:	Magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity
Applicant:	Guerbet LLC, 1185 W. 2 nd Street, Bloomington, IN 47403-2160
Review Division:	Division of Medical Imaging Products (HFD-160)
Reviewer:	Olayinka A. Dina, D.V.M., Ph.D.
Supervisor/Team Leader:	Adebayo Lanionu, Ph.D.
Division Director:	Rafel Dwaine Rieves, M.D.
Project Manager:	James W. Moore, Pharm.D, M.A.

Disclaimer:

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204-781 are owned by Guerbet LLC or are data for which Guerbet LLC has obtained a written right of reference. Any information or data necessary for approval of NDA 204-781 that Guerbet LLC does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204-781.

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Safety Pharmacology

In vitro studies

DGD-2-8-A: In vitro study of the Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine, Gd-EDTA on the coagulation system and platelet function - the role of Calcium

DGD-2-6-A: Study of the effect of the Gadolinium complexes Gd-DOTA meglumine and Gd-DTPA meglumine on erythrocytes

DGD-2-2-A: In vitro study of Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine on the complement system

DGD-2-3-A: In vitro study of the histamine-releasing potential of the Gadolinium complexes Gd-DOTA and Gd-DTPA meglumine

DGD-2-10-A: Study of hemodilution in the conscious rabbit after intravenous injection of G449-06

Pharmacokinetics

Oral Absorption

DGD-0-2-A: Variation in the biodistribution of Gd-DOTA in the rat during the first hour after IV bolus injection

Toxicology

Local Tolerance

DGD-1-14-A: G449-06 - Acute subcutaneous toxicity study in rats

DGD-1-15-A: G449-06 - Acute intramuscular toxicity study in rats

Embryo-fetal Development

99.12.802: Embryotoxicity study of Gadoterate meglumine by the intravenous route in the rabbit (segment II)

1. In-vitro Safety Pharmacology studies

1.1 Report No. DGD-2-8-A: In vitro study of the Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine, Gd-EDTA on the coagulation system and platelet function - the role of Calcium

Rationale: The possibility that Gd ions may be substituted for by calcium ions in the Gd complexes (Gadoterate meglumine and Gd-DTPA) was investigated. In this study coagulation and platelet function tests were carried out to compare the interactions of Gadoterate meglumine (Gd-DOTA) and Gd-DTPA with the hemostatic system.

Methods: The following tests were conducted as summarized below:

- General coagulation test exploring the extrinsic pathway (prothrombin time, PT), the intrinsic pathway (activated partial thromboplastin time, APTT) and fibrin formation (thrombin time, TT; reptilase time, RT)
- Fibrinogen A generation test: To explore the formation of soluble fibrin monomers
- Determination of the optimal level of calcium by APTT determination

- Exploration of the coagulation factors involved in the thrombin activation factors II, V, VII and X
- Exploration of the platelet aggregation induced by collagen and the ATP and ionized calcium excretion functions.
- Investigation of the complexing effect of Gadoterate meglumine and Gd-DTPA with regard to ionized calcium using standard solutions of calcium or ionized plasma calcium

For coagulation tests, Gd complexes were used at concentrations of 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} M. For platelet aggregation tests, Gd complexes were used at 25 and 50 mmol/L.

Results: Under the experimental conditions, Gd complexes a) produced a moderate increase in the coagulation time in the fibrin-forming system, b) induced a partial inhibition of platelet aggregation with no effect on excretion function and c) reduced the concentrations of ionized calcium solutions. All the described effects occurred at concentrations above 10 mmol/L (10^{-2} M) for coagulation tests; 50 mM for the platelet aggregation tests and 25 mM and 50 mM for tests of calcium complexing.

Conclusions There was a moderate increase in the coagulation time in a fibrin-forming system; there was a partial inhibition of platelet aggregation with no effect on excretion function and the concentrations of ionized calcium solutions were reduced by the presence of the Gd complexes. The observed effects occurred at the higher concentrations of Gadoterate meglumine and Gd-DTPA in excess of 10 mM.

Reviewer's comment: I agree.

1.2 Report No. DGD-2-6-A: Study of the effect of the Gadolinium complexes Gd-DOTA meglumine and Gd-DTPA meglumine on erythrocytes

Rationale: Given the intravascular use of Gd complexes in imaging, their potential effect on erythrocytes was investigated *in vitro* using human, rabbit and rat blood. The outer layer of erythrocytes (RBCs) is semi-permeable and in the normal state, has physical properties which ensure the maintenance of intracorpuseular hemoglobin concentration. Depending on the osmotic or molecular environment of the RBCs, a modification of their physical properties may occur. Modifications can include deformability during passage through narrow blood capillaries, morphological changes and the release of hemoglobin due to cell lysis.

Methods: This *in vitro* study of the interaction of Gd complexes (Gadoterate meglumine and Gd-DTPA) on erythrocytes was performed via several tests: (a) test for hemolytic effect and a study of the osmotic resistance of RBCs were performed using human blood (b) test for hemolytic effect and morphology of RBCs were performed using rat samples while filterability test was performed on rabbit blood samples.

Gd complexes were used at the following concentrations depending on the test:

- Hemolysis test on human blood (5, 25, 50 and 125 mmol/L)
- Hemolysis test on rat blood (1, 10, 50 and 125 mmol/L)
- Hemolysis test on rabbit blood (50, 125 and 250 mmol/L)
- Osmotic resistance test on human blood (5, 25, 50 and 150 mmol/L)
- Filterability test and erythrocyte morphology (1, 10, 50 and 125 mmol/L)

Results: The Gd complexes (Gadoterate and Gd-DTPA) had no hemolytic effect on human and rabbit RBCs. At 125 mmol/L, the Gd complexes produced hemolysis on rat erythrocytes. There was no change in the osmotic resistance of human erythrocytes after contact with the Gd complexes. The filterability of rat blood was modified by the Gd complexes at concentrations from 10 mmol/L. A slight crenation (deformation) of erythrocytes was observed only at high concentrations as from 125 mmol/L for the two Gd complexes.

Conclusion: Human RBCs exposed to the Gd complexes did not show any change in osmotic resistance. The test for hemolytic effect was negative for human and rabbit blood, however, hemolysis was observed at 125 mmol/L for both Gd complexes in rat blood as from 125 mM. The filterability index, evaluated using rat erythrocytes, was increased and reflected a reduction in deformability. Overall, the sensitivity of erythrocytes in the presence of Gd complexes varied with species.

Reviewer's comment: I agree.

1.3 Report No. DGD-2-2-A: In vitro study of Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine on the complement system

Rationale: Iodinated urographic and angiographic contrast agents have been described as activators of the complement system. The complement system is an enzyme system involving a large number of plasma proteins the activation of which occurs in a cascade and may be triggered by an immunological phenomenon. Complement activation results in inflammatory and cytolytic reactions. This study aimed to determine the potential of Gadoterate meglumine and Gd-DTPA to act as activators of the complement system.

Methods: The test was carried out using two methods: a) the determination of the hemolytic complement (HC50) using guinea pig serum and human serum, and b) the assay of anaphylatoxins C3a and C5a, using human plasma. In both methods, guinea pig sera and plasma samples were incubated for 1 h at 37⁰C in the presence of dilute solutions of the Gd complexes. The Gd complexes were diluted in a Veronal buffer (pH 7.2) and were tested at final dilutions of 1, 5 and 10% (v/v) or 5, 25 and 50 mmol/L. Plasma anaphylatoxins C3a and C5a are produced when the complement system is activated and can be assayed by radioimmunoassay (RIA).

Results/Conclusion: The two Gd complexes caused a dose-dependent reduction of total hemolytic activity of the complement and reduction in the production of C3a. The production of C5a was not affected by the Gd complexes.

Reviewer's comment: I agree.

1.4 Report No. DGD-2-3-A: In vitro study of the histamine-releasing potential of the Gadolinium complexes Gd-DOTA and Gd-DTPA meglumine

Rationale: Iodinated urographic and angiographic contrast agents can potentially induce histamine release *in vitro*. This study evaluated the potential of the Gd complexes, Gadoterate meglumine and Gd-DTPA, to cause histamine release *in vitro* using rat peritoneal mast cells to quantitatively determine histamine or serotonin release.

Methods: The amine-releasing potential of the Gd complexes (Gadoterate meglumine and Gd-DTPA) was evaluated using a suspension of rat mast cells obtained by peritoneal washing. The test concentrations for determining serotonin release were 10, 50 and 100 mmol/L for serotonin release and 50, 125 and 150 mmol/L for histamine release. Serotonin release was measured by counting the quantity of ³H-serotonin release and histamine release determined by radioimmunoassay.

Results:

Serotonin release – No serotonin release was detected at the concentrations of Gd complexes tested.

Histamine release – Results also indicated no change in histamine level at the concentrations of Gadoterate meglumine and Gd-DTPA tested.

Conclusion: In the range of concentrations of Gd complexes tested to evaluate serotonin or histamine release, both Gd complexes did not produce any release of serotonin or histamine from rat mast cells.

Reviewer's comment: I agree.

1.5 Report No. DGD-2-10-A: Study of hemodilution in the conscious rabbit after intravenous injection of G449-06

Objective: The purpose of this study was to evaluate any potential effects of Gadoterate meglumine on plasma volume in conscious New Zealand rabbits following intravenous injection.

Methods: Gadoterate was administered as a bolus IV injection to two groups of 4 and 8 rabbits at the dose level of 0.1 and 1.0 mmol/kg (or 0.32 – 3.24x the human dose), respectively. The hematocrit was measured before and up to 30 min after injection.

Results: The hematocrit in rabbits which received the low dose (0.1 mmol/kg or 0.32x MHD) fell by a maximum of 5% the pre-dose value (at time zero, T0) within 30 min following administration of Gadoterate meglumine. A reduction of up to 13% of the pre-dose

value occurred 1 min after treatment in rabbits which received the the high dose (1.0 mmol/kg or 3.24x MHD) and fell to 3% of the pre-dose value within 30 min.

Table 2: Influence of Gadoterate meglumine injection on NZW rabbit Hematocrit

Dose	0.1 mmol/kg		1.0 mmol/kg	
	Hematocrit (%)	% Variation in the mean vs. Control	Hematocrit (%)	% Variation in the mean vs. Control
Pre-dose (T0)	38.5±0.88	0	41.0±0.66	0
0.5	38.3±0.59	0.5	36.8±0.80	11.5
1	38.3±1.13	0.5	36.3±0.68	12.7
2	37.8±0.96	1.8	37.3±0.78	10.3
5	37.8±1.05	1.8	39.0±0.60	6.3
7	37.3±0.76	3.1	39.5±0.83	5.0
10	36.8±0.96	4.4	40.1±0.66	3.8
15	36.8±1.27	4.4	40.1±0.84	3.6
20	36.8±0.96	3.9	40.0±0.93	3.8
30	36.5±1.21	5.0	40.3±0.88	3.1

Conclusion: Based on the results, Gadoterate meglumine at the single dose of 0.1 mmol/kg (intended clinical dose) had no effect on the hematocrit in rabbits.

Reviewer's comment: I agree.

2. Pharmacokinetics (Rat / Oral study)

2.1 Report No. DGD-0-8-A: Comparative pharmacokinetics of Gd-DOTA and GdCl₃ administered by the oral route in the conscious rat

Report location:	eCTD Module 4 §4.2.2.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	86.10.2.01
Date of study initiation:	November, 1986
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA (G. 449.06; Gadoterate meglumine), batch No. 202; Gd-DTPA (P. 439), batch No. 6; GdCl ₃ solution % purity – N/A

Following administration, rats were anesthetized using a mixture of halothane and Nitrogen protoxide. Heparinized blood samples were then collected via the abdominal aorta at 0.5, 1, 2, 4 and 6 h following treatment. Urine samples were collected from all rats to determine the excretion of Gadoterate meglumine. Livers were removed from rats treated with GdCl₃. For each test article and each time point, 4 rats (2/sex) were sacrificed.

Treatment of samples: Blood was centrifuged (3000 rpm) and plasma diluted to twice its volume in CsCl₂ at 25 g/L. Livers were weighed and a fraction (approximately 1 g) was mineralized in nitric acid (1mL HNO₃/100 g of tissue).

Assay of Gd: Gd assay was performed using Atomic Emission Spectrophotometry (AES) method at 342.247 nm. Each sample was assayed 3 times to determine the mean concentration. Parameters evaluated include plasma Gd³⁺ (μmol/L), urine Gd³⁺ (mmol/L) and liver Gd concentration (μmolGd/kg). The weight of the liver was expressed in kg and the %recovery of Gd was calculated based on the total weight of the liver in relation to the dose injected.

Results:

1. Plasma Gd concentration: Following oral administration (Table 4), plasma Gd³⁺ attained a C_{max} of approximately 20 μmol/L 1 h after treatment. Peak Gd-DTPA was lower (~9 μmol/L) in the same time period (1 h). There was no detectable trace of Gd in plasma after GdCl₃ administration.

Table 4: Plasma Gd concentrations after oral administrations of Gd-DOTA, Gd-DTPA or GdCl₃ in rats

Sample time (h)	Plasma concentration (μmol/L) (Mean ± SEM)		
	Gd-DOTA	Gd-DTPA	GdCl ₃
0.5	11.37 ± 2.48	8.94 ± 4.35	0
1	19.67 ± 9.40	6.36 ± 2.45	0
2	11.77 ± 4.35	3.08 ± 1.94	0
4	1.55 ± 0.70	0.18 ± 0.25	0
6	1.67 ± 0.54	0.00 ± 0.00	0

Sponsor's Table (Table 1; PK summary report 3_01_010029, page 4 of 22)

2. Urinary Gd concentration: Gd was rapidly excreted in urine after oral administration of Gadoterate meglumine or Gd-DTPA. Approximately 6 mmol/L of Gadoterate meglumine was excreted in the urine after 1 h. After 6 h, urinary excretion of Gadoterate meglumine (0.694 mmol/L) was excreted compared to excretion at the 1 h time point. Similar results were obtained with Gd-DTPA.

Table 5: Mean urinary Gd concentrations following oral administration of Gadoterate meglumine (Gd-DOTA) or Gd-DTPA) in rats

Sample time (h)	Urinary concentration (mmol/L)	
	Gd-DOTA	Gd-DTPA
0.5	7.256	3.227
1	5.555	7.354
2	2.631	3.708
4	2.909	3.446
6	0.694	0.297

Table (Table 3; PK summary report 3_01_010029, page 5 of 22)

3. Liver Gd concentration: The levels of Gd in the liver following oral administration of GdCl₃ are shown in Table 6:

Following oral administration GdCl₃, Gd was not detected in the plasma or urine. However, approximately 1.5 µmol/kg of liver Gd was measured in the liver after a Tmax of 1-2 h. This result indicated a poorly absorbed after from the gastrointestinal tract but was concentrated in the liver.

Table 6: Mean Gd levels in the liver after oral administration of GdCl₃ in rats

Time after treatment (hour)	Mean Gadolinium (GdCl ₃) level (µmol/kg liver)	% injected dose recovered
0.5	0.405	0.005
1	1.430	0.022
2	1.480	0.022
4	0.055	0.001
6	0.0	0.0

Table (Table 2; PK summary report 3_01_010029, page 5 of 22)

Discussion

The findings of this oral absorption study of Gadoterate meglumine, Gd-DTPA and GdCl₃ indicated that Gadoterate meglumine and Gd-DTPA were poorly absorbed from the gastrointestinal tract reaching their peak concentrations in plasma within the first hour. Both compounds were excreted via the urinary route. In contrast, following oral administration, GdCl₃ was not detected in plasma but was taken up by the liver. GdCl₃ was not excreted in the urine in detectable proportions.

Conclusion

The results showed that the absorption of Gadoterate meglumine and Gd-DTPA was low following oral administration and no detectable amounts of Gd Chloride (GdCl₃) were found in the plasma.

Reviewer's comments

I agree with the findings of the study.

3. Local Tolerance

3.1. DGD-1-14-A: Acute subcutaneous toxicity study in rats

Objective: The purpose of this study was to evaluate the local tolerance of Gadoterate meglumine (G 449.06) after subcutaneous administration.

Methods: Gadoterate meglumine was administered at the single dose level of 2.5 mmol/kg (2.5 mL/kg) to 12 male and 12 female Sprague-Dawley rats. Three rats per sex were sacrificed 6 h, 3, 8 and 29 days after dosing. A saline solution (0.9% NaCl) was administered to control animals and clinical signs and body weight observed for 28 days. At necropsy, the injection site was preserved and submitted for microscopic examination.

Results: There was no mortality and no treatment-related effect was observed on body weight or behavior. At the injection site, macroscopic and microscopic changes observed on days 1 and 3 were similar between control and treated rats. The changes which were no longer present in animals sacrificed on days 8 and 29 were attributed to mechanical injury during the injection procedure.

Conclusion: Under the experimental condition of this study, Gadoterate was well tolerated locally in rats following a single subcutaneous injection.

Reviewer's comments: I agree.

3.2. DGD-1-15-A: Acute intramuscular toxicity study in rats

Objective: The purpose of this study was to evaluate the local tolerance of Gadoterate meglumine (G 449.06) after intramuscular administration.

Methods: Gadoterate meglumine was administered at the single dose level of 2.5 mmol/kg (2.5 mL/kg) to 12 male and 12 female Sprague-Dawley rats. Three rats per sex were sacrificed 6 h, 3, 8 and 29 days after dosing. A saline solution (0.9% NaCl) was administered to control animals and clinical signs and body weight observed for 28 days. At necropsy, the injection site was preserved and submitted for microscopic examination.

Results: There was no mortality and no treatment-related effect was observed on body weight or behavior. At the injection site, macroscopic and microscopic changes observed on days 1 and 3 were similar between control and treated rats. The changes which were no

longer present in animals sacrificed on days 8 and 29 were attributed to mechanical injury during the injection procedure.

Conclusion: Under the experimental condition of this study, Gadoterate was well tolerated locally in rats following a single intramuscular injection.

Reviewer's comments: I agree.

4. Embryonic Fetal Development in Rabbits (Segment II)

4.1 Report No. 99.12.802

Study Title: Gadoterate meglumine (Dotarem) – Embryotoxicity study by the intravenous route in the rabbit (segment II)

Study no.: 848/076
Study report location: eCTD Module 4 §4.2.3.5.2.1
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: January 14, 2000
GLP compliance: Yes (x), No (); page 5 of 263
QA statement: Yes (x), No (); pages 6 of 263
Drug, lot #, and % purity: Test article: Gadoterate meglumine (Gd-DOTA), supplied by Guerbet, France; batch No. 99 M 065, % purity – 100%
Control article: Sterile physiological saline (0.9 % NaCl), Supplier: [REDACTED] (b) (4)
[REDACTED] batch Nos: 9563 & 8435

Objective

The purpose of this study was to evaluate the effects of intravenously administered doses of Gadoterate meglumine on embryonic development during the organogenesis phase of pregnancy in the rat.

Key findings

Based on the incidence of mortality in five pregnant rabbits administered the high dose (7 mmol/kg/day or 23 times the intended human dose), significant reduction in food consumption and body weight gain, maternal toxicity was established as the high dose. There was no evidence of embryotoxicity at the intermediate dose level (3 mmol/kg/day or 10 times the human dose). Based on the absence of embryotoxicity at the intermediate dose, NOAEL was established at 3 mmol/kg/day (or 10 times the human dose).

Methods

Doses: 0 (control/saline vehicle), 1, 3 and 7 mmol/kg (i.e., 3.24, 9.72 and 22.7-fold the human dose adjusted for body surface area, respectively)

Frequency of dosing: Repeated doses on Gestation day (GD) 6 -19

Dose volume (mL/kg/day): 14 (control/saline), 2, 6 and 14 (for low, intermediate and high doses levels, respectively)

Route of administration: Intravenous

Formulation/Vehicle: Aqueous solution

Species/Strain: Rabbit / New Zealand White rabbit (NZW), (b) (4)

Number/Sex/Group: F₀ dams: 80 pregnant females/group were administered intravenously with Gadoterate meglumine from GD 6 - GD19 inclusive. All surviving females were sacrificed for examination of their uterine contents on GD29

Initial age: 16-18 weeks; Weight at Day 0 of gestation day (G0): 3.0 to 4.0 kg

Satellite groups: none

Study design: See Table 7 below

Deviation from study protocol: The study was performed in conformity with the original protocol no. 848/076-D. Minor protocol deviations did not affect the integrity or outcome of the study

Methods

Dose selection

Dose levels were selected on the basis of the results of a dose range-finding conducted in rabbits (Study No. 848/075; Report No. 99.12.801) in which gadoterate meglumine was administered intravenously to 6 pregnant female NZW rabbits at dose levels of 0, 1, 3 and 6 mmol/kg/day (or 3.2, 10 and 20 times the human dose based on body surface area). There were no deaths or treatment-related clinical signs in any group except a report of rapid breathing on gestation day (GD) 8 for high dose (6mmol/kg/day) animals. A transient reduction in body weight gain on GD9 to GD11 was observed in females administered the high dose (6 mmol/kg/day). Food consumption was also reduced in the same group throughout the treatment period when compared to controls. No treatment-related effects were observed in the post-implantation and fetal data. External examination of the fetuses showed some abnormalities including acaudia, spina bifida and short tail in 5 fetuses from one high dose dam. 1 fetus from another high dose dam showed forepaw hyperflexion; 1 fetus with abnormality was observed in the control group. Based on the result of this study, doses of 1, 3 and 7 mmol/kg/day were selected for the reviewed study 99.12.802.

Study Design

Table 7: Study Design and Dose groups (99.12.802)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg/day)	Number of rats Females only
1 (veh; control)	0	20
2 (LD)	1	20
3 (MD)	3	20
4 (HD)	7	20

Source: Reviewer's Table adapted from Sponsor's Study Design veh = vehicle; LD, MD, and HD = low, intermediate and high dose, respectively of Gadoterate meglumine (Gd-DOTA)

Table 8: Gadoterate meglumine human dose multiples (99.12.802)

Administration	Vehicle	Low Dose	Intermediate Dose	High Dose
Dose (mmol/kg)	0	1	3	7
Dose (mmol/m ²)	0	3.24	9.72	22.7
Dose multiples (based on BSA)	N/A	3.2x	10x	23x

Reviewer's Table; BSA = body surface area

The rats were dosed daily via the IV route from day 6 to day 19 (inclusive) of gestation. Control rats received sterile isotonic saline during the same treatment period.

Observations and Results

Observations

Table 9: Summary of Methods – Rabbit Segment II (99.12.802)

Protocol	Method, frequency and/or objectives
Clinical observations	Monitored throughout gestation. All animals were observed twice/day to detect mortality
Body weight	All animals were weighed individual on days 0, 6, 9, 13, 16, 20, 24 and 29 of gestation
Food consumption	Food consumption of each animal was recorded daily from the day of arrival to G29. The mean (g/animal/day) was calculated for the periods G0-G6, G6-G9, G9-G13, G13-G16, G16-G20, G20-G24 and G24-G29
Necropsy	All surviving females were sacrificed (intravenous sodium pentobarbital and exsanguination) and necropsied/C-section on GD 29 and uterine contents including placentae examined according to SOP. Each animal was examined and live fetuses weighed
Fetal examination	The fetuses were examined for external and visceral abnormalities and sexed. Other examinations were performed according to SOP

Source: Reviewer's Table constructed from sponsor's data

Results

F₀ generation (Dams)

Mortality: 5 pregnant rabbits administered the high dose (7 mmol/kg/day or 23 times the human dose based on body surface are) died or were sacrificed moribund between GD10 and GD22. All the animals involved showed anorexia and body weight loss. Necropsy did not reveal any treatment-related lesions.

Clinical signs: Clinical signs observed in some rabbits before death included convulsions, tremors and loss of balance. Other signs included swelling and redening of the pinnae especially in rabbits administered the high dose (7 mmol/kg/day). There was a dose-related reduction in fecal output.

Body Weight: A reduction in body weight was observed in rabbits treated with the high dose. The decrease in body weight was not statistically significant. No reduction in body weight was observed in the low and intermediate dose groups (Table 10 below).

Feed Consumption: There was a dose related decrease in food consumption throughout the treatment period. This decrease was statistically significant in the high dose ($p < 0.01$) and intermediate dose ($p < 0.01$) groups compared to controls. A minimal, statistically insignificant decrease in food consumption was observed in the low dose group.

Toxicokinetics: Not performed

Necropsy: Rabbits in the high dose group (7 mmol/kg/day) had significantly reduced ($p < 0.05$) gravid uterine weights when compared with control group rabbits. Animals in the 1 and 3 mmol/kg/day groups were not affected. Ovary weights were comparable in all dose groups.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
 (Table 10):

Table 10: The effect of Gadoterate meglumine on embryo-fetal development (99.12.802)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	1 (LD)	3 (MD)	7 (HD)
Dams (F₀)				
No. Pregnant	19	19	19	19
No. dead/sacrificed moribund	0	0	0	5
No. Aborted or with Total Resorption of Litter	0	0	1 ^b	11
No. of females evaluated	19	19	18	11
Clinical signs (reduced fecal output)	-	+	+	++
Body weight (%) ^a	3.8 kg	0	0	↓ -5.3
Food consumption (%) ^a	148 g/day	↓ -14.9	↓ -20.9	↓ -31.1**
Gravid Uterus Weight (g)	485	484	478	355*

Avg. No. of corpora lutea	11.7	11.7	11.5	9.0*
Avg. No. of implantations	10.2	10.0	9.9	7.2**
Mean pre-implantation loss (%)	11.8	14.0	13.9	18.7
F₁ litters (from females killed on GD29)				
No. of litters examined	19	19	18	11
No. live fetuses	170	172	162	71 ^{a*}
Mean No. of resorptions (early + late)	1.3	0.9	0.9	0.8
No. of dead fetuses	0	0	0	0
Mean post-implantation loss (%)	12.4	9.5	9.0	10.1
Mean fetal body weight (g)	39.9	38.6	38.7	40.1
Fetal sex ratio (% males)	54.3	42.8	50.2	45.6
Fetal anomalies				
Gross external, visceral and skeletal anomalies	-	-	-	-

Reviewer's Table based on sponsor data (Table 2.6.7.13C, Pages 6 & 7 of 7, Tabulated summaries, Table 3_10_00103).

For controls, values indicate group means (For treated groups, values indicate percent differences from controls); LD, MD, HD = Low, Mid (intermediate) and high dose levels; - = no noteworthy findings

*, ** = $p < 0.05$; $p < 0.01$ (Statistical differences were based on actual data and not on the percentage differences); ↑, ↓ = increase or decrease in the value assessed; +, ++ = mild, moderate; ^a = At the end of dosing period; ^b = sacrificed after abortion.

a. Incidence of pregnancy: An incidence of one non-pregnant rabbit was reported in each of the control, low dose (1 mmol/kg/day) and intermediate dose (3 mmol/kg/day) level. 4 non-pregnant rabbits were noted in the 7 mmol/kg/day group.

b. Pre-implantation data: The average number of corpora lutea and implantation sites in rabbits treated at the high dose level, were significantly lower when compared to controls.

Pre-implantation loss was comparable in all groups.

c. Post-implantation data: The number of resorptions (1.3) and the mean post-implantation loss (12.4%) was highest in the control groups, no clear effect of treatment on the incidence of resorption and post-implantation loss was demonstrated.

The mean live litter size was significantly decreased (71; $p < 0.05$) in the high dose (7 mmol/kg/day) group when compared to the control group (170). There were no dead fetuses at any dose level.

Offspring (Malformations, Variations, etc.)

Fetal data: There was no treatment-related effects on fetal weight and fetal ratio.

Fetal examinations (External and Visceral). Table 11 indicates the number of fetuses and litters examined:

Table 11: Number of fetuses and litters (in parentheses) examined (99.12.802)

	Group 1 Control	Group 2 1 mmol/kg/day	Group 3 3 mmol/kg/day	Group 4 7 mmol/kg/day
External examination	170 (19)	172 (19)	162 (18)	71 (10)
Internal examination:				
- thoracic and abdominal cavities only	90 (19)	90 (19)	85 (18)	38 (10)
- thoracic and abdominal cavities with head	80 (18)	82 (19)	77 (18)	33 (10)
Skeletal examination:				
- with head	90 (19)	90 (19)	85 (18)	38 (10)
- without head	80 (19)	82 (19)	77 (18)	33 (10)

At least 18 litters were examined in each of the 1 and 3 mmol/kg/day and control groups. Only 10 litters were available in the 7 mmol/kg/day group owing to the observed mortality and lower pregnancy rate (see above).

External: Observed malformations include spina bifida in 1 fetus in the control group. Spina bifida was not observed in any of fetus in the treatment groups. Besides one fetus in the high dose group with a flexed paw, no other fetuses had external findings in any group.

Visceral: One malformed fetus with cardiac defects was observed in each of control and low dose (1 mmol/kg/day) groups. No similar defect was observed at the intermediate and high dose levels. There were no malformations in the fetal heads examined in any study group.

Skeletal: One fetus in the high dose group had ectrodactily (missing first digit in both forepaws. A fetus in the high dose group with a flexed forepaw was noted earlier.

Conclusions: Five rabbits administered the high dose (7 mmol/kg/day) died. There was a slight maternal toxicity at the intermediate dose (3 mmol/kg/day) resulting from a reduced maternal food consumption and in body weight gain. However, there was no evidence of embryotoxicity at the intermediate dose level (3 mmol/kg/day).

Reviewer’s comments: Based on the incidence of mortality in 5 pregnant rabbits administered the high dose, significant reduction in food consumption and body weight gain, maternal toxicity was established as the high dose (7 mmol/kg/day or 23 times the intended human dose). There was no evidence of embryotoxicity at the intermediate dose (3 mmol/kg/day or 10 times the human dose). Based on the absence of embryotoxicity at the intermediate dose, NOAEL was established at 3 mmol/kg/day (or 10 times the human dose). Overall, I agree with the findings.

NDA #: 204-781 (Gadoterate meglumine) Reviewer: Olayinka A. Dina, DVM, Ph.D.
PT Review 2 (Addendum to NDA Review)

Concluding Reviewer's Remarks: The review of *in vitro* Safety Pharmacology studies DGD-2-8-A, 2-6-A, 2-2-A, 2-3-A and 2-10-A; Pharmacokinetic study DGD-0-8-A; Local Tolerance studies DGD-1-14-A and 1-15-A and Embryofetal study of Gadoterate meglumine in Rabbits (99.12.802) in Pharm/Tox review 2 complement the Pharmacology/Toxicology review of Gadoterate meglumine (NDA 204-781) archived in DARRTS on February 21, 2013 (Reference ID No. 3264990).

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/s/

OLAYINKA A DINA
03/05/2013

ADEBAYO A LANIYONU
03/05/2013

Supervisory Pharmacologist Memo

NDA: 204-781
Drug: DOTAREM® (gadoterate meglumine)
Sponsor: Guerbet LLC.

Gadoterate meglumine Injection for intravenous use is a paramagnetic macrocyclic ionic contrast agent for magnetic resonance imaging (MRI). The proposed indication is for use in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity. The proposed dose is 0.1 mmol/kg body weight as an intravenous bolus injection, manually or by power injector, at a flow rate of approximately 2 mL/second for adults and 1-2 mL/second for children.

Dr. Dina conducted the Pharmacology/Toxicology primary review of the NDA. Dr. Dina concluded that the results of the nonclinical studies indicate no significant safety concerns for adults and pediatric patients ≥ 2 , and recommended approval for these age groups from pharmacology/Toxicology perspectives. Dr. Dina noted the absence of juvenile animal toxicity study to support the safe use of Dotarem in pediatric patients ages ≤ 2 . Of note is that the Medical Imaging Drugs Advisory Committee meeting on Dotarem held on February 14, 2013 extensively discussed the value of a juvenile animal study to support approval in this age group. Dr. Dina did not recommend approval for this age group. He proposed changes in the label.

I concur with his approval recommendations.

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/s/

ADEBAYO A LANIYONU
02/21/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 204-781
Supporting document/s:	SD-1 / eCTD sequence No. 0000
Applicant's letter date:	September 20, 2012
CDER stamp date:	September 20, 2012
Product:	Gadoterate meglumine Injection
Indication:	Magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity
Applicant:	Guerbet LLC, 1185 W. 2 nd Street, Bloomington, IN 47403-2160
Review Division:	Division of Medical Imaging Products (HFD-160)
Reviewer:	Olayinka A. Dina, D.V.M., Ph.D.
Supervisor/Team Leader:	Adebayo Lanijonu, Ph.D.
Division Director:	Rafel Dwaine Rieves, M.D.
Project Manager:	James W. Moore, Pharm.D, M.A.

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204-781 are owned by Guerbet LLC or are data for which Guerbet LLC has obtained a written right of reference. Any information or data necessary for approval of NDA 204-781 that Guerbet LLC does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204-781.

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1 Executive Summary

1.1 Introduction

Gadoterate meglumine (Dotarem), a paramagnetic, macrocyclic ionic gadolinium (Gd) compound is proposed for use as a contrast agent for magnetic resonance imaging (MRI). As a macrocyclic compound, the gadolinium ion (Gd^{3+}) is bound in a stable complex. The macrocyclic structure in Gadoterate meglumine is 1,4,7,10-tetraazacyclododecane-N, N',N'',N'''-tetraacetic acid or DOTA).

Gadoterate meglumine is intended for intravenous use in adults and pediatric patients (neonates to 17 years) at a recommended dose of 0.1mmol/kg (or 3.7 mmol/m² based on body surface area) to detect and visualize areas with disrupted blood brain barrier (BBB) and/or abnormal vascularity. Following administration, Gadoterate meglumine rapidly distributes to the extracellular fluid. Similar to other MRI contrast agents, Gadoterate meglumine is water-soluble, does not cross the intact blood-brain barrier and is excreted unchanged in the urine.

1.2 Brief Discussion of Nonclinical Findings

1.2.1 Pharmacology

Primary pharmacodynamics: No study conducted

Secondary pharmacodynamics: No study conducted

Safety pharmacology:

CNS safety: Behavioral tests were conducted in conscious animals to evaluate the neurological safety of Gadoterate meglumine. There was no effect on motility. No central depressant, extrapyramidal or cataleptic effect was observed. Gadoterate meglumine did not alter the body temperature. Gadoterate meglumine solution (1350mOsm/kg) had a pro-convulsive effect following an intravenous injection of 4mmol/kg in picrotoxin-treated mice and after an intracisternal administration of Gadoterate meglumine in pentylenetetrazole-treated rats.

CVS safety: In a pivotal cardiovascular study in conscious telemetered beagle dogs, Gadoterate meglumine was administered intravenously at doses of 0.6, 2.4, 3.6 and 5.5mmol/kg (or 3.2x, 13x, 20x and 30x the clinical dose adjusted for body surface area, respectively). ECG (Lead II) was recorded continuously for up to 24 hours. At 0.6mmol/kg, no remarkable effects were observed on the heart rate (HR), arterial blood pressure (BP) and ECG parameters (PR, QT and QRS complex). There was a slight increase in HR and arterial BP at 2.4 and 3.6mmol/kg compared to the saline control. At 4h following the 3.6 mmol/kg dose, QTc was slightly reduced (-2 msec). At the highest dose (5.5.mmol/kg), there was a reduction in QTc (-8 to -13msec or 2-6% change) at 1-4 hours after dosing. NOAEL was established as 0.6mmol/kg (or 3.2x the clinical dose).

The potential effect of Gadoterate meglumine on cardiac action potential was evaluated *in vitro* on isolated canine Purkinje fibers. The results showed that Gadoterate meglumine had no effect on action potential duration in Purkinje fibers.

Respiratory system: Gadoterate meglumine was administered intravenously to mongrel dogs at doses of 0.1 to 1.0 mmol/kg (or 0.54x to 5.4x the human dose). The high dose (1.0 mmol/kg) resulted in a 4-16% increase in respiratory rate. Lower doses (0.1 and 0.5 mmol/kg) did not elicit any pulmonary effects. Based on the results, the NOAEL was determined as 0.5 mmol/kg (or 2.7x the human dose).

Renal system – The effect of Gadoterate meglumine on renal function was evaluated in a glycerol-induced renal failure model in the rat. Gadoterate meglumine was administered as single intravenous dose of 2 mmol/kg (or 3.2x the human dose). Gadoterate meglumine (or its comparator Magnevist administered at the same dose), did not cause an exacerbation of the impairment of renal function induced by glycerol in rats. As expected, intramuscular injection of glycerol caused a significant increase in urinary excretion of proteins, plasma creatinine, and urea.

A safety pharmacology study was conducted to evaluate potential effects of Gadoterate meglumine on renal function in normal rats and rats pretreated with (L-NAME (N- ω -nitro-L-arginine methyl ester). The animals were administered Gadoterate meglumine (2 mmol/kg or 3.2x the clinical dose adjusted for body surface area) intravenously for 14 days. Gadoterate meglumine was well tolerated with or without L-NAME. L-NAME is an inhibitor of nitric oxide. Inhibition of nitric oxide and prostanoids may result in renal vascular effects including nephrotoxicity. Results showed no adverse effect on renal function. Renal kidney hypertrophy observed 24h following the last treatment was no longer evident after a 28-day treatment-free period. Proximal convoluted tubule vacuolation, seen in all animals euthanized 24h after the last treatment with Gadoterate meglumine, was also not evident after the recovery period. By the end of the treatment-free period, all notable and significant changes in plasma and urinary parameters and histological findings were reversible.

1.2.2 Pharmacokinetics

Studies were conducted to determine the PK profile of Gadoterate meglumine when administered in single- or repeat-dose intravenous administration to mice, rats, rabbits, dogs and goats. Gadolinium (Gd) retained in the body was determined in tissues and biological samples using the Atomic Emission Spectrophotometry (AES) method. The Gadoterate meglumine formulation used in animal PK studies was the same as the to-be-marketed formulation tested in clinical trials. In multiple studies, the PK of Gadoterate meglumine (Gd-DOTA) was compared to that of Magnevist (Gd-DTPA), the gadolinium-based contrast agent that was available at the time these PK studies were performed.

Single-dose PK: Results of nonclinical PK single-dose studies of Gadoterate meglumine in different animal species revealed: 1) a rapid distribution of Gd-DOTA in several organs, 2) the highest Gd concentrations were observed in the kidneys and bone, 3) half-life ($t_{1/2}$) across species was rapid (approximately 1 hr), 4) there was no protein binding or metabolism of Gadoterate meglumine, 5) there was a rapid urinary elimination and a low biliary excretion of Gd, 6) results indicated negligible excretion of Gadoterate meglumine in milk, 7) there was evidence of

transplacental transfer of Gadoterate meglumine, 8) Gadoterate meglumine was poorly absorbed via the oral route.

Repeat-dose PK: Following repeated dose administration of Gadoterate meglumine at doses of 0.5, 0.7 and 1.5 mmol/kg in rats (or 0.8, 1.14 and 2.43-fold MHD) over a period of 28 days followed by a 28-day treatment-free period. Gadolinium was detected in the kidneys, liver and femur 1 day after the end of the 28-day treatment period.

Gd concentration was dose-dependent and highest amounts were obtained in the kidney. A linear relationship was obtained between Gd concentration in tissues and the dose administered. Gd concentration was considerably decreased following the treatment-free period with slight amounts measurable in high dose animals. The low and mid dose groups were not evaluated for Gd content after the treatment-free period.

Gadoterate meglumine was administered to dogs in doses of 0.5, 0.7 and 1.5 mmol/kg (or 2.70, 3.78, and 8.11-fold MHD) over 28 days of treatment followed by a 28-day treatment-free period. Similar to the finding in rats, Gadolinium was detected in the kidneys, liver and femur. Samples were obtained 24 h following the first injection, after the last injection, and at the end of the 28-day treatment-free period.

As for rats, PK was linear. At the end of the treatment-free period, highest amounts of Gd were obtained in the kidneys. The repeat dose PK studies confirmed the findings of the single-dose PK studies. Of note, in the single dose studies, a small fraction of Gd was detected in the liver and bone.

Under conditions of repeated exposure, higher levels of tissue Gd were obtained with implications for a greater, long-lasting retention of Gadolinium in the body. It has been shown in the literature that bone tissue may serve as a site for Gd storage. Long-term persistence and slow release of Gd^{3+} from bone stores could therefore enhance Gd-associated toxicity.

It is also noteworthy that the skin was not evaluated for Gd content in view of the importance of the role of skin Gd content in the pathophysiology of the onset and propagation of NSF.

1.2.3 Toxicology

Single-dose toxicity: Expanded single-dose toxicity studies were performed in rats and dogs.

In rats, Gadoterate meglumine was administered intravenously at dose levels of 7, 10.1, and 14.5mmol/kg (or 11x, 16x or 24x the human dose, respectively). There were no treatment-related mortalities. Treatment-related clinical signs included piloerection and half-closed eyes were observed in rats of both sexes; swollen muzzle was observed in 6/10 males and 6/10 females, decreased physical activity (3/10 males) and respiratory difficulties (2/10 males). Gadoterate meglumine did not cause remarkable findings in hematology, ophthalmoscopy, food consumption or body weight. Biliary proliferation was observed in animals administered the high dose (14.5 mmol/kg or 24x the human dose). Findings were reversible at post-recovery day 15 sacrifice. Based on these results, the NOAEL for the single-dose toxicity study in rats was established at 7mmol/kg (or 11x the clinical dose).

In dogs, Gadoterate meglumine was administered as a single intravenous dose at the dose levels of 2.5, 5 or 7.5 mmol/kg. There was no mortality. Treatment-related clinical signs of vomiting and urination were observed in males and female dogs following injection at all administered doses. The vomiting and urination were dose-related. According to the sponsor, the urination was due to the high osmolarity of the test article. There were no remarkable effects on body weight, food consumption, ocular signs, hematology, and serum chemistry parameters. Urinalysis showed an increased urinary volume on day 2 in males administered the high dose (7.5mmol/kg). High urine volume was associated with reduced sodium and chloride excretion. No abnormalities were observed in urine volume, sodium, or chloride at the end of the observation period. There were also no treatment-related changes in organ weight. On day 2, vacuolation of renal tubules occurred at the mid and high dose levels and in hepatocytes at the high dose level. The vacuolation, which was no longer evident at day 15 in males and females, was not associated with degeneration or tubular necrosis. Based on the results, NOAEL for single-dose toxicity in dogs was established at 2.5 mmol/kg (or 14-fold MHD) since no renal tubular vacuolation was observed at this dose level.

Repeat-dose toxicity: Following repeated administration of Gadoterate meglumine in the rat at dose levels of 2, 4 and 8 mmol/kg (or 3.2x, 6.5x and 13x the human dose) over a 4-week period followed by a 13-week treatment-free period, no test article-induced mortality was reported. Findings, where present, were observed at the mid (4mmol/kg) and high dose (8mmol/kg) levels. The findings were mostly reversed after the 13-week post-treatment, treatment-free period. NOEL was not established since vacuolation was also observed at the low dose (2 mmol/kg/day or 3.2x MHD).

Similar to the rat, Gadoterate meglumine was administered in repeated doses (0.3, 0.7 and 1.5mmol/kg (or 1.6x, 3.8x and 8.1x the human dose) over a 4-week period followed by a 13-week treatment-free period in the dog. Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. There was also a dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility/recovery period. Based on the finding of renal tubular vacuolation in all treated animals, NOEL was not established in this study.

Genetic toxicity: Gadoterate meglumine was not mutagenic (with or without metabolic activation) in the Ames test, Chromosomal aberration test on Chinese Hamster ovary cells, a gene mutation test in Chinese Hamster lung cells and in the *in vivo* micronucleus test in mice.

Carcinogenicity: No studies were conducted.

Reproductive toxicity: i). Fertility and early embryonic development: In a study to evaluate the effect of Gadoterate meglumine on fertility and early embryonic development, Gadoterate meglumine was administered intravenously to rats at the doses of 2, 4 and 10 mmol/kg/day (or 3.2, 6.5 and 16-fold the clinical dose adjusted for body surface area, respectively). Males were treated for 63 days prior to mating and throughout mating while females were treated 2 weeks prior to mating and throughout mating until gestation day (GD) 17.

Renal tubular vacuolation was observed in rats in all the treatment groups. Pale and enlarged kidneys were observed in males and females administered the mid and high dose levels. There

was a reduction in the rate of body weight gain during gestation in the 4 and 10 mmol/kg dose groups.

Based on these findings, the no-observed-adverse-effect-level (NOAEL) in this study was established as 2 mmol/kg (or 3.2-fold the human dose based on body surface area) for general toxicity in F₀ males and females and F₁ litters and ≥ 10 mmol/kg (or 16.2-fold human dose) for fertility and reproductive performance. Overall, there were no adverse effects on fertility or reproductive function/performance. There was no evidence of teratogenic effects following the daily intravenous administration of Gadoterate meglumine to male and female rat fetuses.

In rabbits, the NOAEL for F₀ females (dams) was 0.8 mmol/kg/day (or 2.6x-MHD) and for F₁ litters NOAEL was 0.8 mmol/kg/day (or 2.6x-MHD). There was no evidence of maternotoxicity, embryotoxicity or teratogenicity in rabbits.

ii). Pre- and postnatal development in the rat: Gadoterate meglumine did not appear to have any effect on breeding performance, fertility or reproductive performance in the F₁ litters. It did not demonstrate any adverse effects on the progress and outcome of pregnancy or on the development of the F₁ litters in the period of organogenesis. At the high dose of 0.8 mmol/kg (or 1.3-fold MHD) administered to F₀ dams, Gadoterate meglumine caused a slight toxic effect notably on body weight of the F₁ generation hence the NOAEL for F₀ dams was determined as 0.4 mmol/kg/day (or 0.7-fold MHD).

Studies in Juvenile animals: Juvenile animal toxicity studies were not submitted in support of the pediatric indication in patients from neonates 0-2 years of age. The agency has recommended that sponsors of all gadolinium-based contrast agents proposing a pediatric indication conduct a juvenile animal study to support their application. The sponsor subsequently submitted a juvenile animal study protocol for review and comments during the review cycle.

1.2.4 Special Toxicology

i). Nephrogenic Systemic Fibrosis (NSF): Nephrogenic systemic fibrosis (NSF) is a recently described, serious, fibrotic and highly debilitating disorder most frequently described in patients with end-stage renal disease or acute renal failure. While NSF primarily affects the skin, other organ compartments may also be involved. Although the etiology of NSF is not fully understood, exposure to gadolinium-containing contrast agents has been associated with the onset of symptoms of NSF. Considerable progress towards a better understanding of this disease has occurred since the first association between the clinical use of GBCAs and NSF was made. Consequently, there are several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate < 30 mL /min/1.73m²). The commonly reported hypothesis for the involvement of gadolinium in the pathomechanism of NSF is the in vivo dechelation of GBCAs. Although all GBCAs have the potential to release gadolinium ion (Gd³⁺), which in the context of renal failure, is an important trigger for NSF, such a release is thought to more readily occur among the less stable gadolinium chelates. Current understanding seems to indicate reasonably that Gadolinium complexes play a causative role in the pathophysiology of nephrogenic systemic fibrosis or nephrogenic fibrosing dermopathy. Still, the exact pathogenesis and the risk for patients are unclear.

Two published articles (Fretellier et al, 2011a, b) were reviewed to provide a better understanding of the disease mechanism in Nephrogenic Systemic Fibrosis (NSF).

In the first study, following intravenous injection in rats with impaired renal function, neither Gadoterate meglumine nor gadodiamide induced macroscopic skin lesions, in contrast with nonformulated gadodiamide which was associated with systemic toxicity. It was concluded that the stable gadoterate was associated with less pathologic and biochemical effects than linear ionic compound, gadodiamide.

In the second study, the levels of dissociated versus chelated Gadolinium (Gd) in plasma, skin, and bone was assessed in Wistar rats with impaired renal function following intravenous administration of Gadoterate meglumine, Gadodiamide (Omniscan) or nonformulated gadodiamide (without excess caldiameter ligand). Omniscan and gadodiamide, unlike Gadoterate meglumine induced microscopic skin lesions renally-impaired rats. A higher Gd concentration was observed in the skin and bone (femur) in Omniscan- and nonformulated gadodiamide-treated rats when compared to rats treated with Gadoterate meglumine.

ii). Antigenicity: Gadoterate meglumine did not induce any immediate hypersensitivity reactions.

iii). Toxicological Characterization of Impurities: Gadoterate meglumine consists of DOTA (b) (4) gadolinium oxide (paramagnetic agent) and meglumine (b) (4)

Since gadolinium oxide and meglumine are not known sources of impurity, the discussion on impurities in the drug product is focused on DOTA (b) (4) (b) (4)

Impurities that could arise during manufacture or during storage of Gadoterate meglumine include (b) (4)

(b) (4)

1.2.5 Local Tolerance

Studies were performed in rats to evaluate the local tolerance of Gadoterate meglumine (2.5mmol/kg) following subcutaneous or intramuscular injection, respectively. Macroscopic and microscopic changes at the injection site were similar between control and test article-treated animals on days 1 and 3.

Transient local inflammatory reactions (hematoma, edema and mononuclear/polynuclear cell aggregation) were seen at 6 hours post-dose. Gadoterate meglumine was well tolerated in rats after a single subcutaneous or intramuscular injection.

In another study conducted in rabbits, Gadoterate meglumine was administered at 0.9, 0.9 and 0.25 mmol/animal via intravenous, intra-arterial or perivenous routes respectively. Microscopic findings at the different sites of administration in control and Gadoterate meglumine-treated rabbits included in varying degrees erythema, hemorrhagic infiltration, epidermal vacuolation, presence of inflammatory cells, ulceration of the outer skin and perivascular hemorrhage. The findings were consistent with histopathological findings following the intravenous administration of Gadoterate meglumine in single-dose and repeat-dose toxicity reports.

1.3 Recommendations

1.3.1 Approvability

The proposed indication for Gadoterate meglumine (Dotarem) – “Magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity” involves both adult and pediatric components.

Pharmacology/Toxicology is recommending approval meglumine in adults and children 2-17 years of age based on the sufficiency of nonclinical data to support the use of Gadoterate meglumine in these age groups. Nonclinical juvenile animal toxicity studies to support proposed indications in pediatric population aged 0-2 years were not provided. The potential recommendation from a nonclinical perspective is to approve Gadoterate meglumine for use in adults and children 2-17 years of age.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

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

8.1 Pregnancy

Pregnancy Category C:

Label proposed by sponsor:

There are no adequate and well-controlled studies conducted in pregnant women. (b) (4)

[REDACTED]

[REDACTED] (b) (4)

. Maternal toxicity was observed in rats at 10mmol/kg/day (16 times the human dose based on body surface area) and in rabbits at 7mmol/kg/day (23 times the human dose based on body surface area).

Recommended label:

There are no adequate and well-controlled studies conducted in pregnant women. DOTAREM Injection should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

DOTAREM Injection was not embryotoxic or teratogenic in rats and rabbits. While it is unknown if DOTAREM crosses the human placenta, other gadolinium-based contrast agents (GBCAs) do cross the placenta in humans and result in fetal exposure. Limited published human data on exposure to other GBCAs during pregnancy did not show adverse effects in exposed neonates. DOTAREM should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. (b) (4)

[REDACTED]

8.3 Nursing mothers

Label proposed by sponsor:

It is not known whether DOTAREM is excreted in human milk. Because many drugs are excreted in human milk, exercise caution when DOTAREM is administered to a nursing woman.

Nonclinical data show that DOTAREM is excreted into breast milk in very small amounts (<0.1% of the dose intravenously administered) and the absorption via the gastrointestinal tract is poor.

Recommended label:

It is not known whether DOTAREM is excreted in human milk. Limited case reports on use of GBCAs in nursing mothers indicate that 0.01 to 0.04% of the maternal gadolinium is excreted in

human milk. [REDACTED] (b) (4)

[REDACTED] Nonclinical data show that DOTAREM is excreted into breast milk in very small amounts (<0.1% of the dose intravenously administered) and the absorption via the gastrointestinal tract is poor. Because many drugs are excreted in human milk, exercise caution when DOTAREM is administered to a nursing woman.

8.4 Pediatric Use

The safety and efficacy of DOTAREM at a single dose of 0.1 mmol/kg have been established in children from 2 to 17 years of age. No dosage adjustment according to age is necessary in this population. [See *Clinical Studies (14) and Dosage and Administration (2.1)*]. The safety and efficacy of DOTAREM have not been established in children below 2 years of age.

Recommended label:

No labeling changes recommended.

(Section 13 of the proposed label) 13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Label proposed by sponsor

Long-term animal studies have not been performed to evaluate the carcinogenic potential of [REDACTED] (b) (4).

[REDACTED] (b) (4) did not demonstrate mutagenic potential in vitro bacterial reverse mutation assays (Ames test) using *Salmonella typhimurium*, in an in vitro chromosome aberration assay in Chinese hamster ovary cells, in an in vitro gene mutation assay in Chinese hamster lung cells, nor in an in vivo mouse micronucleus assay.

No impairment of male or female fertility and reproductive performance was observed in rats after intravenous administration of [REDACTED] (b) (4) at the maximum tested dose of 10 mmol/kg/day (16 times the maximum human dose based on surface area), given during more than 9 weeks in males and more than 4 weeks in females. Sperm counts and sperm motility were not adversely affected by treatment with [REDACTED] (b) (4) Injection.

Recommended label:

Long-term animal studies have not been performed to evaluate the carcinogenic potential of [REDACTED] (b) (4) gadoterate meglumine.

Gadoterate meglumine [REDACTED] (b) (4) did not demonstrate mutagenic potential in vitro bacterial reverse mutation assays (Ames test) using *Salmonella typhimurium*, in an in vitro chromosome aberration assay in Chinese hamster ovary cells, in an in vitro gene mutation assay in Chinese hamster lung cells, nor in an in vivo mouse micronucleus assay.

No impairment of male or female fertility and reproductive performance was observed in rats after intravenous administration of gadoterate meglumine [REDACTED] (b) (4) at the maximum tested dose of 10 mmol/kg/day (16 times the maximum human dose based on surface area), given during more than 9 weeks in males and more than 4 weeks in females. [REDACTED] (b) (4)

13.2 Animal Toxicology and/or Pharmacology

Label proposed by sponsor

[REDACTED] (b) (4)

Recommended label:

[REDACTED] (b) (4)

Local intolerance reactions, including moderate irritation associated with infiltration of inflammatory cells was observed after subcutaneous or intramuscular injection in rats and after intravenous, intra-arterial or perivenous injection in rabbits.

2 Drug Information

2.1 Drug

2.1.1 Introduction

Gadoterate meglumine is an aqueous solution. It is an ionic macrocyclic gadolinium (Gd) complex used as a non-specific contrast agent for magnetic resonance imaging (MRI).

The mechanism of pharmacodynamic effect of Gadolinium (Gd) on MRI signal is well documented. The Gd^{3+} ion has paramagnetic properties due to its 7 unpaired electrons leading to a high magnetic moment and labile water coordination properties. Gd enhances MR signal by modifying relaxation times of water protons in blood and tissues - a mechanism also referred to as 'shortening'. Shortening results in increased signal intensity in T_1 -weighted (spin-lattice) sequences and a reduced signal intensity in T_2 -weighted (spin-spin) sequences.

Unbound (or 'free') gadolinium ion (Gd^{3+}) is toxic and is bound to a ligand to form a chelate in all approved gadolinium-based contrast agents (GBCAs). The structure of the chelated Gd can be open-chain (linear) or ring-bound (macrocyclic). GBCA molecules can be defined further according to charge as nonionic or ionic. Gadoterate meglumine is an ionic macrocyclic gadolinium complex. Macrocyclic GBCAs are generally believed to have a lesser propensity to release the Gd ion that is tightly and rigidly bound in the ligand ring.

Gadoterate meglumine is soluble in water and a 0.5M aqueous solution has a pH between 6.5 and 8.0

CAS Registry Number (Optional)

Gadoterate meglumine: 92943-93-6; Gadoteric acid: 72573-82-1; Meglumine: 6284-40-8

Generic Name

Gadoterate meglumine (Other names include: Gadoterate meglumine; DOTA-Gd meglumine; Meglumine salt of Gadoteric acid)

Code Name

P 466

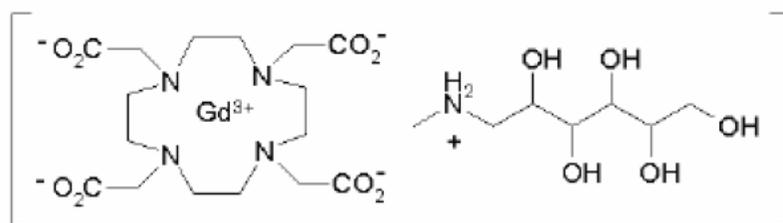
Chemical Name

Gd-DOTA or Meglumine salt of 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid

Molecular Formula/Molecular Weight

$C_{23}H_{42}O_{13}N_5Gd$ [$HOCH_2-(CHOH)_4-CH_2NHCH_3$] / 753.86 g/mol

Structure or Biochemical Description

Figure 1: Structure of Gadoterate dimeglumine

Source: Sponsor's submission (§ 2.3.S, page 3 of 47)

Pharmacologic Class

Diagnostic MRI contrast agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 65,041 (Gadoterate meglumine)

2.3 Drug Formulation

Dotarem (Gadoterate meglumine) is a clear colorless to slightly yellow solution. The quantitative composition of the drug product is provided in Table 1 below:

Table 1: Quantitative Composition of Gadoterate meglumine

Name of Ingredient	Function	Quantity
Active Ingredient: Gadoterate meglumine comprising of:	Contrast agent	376.93 mg
• DOTA*	(b) (4)	(b) (4)
• Gadolinium oxide	Paramagnetic agent	
• Meglumine	(b) (4)	
Excipient:		
Water for injection	(b) (4)	

Source: Reviewer's Table adapted from sponsor's data (Table 1, page 2 of 53, Report No. 2_12_00700-2.0, Description and composition of Drug product; Common Technical Document Summaries § 2.3.P);

(b) (4)

All nonclinical studies, excluding those conducted to characterize impurity toxicity, were performed with the same formulation that was used in the clinical trials and the proposed to-be-marketed drug product. This Gadoterate meglumine formulation was also referred to by the code name G449.06 or EK-5504 in some study reports. A Gadoterate meglumine formulation with the code name (b) (4) (b) (4) to evaluate impurities.

2.3.1 Route of administration

Bolus Intravenous

2.4 Comments on Novel Excipients

Water for injection is the only excipient included in Gadoterate meglumine.

2.5 Comments on Impurities/Degradants of Concern

Impurities in Gadoterate meglumine include (b) (4) and (b) (4)

Potential impurities from Gadoterate meglumine components were described in section 2.3.S.3.2 captioned "Impurities" in the submission. According to the sponsor, these impurities are controlled before manufacture of Gadoterate meglumine and do not require additional control in Gadoterate meglumine. Impurities that can arise during manufacture or storage of Gadoterate meglumine include (b) (4)

According to ICH guideline Q3A(R2) (Impurities in new drug substances, October 2006), for a compound given at a daily dose greater than 2 g, all impurities present at a level strictly greater than 0.05 % must be qualified (qualification threshold). This applies to Gadoterate meglumine which is given in patients usually at a dose of 0.1mmol/kg (i.e. 7 mmol for 70 kg BW). As the molecular weights of Gd-DOTA and DOTA are 558.7 and 404.4, respectively, 7 mmol correspond to a maximum dose of 4 g of Gd-DOTA or 2.8 g of DOTA. However, the limits proposed by the guideline are for products with chronic use. Qualification of impurities is discussed in Section 10.4 of the review.

2.6 Proposed Clinical Population and Dosing Regimen

2.6.1 Proposed Diagnostic Indication and Clinical Population

According to the sponsor, Gadoterate meglumine is a gadolinium-based contrast agent indicated for intravenous use with magnetic resonance imaging (MRI) in the brain (intracranial), spine and associated tissues in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity.

2.6.2 Proposed Dose

The proposed dose is 0.1 mmol/kg body weight to be administered as an intravenous bolus injection, manually or by power injector, at a flow rate of approximately 2 mL/second for adults and 1-2 mL/second for children.

2.7 Regulatory Background

Gadoterate meglumine (Dotarem) is an ionic-macrocylic gadolinium (Gd) containing contrast agent developed as an injectable solution to be administered intravenously and intended for the diagnostic examinations carried out by Magnetic Resonance Imaging (MRI). Gadoterate meglumine was developed by Guerbet in the 1980s and was first approved in France in 1989 for

use in intracranial and spinal MRI. Gadoterate meglumine has been approved in Europe and Japan under the name MagneScope[®]. Guerbet submitted an initial Investigational New Drug (IND) application (65,041) to the FDA on June 12, 2002. Pursuant to 505(b) of the Federal Food, Drug, and Cosmetic Act and 21 CFR §314.1, the sponsor submitted NDA 204-781 for Gadoterate meglumine to the Agency on September 20, 2012.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Primary Pharmacology: No studies submitted

Secondary Pharmacology: No studies submitted

Safety Pharmacology

DGD-2-5-A: Neurological safety of G449.06 in the rat and the mouse (behavioral study)

99-12-809: Evaluation of effects on blood pressure, heart rate, and electrocardiogram after single intravenous dosing in conscious dogs

DGD-33-005: Evaluation of pro-arrhythmic effects after intravenous administration in methoxamine pretreated anesthetized rabbits

DGD-33-001: Evaluation of the effect on cardiac action potential in isolated canine Purkinje fibers

DGD-2-12-A: Cardiovascular and respiratory safety of G449-06 administered IV to anesthetized dogs. Study of dose-dependent effects

DGD-33-006: Evaluation of effect on renal function in glycerol-induced renal failure in the rat following single intravenous administration

DGD-33-007: Evaluation of effect on renal function in normal rats and L-NAME-treated rats following repeated intravenous administration for 14 days

Pharmacodynamic Drug Interactions

Pharmacokinetics

Distribution

DGD-0-2-A: Variation in the biodistribution of Gd-DOTA in the rat during the first hour after IV bolus injection

DGD-0-1-A: Plasma, urinary and biliary kinetics of Gd-DOTA compared with Gd-DTPA and distribution in the organism at 3 hours after intravenous administration in the anesthetized rabbit

DGD-0-3-A: Plasma, urinary and biliary kinetics of Gd-DOTA after intravenous administration in the anesthetized dog

DGD-0-9-A: Excretion in milk and plasma kinetics of Gadolinium after intravenous injection of Gd-DOTA in the conscious goat

DGD-0-6-A: Binding of Gadolinium complexes Gd-DOTA and Gd-DTPA to human albumin

DGD-0-5-A: Comparative biodistribution of Gd-DOTA and Gd-DTPA in the anesthetized rabbit 1 hour after bolus injection

DGD-0-15-A: Whole body retention of Gd-DOTA in the hairless mouse over 21 days - A comparative study versus Gd-DTPA

DGD-0-4-A: Comparative biodistribution of Gd-DOTA and Gd-DTPA in the conscious rat from 24 hours to 7 days after an IV bolus injection

DGD-0-13-A: Distribution of G449-06 in the body during intravenous subacute toxicity studies with a 28-day reversibility period in the rat

DGD-0-12-A: Distribution G449-06 in dogs during a subacute toxicity study by IV administration followed by a 28-day reversibility period

DGD-0-14-A: Transplacental transfer of G449-06 administered at 0.5 mmol per kg to female rats after 15 days of gestation

Metabolism

DGD-0-10-A: Detection of metabolites of Gd-DOTA after IV bolus in the rat, the dog and the rabbit

Excretion

DGD-0-16-A: Comparative study of the excretion of Gd-DOTA and Gd-DTPA administered intravenously in the anesthetized rat with renal failure

Toxicology

99-12-807: Single dose toxicity study by intravenous route in rats

99-12-808: Single dose toxicity study by intravenous route in Beagle dogs

Rat Intravenous

99-12-806: 4 week toxicity study by intravenous route in rats followed by a 13-week treatment free period

DGD-1-10-A: G449-06 - Toxicity study by intravenous administration to dogs for 4 weeks followed by a 4-week reversibility period

Genotoxicity

DGD-33-004: Bacterial reverse mutation test (Plate incorporation and Preincubation methods)

DGD-12-A: G449-06 - Chromosomal aberration study in CHO Chinese Hamster cells

DGD-1-13-A: G449-06 - Gene mutation assay in vitro in V79 Chinese Hamster cells

DGD-1-11-A: G449-06 - Micronucleus test in mice

Reproductive and Developmental Toxicity

Embryo-fetal Development

DGD-1-8-A: G449-06 -Teratology study in the rat (segment II)

99-12-804: Gadoterate meglumine (Dotarem) - Combined fertility and embryotoxicity study by the IV route in the rat (Segment I and II)

DGD-1-6-A: G449-06 - evaluation of the embryotoxic or possible teratogenic effects in the rabbit (intravenous route)

Local Tolerance

DGD-1-14-A: G449-06 - Acute subcutaneous toxicity study in rats

DGD-1-15-A: G449-06 - Acute intramuscular toxicity study in rats

DGD-33-002: Local tolerance in the rabbit by intravenous, perivenous and intra-arterial routes

Other Toxicity Studies

Immunotoxicity

DGD-33-003: Evaluation of the potential to induce immediate hypersensitivity-induced anaphylactic shock and passive cutaneous anaphylaxis in the guinea pig

Nephrogenic Systemic Fibrosis (NSF)

No studies submitted. Publications cited and reviewed (see Bibliography)

Impurities

(b) (4)

3.2 Studies Not Reviewed**Table 2: Studies not reviewed**

<u>Study No.</u>	<u>Title of Study</u>	<u>Reason(s) study was not reviewed</u>
DGD-2-1-A	Cardiac tolerance of G449-06 after intravenous injection in the anesthetized dog (open thorax) - Study of the dose-dependent effect	Older non-GLP; A more recent pivotal GLP study (99.12.809) was reviewed
DGD-2-9-A	Effects of G449-06 on cardiovascular function after intravenous injection in the anesthetized dog (open thorax) - Study of the injection rate-dependent effect	Older non-GLP; A more recent pivotal GLP study (99.12.809) was reviewed
DGD-2-11-A	Effects of G449-06 on general and respiratory function after intravenous injection in the anesthetized dog - Study of the injection rate-dependent effect	Older non-GLP single-dose trial; GLP study DGD-12-A in the same species using 3 doses was reviewed
DGD-2-4-A	Preliminary study of the hemodynamic and functional renal safety of G449-06 in the anesthetized dog after a single intravenous injection	Preliminary non GLP single-dose trial study; Study DGD-33-007 (GLP) was reviewed
DGD-2-7-A	In vitro study of the Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine, Gd-EDTA on Calcium-dependent and non-dependent enzymes systems	Early non-GLP studies using older formulation
DGD-2-8-A	In vitro study of the Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine, Gd-EDTA on the coagulation system and platelet function - the role of Calcium	
DGD-2-6-A	Study of the effect of the Gadolinium complexes Gd-DOTA meglumine and Gd-DTPA meglumine on erythrocytes	
DGD-2-2-A	In vitro study of Gadolinium complexes Gd-DOTA meglumine, Gd-DTPA meglumine on the complement system	
DGD-2-3-A	In vitro study of the histamine-releasing potential of the Gadolinium complexes Gd-DOTA and Gd-DTPA meglumine	
DGD-2-10-A	Study of hemodilution in the conscious rabbit	Preliminary Non-GLP

	after intravenous injection of G449-06	study
DGD-0-8-A	Comparative pharmacokinetics of Gd-DOTA and GdCl ₃ administered by the oral route in the conscious rat	Early non-GLP oral study
DGD-0-7-A	Comparative excretion of Gd-DOTA and Gd-DTPA in the rat metabolism cage over 14 days	Early non-GLP study; Later, Pivotal GLP study was reviewed
DGD-1-2-A	Acute toxicity of G449-06 injected by the intravenous route in the mouse	Early non-GLP study; Later, Pivotal GLP studies were reviewed
DGD-1-3-A	Acute toxicity of G449-06 administered by oral route in the mouse	Early non-GLP oral toxicity study
99.12.810	Acute intravenous toxicity study in rats	Pivotal study was reviewed
DGD-1-4-A	Acute toxicity of G449-06 administered by the oral route in the rat in a volume of 30 mL per kg	Early non-GLP oral toxicity study
DGD-1-7-A	Acute toxicity of G449-06 administered by intracisternal route in the rat	Early non-GLP intracisternal acute toxicity study
99.12.811	Dose range finding toxicity study by intravenous route in Beagle dogs	Dose-range finding study
DGD-1-9-A	Toxicity study by intravenous administration to rats for 4 weeks followed by a 4-week reversibility period	Early study; Pivotal repeat-dose toxicity study was reviewed
99.12.805	Gadoterate meglumine (Dotarem) - Seven-day toxicity study by intravenous route in rats	Non-pivotal study. Pivotal study at the same doses was reviewed
99.12.803	Dose range finding fertility and embryofertility study by the IV route in the rat	Dose-range finding study
99.12.801	Gadoterate meglumine (Dotarem) dose range finding study by the IV route in the pregnant rabbit	Non-pivotal, dose-range finding study
99.12.802	Gadoterate meglumine (Dotarem) – Embryotoxicity study by the intravenous route in the rabbit (segment II)	GLP Segment II embryotoxicity study; Pivotal study was reviewed

3.3 Previous Reviews Referenced

None

4 Pharmacology

Mechanism of action

The mechanism of pharmacodynamic effect of Gadolinium (Gd) on Magnetic Resonance Imaging (MRI) signal is well documented. Gd ion has paramagnetic properties due to its 7 unpaired electrons leading to a high magnetic moment and labile water coordination properties. Gd enhances MR signal by modifying relaxation times of water protons in blood and tissues - a

mechanism also referred to as ‘shortening’. Shortening results in increased signal intensity in T₁-weighted (spin-lattice) sequences and a reduced signal intensity in T₂-weighted (spin-spin) sequences.

4.1 Primary Pharmacology

No studies were submitted.

4.2 Secondary Pharmacology

No studies were submitted

4.3 Safety Pharmacology

4.3.0 Introduction

The sponsor conducted safety pharmacology studies in rabbits, rats and dogs to evaluate Cardiovascular, Respiratory, Renal and CNS effects of Gd-DOTA (or Gadoterate meglumine). Table 3 provides an overview of Safety Pharmacology studies submitted to this NDA.

Table 3: Overview of Safety Pharmacology Studies (including in vitro Pharmacology studies)

System	Species/Strain/ Tissue	Route of Admin.	Report No.	Dose (mmol/kg)	GLP (Yes/No)	Reviewed (Yes/No)
CNS	Mice	IV	DGD-2-5-A	(0,1), (0,8), (0,1, 4)	No	Yes
	Rats	IV, IC, IA	DGD-2-5-A	IV (0,1), IA (0,0.5 mL/rat), IC (0,1, 2 µmol/L)	No	Yes
CVS	Dogs (Telemetry)	IV	99-12-809	0, 2.4, 3.6, & 5.5	Yes	Yes
	Rabbits (Proarrhythmic effect)	IV	DGD-33-005	0, 1.0, 2.0 & 4.0	Yes	Yes
	Canine purkinje fibers	In vitro	DGD-33-001	0, 2.5, 5 & 10	Yes	Yes
	Dogs	IV	DGD-2-1-A	0.1, 0.5 & 1.0	No	No
Respiratory	Dogs	IV	DGD-2-9-A	0.1	No	No
	Dogs	IV	DGD-2-12-A	0.1, 0.5 & 1.0	No	Yes
Renal	Dogs	IV	DGD-2-11-A	0.1	No	No
	Rats	IV	DGD-33-006	0, 2	Yes	Yes
	Dogs	IV	DGD-33-007	0, 2	Yes	Yes
	Dogs	IV	DGD-2-4-A	0, 2	No	No

In-vitro						
Enzymes	Homogenized rat striatum	In vitro	DGD-2-7-A	N/A	No	No
Coagulation	Human plasma	In vitro	DGD-2-8-A	N/A	No	No
Erythrocytes	Human, rabbit, rat blood	In vitro	DGD-2-6-A	N/A	No	No
Complement	G/pig serum, human plasma	In vitro	DGD-2-2-A	N/A	No	No
Histamine, Serotonin release	Rat peritoneal mast cells	In vitro	DGD-2-3-A	N/A	No	No

Reviewer's Table based on sponsor's data; IV, IC, IA = intravenous, intracisternal, intra-arterial, respectively

4.3.1 Summary of Safety Pharmacology findings

CNS safety: Behavioral tests were conducted in conscious animals to evaluate the neurological safety of Gadoterate meglumine. There was no effect on motility. No central depressant, extrapyramidal or cataleptic effect was observed. Gadoterate meglumine did not alter the body temperature. Gadoterate meglumine solution (1350mOsm/kg) had a pro-convulsive effect following an intravenous injection of 4mmol/kg in picrotoxin-treated mice and after an intracisternal administration of Gadoterate meglumine in pentylenetetrazole-treated rats.

CVS safety: In a pivotal cardiovascular study in conscious telemetered beagle dogs, Gadoterate meglumine was administered intravenously at doses of 0.6, 2.4, 3.6 and 5.5mmol/kg (or 3.2x, 13x, 20x and 30x the clinical dose adjusted for body surface area, respectively). ECG (Lead II) was recorded continuously for up to 24 hours. At 0.6mmol/kg, no remarkable effects were observed on the heart rate (HR), arterial blood pressure (BP) and ECG parameters (PR, QT and QRS complex). There was a slight increase in HR and arterial BP at 2.4 and 3.6mmol/kg compared to the saline control. At 4h following the 3.6 mmol/kg dose, QTc was slightly reduced (-2 msec). At the highest dose (5.5.mmol/kg), there was a reduction in QTc (-8 to -13msec or 2-6% change) at 1-4 hours after dosing. NOAEL was established as 0.6mmol/kg (or 3.2x the clinical dose).

The potential effect of Gadoterate meglumine on cardiac action potential was evaluated *in vitro* on isolated canine purkinje fibers. The results showed that Gadoterate meglumine had no effect on action potential duration in Purkinje fibers.

Respiratory system: Gadoterate meglumine was administered intravenously to mongrel dogs at doses of 0.1 to 1.0mmol/kg (or 0.54x to 5.4x the human dose). The high dose (1.0 mmol/kg) resulted in an increase of 4-16% increase in respiratory rate. Lower doses (0.1 and 0.5 mmol/kg) did not elicit any pulmonary effects. Based on the results, the NOAEL was determined as 0.5mmol/kg (or 2.7x the human dose).

Renal system – The effect of Gadoterate meglumine on renal function was evaluated in a glycerol-induced renal failure model in the rat. Gadoterate meglumine was administered as single intravenous dose of 2mmol/kg (or 3.2x the human dose). Gadoterate meglumine (or its comparator Magnevist administered at the same dose), did not cause an exacerbation of the

impairment of renal function induced by glycerol in rats. As expected, intramuscular injection of glycerol caused a significant increase in urinary excretion of proteins, plasma creatinine, and urea.

A safety pharmacology study was conducted to evaluate potential effects of Gadoterate meglumine on renal function in normal rats and rats pretreated with (L-NAME (N- ω -nitro-L-arginine methyl ester). The animals were administered Gadoterate meglumine (2 mmol/kg or 3.2x the clinical dose adjusted for body surface area) intravenously for 14 days. Gadoterate meglumine was well tolerated with or without L-NAME. L-NAME is an inhibitor of nitric oxide. Inhibition of nitric oxide and prostanoids may result in renal vascular effects including nephrotoxicity. Results showed no adverse effect on renal function. Renal kidney hypertrophy observed 24h following the last treatment was no longer evident after a 28-day treatment-free period. Proximal convoluted tubule vacuolation, seen in all animals euthanized 24h after the last treatment with Gadoterate meglumine, was also not evident after the recovery period. By the end of the treatment-free period, all notable and significant changes in plasma and urinary parameters and histological findings were reversible.

4.3.2 Central Nervous System

4.3.2.1 Report DGD-2-5-A: Neurological safety of Gadoterate meglumine (G 449.06) in the rat and the mouse (behavioral study)

Report location:	eCTD Module 4 §4.2.1.3.1
Conducting laboratory and location:	Laboratoire GUERBET Unite de Pharmacodynamie I 16-24, rue Jean Chaptal, 91601 Alnay Sous Bois, Cedex, France
Study #:	87.01.104
Date of study initiation:	January, 1987
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadoterate meglumine (formulation G 449.06), Batch No. 202, % purity: N/A; Saline (0.9% NaCl) was used as control
Animal species/strain/sex per dose:	Mice: Female Swiss (b) (4) (b) (4) (b) (4) 3 groups of 10 mice per group per study except for 1 test (details in methods); only 1 dose (1 mmol/kg) was tested Rats: Male Sprague-Dawley (b) (4) (b) (4) (b) (4)
Age:	N/A
Weight:	Mice: ~20 g / animal; Rats: ~170 g / animal
Doses:	1 mmol/kg 9or 0.8 mmol/m ² , based on body surface area)
Duration/route:	Intravenous

Objective

10 sub-studies (7 in mice and 3 in rats) were conducted to evaluate the safety of Gadoterate meglumine on the central nervous system (CNS) following intravenous administration.

Key findings

In the various behavioral tests conducted in conscious animals to evaluate the neurological safety of Gadoterate meglumine, there was no effect on motility; no central depressant effect was identified; there was no extrapyramidal effect and no cataleptic effect was observed. Gadoterate meglumine did not alter the body temperature following its intravenous administration. Method-control (reference) substances were used to validate the various experiments. Reference substances produced the expected results. Gadoterate meglumine, in most of the studies, did not induce any effect. However, noteworthy findings included a painful intra-arterial administration in rats due to the hyperosmolarity of the injected Gadoterate meglumine solution (1350mOsm/kg) and a pro-convulsive effect following an intravenous injection of 4mmol/kg in picrotoxin-treated mice and after an intra-cisternal administration of Gadoterate meglumine in pentylenetetrazole-treated rats.

List of CNS sub-studies: The following are the studies conducted in report DGD-2-5-A. Methods, Results, Conclusions and Reviewer's comments were described for each sub-study:

Table 4: Neurological studies to evaluate the neurological safety of Gadoterate meglumine in mice and rats

Species	Route of administration	Sub-studies
A. Mice		
1	IV	Study of the spontaneous effect of Gadoterate meglumine on spontaneous motility in the mouse after intravenous administration
2		Study of the effect of intravenously administered gadoterate meglumine on traction, righting reflex and tail squeeze tests in the mouse
3		Study of the general depressant effect due to a potentiation of barbiturate-induced hypnosis in the mouse after intravenous administration
4		Study of thermoregulation in the mouse after intravenous administration of test article (Gadoterate meglumine)
5		Study of the Antinociceptive effect in the Hot Plate Test in the mouse after intravenous administration of gadoterate meglumine
6		Study of the pro-convulsive stimulant effect by potentiation of pentylenetetrazole
7		Study of the pro-convulsive stimulant effect by potentiation of picrotoxin-induced convulsions in the mouse after intravenous administration
B. Rats		
1	IV	Study of the Extrapyramidal effect through the study of catalepsy in the rat after intravenous

		administration
2	IA	Study of the Algogenic effect in the rat after intra-arterial administration
3	IC	pro-convulsive stimulant effect by potentiation of pentylenetetrazole-induced convulsions in the rat after intracisternal administration

Reviewer's Table based on sponsor's data; IV, IC, IA = intravenous, intracisternal, intra-arterial, respectively

Each test was conducted in a specially-designated room with artificial lighting and 22⁰C ambient temperature. Animals were individually-weighed and food withdrawn until study began. Each sub-study was reviewed as a stand-alone study. The "Discussion and Key findings" sections reflected findings from study DGD-2-5-A as a whole.

Study A1: Study of the spontaneous effect of Gadoterate meglumine on spontaneous motility in the mouse after intravenous administration

Objective: The objective of this test was to determine the potential effect of Gadoterate meglumine (G 449.06) on spontaneous motility in the mouse using the circle test.

Methods:

Principle of test: The test (Morpuigo, 1971) allowed the recording of spontaneous motility in mice after being placed singly in the center of concentric circles (diameter: 5, 11 and 18 cm). The position of the animal was recorded 5 sec after placing the mouse and motor activity rated as 0 (total immobility) or 4 (normal motility) when the animal leaves the circle. Intermediate scores were obtained when the animal moves around inside the circle. The test assessed the effect of saline (control substance), Gadoterate meglumine or Diazepam on spontaneous motility. The score of the 10 mice in each group was added and the effect of each treatment compared with the saline group.

Control article: Pyrogen-free injectable isotonic saline (0.9% NaCl; (b) (4) Batch No. 81 625)

Test article: Gadoterate meglumine (G 449.06, Batch No. 202) diluted 1:5 with normal saline ((b) (4) Batch No. 81.625)

Reference compound: The sedative agent, diazepam (Valium, 50 mg/kg, orally, (b) (4) Batch No. FO27P) was used as a reference compound.

Animals and dose groups: 30 female mice (19-23 g) divided into 3 dose groups of 10 mice per group:

- I. Negative control: 10 ml/kg saline
- II. Positive control: 50 mL/kg diazepam, per os (equivalent to 10 mL/kg of commercial diazepam solution containing 10 mg per 2 mL)
- III. Test compound: 1 mmol/kg (or 0.8 mmol/m²) of Gadoterate meglumine (G 449.06) equivalent to 10 mL/kg)

Results: All 10 mice in the saline group left the circles. 4 mice with maximal sedation remained immobile in the center of the circles; 3 moves slightly (partially-sedated) and 3 mice left the

circles. 9 mice in the Gadoterate meglumine-treated group left the circles in the stipulated time while 1 mouse took 5 seconds longer to leave the circles. These findings are described in Table 5:

Table 5: Spontaneous effect of Gadoterate meglumine on spontaneous motility in the mouse

Group	Observations	Circle Test score	p-value
I – Saline control	All 10 mice left the circles	40	-
II – Diazepam	- 4 mice stayed in immobile in the center of the circles (maximum sedation) - 3 mice moved slightly (partial sedation) - 3 mice left the circles	19	$p \leq 0.05$
III - Gadoterate meglumine	- 9 mice left the circles in the stipulated time - 1 mouse took 5 sec longer to leave the circles	39	NS

Reviewer's Table based on sponsor's data (page 10/135); NS = not significant

Conclusions: Gadoterate meglumine, administered intravenously at a dose of 1 mmol/kg (or 0.8 mmol/m² adjusted for body surface area), did not have an effect on spontaneous activity in mice. As expected, orally administered Diazepam was used as a reference compound at a dose of 50 mg/kg. By producing sedation, Diazepam caused a significant inhibition of spontaneous motility when compared to saline control-treated mice.

Reviewer's comments: I agree with the objective, design and conclusions of the study to screen the test article (Gadoterate meglumine) for CNS activity.

Study A2: Study of the effect of intravenously administered Gadoterate meglumine on Traction, Righting reflex and Tail squeeze Tests in the mouse

Objective: The objective of this test was to determine the potential sedative or stimulant effect of Gadoterate meglumine on traction, righting reflex and tail squeeze in the mouse.

Methods: The traction and righting reflex tests were performed before injection of test or control substances and then at 15, 30, 45 and 60 min after injection. The squeeze test was carried out only on naïve mice and was consequently performed only once per mouse 30 min following injection of test or control articles.

Principle: The principle of the tests is described below:

Table 6: Principle and value of the traction, righting and tail squeeze test (DGD-2-5-A)

Test	Principle
Traction	When a 35 cm-long metal wire was held horizontally at a height of 40 cm above the working plane was presented to the mouse, the normal reaction (positive response) of the animal was to suspend itself from the wire by the forelegs and then draw up the hind legs for traction in a time less than or

	<p>equal to 5 seconds</p> <p><u>Potential outcomes:</u> 1) the mouse did not hang by the front paw on the wire or 2) no attempt made to pull itself up by the hind limb. The mouse then stayed suspended after the 5 sec or releases itself and dropped before the end of the 5 sec. Both responses were considered negative</p> <p><u>Value of test:</u> To determine the degree of motor activity due to administered test substances</p>
Righting reflex	<p>When an animal (including the mouse) is placed on its back (dorsal decubitus position), it will resume its normal position within less than 5 sec – a response known as the righting reflex.</p> <p><u>Potential outcomes:</u> In this test, it was considered a negative response if the mouse righted itself after 5 sec or failed to do so</p> <p><u>Value of test:</u> To determine if test substances had any CNS depressant effects</p>
Tail squeeze	<p>When the top part of the mouse tail is squeezed, the animal squeaked in response to the pain and turned in the direction of the aggression. This response was considered as positive in this test. The tail squeeze test was conducted in parallel with the traction test.</p> <p><u>Possible outcome:</u> Mouse squeaked in response to pain</p> <p><u>Value of test:</u> to determine the analgesic effect, if any, of the test substances</p>

Reviewer's Table based on sponsor's data

Control article: Pyrogen-free injectable isotonic saline (0.9% NaCl; (b) (4) Batch No. 81 625)

Test article: Gadoterate meglumine (G 449.06; Batch No. 202) diluted to 1:5 with isotonic saline ((b) (4) Batch No. 81 625)

Reference compounds: The sedative-anxiolytic compound – diazepam (Valium, 50 mg/kg, orally, (b) (4) Batch No. FO27P) was used as a reference compound for the traction and tail squeeze tests. 6% sodium pentobarbitone (Batch No. 56.051) diluted to 1:50 with distilled water was used for the righting reflex test.

Study Design:

Animals and dose groups: 30 female mice (19-25 g) divided into 3 dose groups of 10 mice per group/test:

Table 7: Study design and dose groups for the traction, righting reflex and tail squeeze tests

	Tests		
	Tracking	Righting reflex	Tail squeeze
No. of mice	10/group; 18-24g	10/group; 19-24g	10/group; 19-25g
Control article	10	10 mL/kg/mouse/group	10

(Saline)	mL/kg/mouse/group		mL/kg/mouse/group
Reference compound	Diazepam: 12.5 mg/kg (2.5mL/kg)	Sodium pentobarbitone: 40 mg/kg (33.5 mL/kg of diluted solution)	Diazepam: 25 mg/kg (5 mL/kg of diluted solution)
Test article (Gadoterate meglumine)	1 mmol/kg		

Reviewer's Table based on sponsor's data

Results: For each of the 3 tests, the number of animals giving a negative response was counted and frequency was compared using the chi squared (χ^2) test with Yates correction.

Traction test: The results of the traction test are shown in Table 8:

Table 8: Results of the Traction test (number of animals giving a negative response)

	Time zero (T ₀) min	15 min	30 min	45 min	60 min
Isotonic saline	0/10	0/10	0/10	0/10	0/10
Diazepam	0/10	10/10 ^a 9/10 ^b 1/10 $p \leq 0.01$	9/10 ^a 7/10 ^b 2/10 $p \leq 0.01$	9/10 ^a 8/10 ^b 1/10 $p \leq 0.01$	10/10 $p \leq 0.01$
Gadoterate meglumine	0/10	0/10 NS	2/10 ^a 0/10 ^b 2/10 NS	1/10 ^a 0/10 ^b 1/10 NS	0/10 NS

Reviewer's Table adapted from sponsor's Table on p.17 of 135; ^a, Number of mice which did not achieve a good grip; ^b, Number of which did not pull up within the 5 min set time of test; NS, not significant.

All saline-treated control mice had normal activity. Diazepam-treated mice could not achieve a good grip and 9/10 mice fell off and 1/10 remained suspended. Findings of the traction test were significant ($p \leq 0.01$) at each time point from 15 – 60 min when diazepam was compared with isotonic saline solution. The effect of Diazepam persisted until 60 min post-administration. The tail squeeze test conducted in parallel with the traction test at 30 min demonstrated the absence of reaction to pain in 3/10 animals administered Diazepam.

There was normal activity in mice treated with Gadoterate meglumine and the results were not significant (NS) when Gadoterate meglumine (G 449.06) was compared with the saline control. In 3/10 Gadoterate meglumine -treated animals, there was a slight lengthening of the pull-up time (7 sec) at 30 and 45 min after injection.

Conclusion: Diazepam had a significant effect on the response of mice in the traction test. Gadoterate meglumine had no effect and saline-treated rats had normal activity.

Reviewer's comments: I agree.

Righting reflex test: The results of the Righting reflex test are shown in Table 9:

Table 9: The Righting reflex test (number of animals showing a negative response)

	Time zero (T ₀) min	15 min	30 min	45 min	60 min
Isotonic saline	0/10	0/10	0/10	0/10	0/10
Na Pentobarbitone	0/10	6/10 p ≤ 0.02	6/10 p ≤ 0.02	5/10 p ≤ 0.05	0/10 NS
Gadoterate meglumine	0/10	0/10 NS	0/10 NS	0/10 NS	0/10 NS

Reviewer's Table adapted from sponsor's Table on p.20 of 135; NS, not significant

All saline-treated control mice had positive response (normal righting reflex present). 6/10 sodium pentobarbitone-treated mice remained on their back (dorsal decubitus position) at 15 min post-administration. The effect of pentobarbitone was still present in 5/10 at 45 min and 2 of the 5 mice took longer than the set time to righting themselves. At 60 min, no further effect of pentobarbitone was evident. All of the mice administered Gadoterate meglumine had a positive response (righting reflex positive).

Conclusion: The tranquillizing effect of pentobarbitone on the righting reflex test was highly significant at 15 - 45 min following oral administration.

Reviewer's comments: I agree

Tail Squeeze Test:

Results: 0/1, 6/10 or 0/10 mice had a negative response 30 min after IV 0.9% NaCl (control) solution, Diazepam or Gadoterate meglumine, respectively. Compared to saline treatment - Diazepam, unlike Gadoterate meglumine - had a statistically significant sedative effect (loss of pain sensation) on mice in the tail squeeze test.

Conclusion: Due to its strong sedative effect, diazepam significantly inhibited the response to the pain sensation provoked by the squeezing. A similar effect was not observed in the animals which received Gadoterate meglumine.

Reviewer's comments: I agree

Study A3: Study of the general depressant effect due to a potentiation of Barbiturate-induced hypnosis in the mouse after intravenous administration

Objective: The purpose of this test was to determine the effect of an intravenous injection of Gadoterate meglumine (449.06) preceding an intraperitoneal administration of Pentobarbitone in mice.

Methods:

Principle and value of the test: Mice were administered a dose of pentobarbitone intraperitoneally. Some mice failed to respond to the righting reflex test. The duration of the lack of response was recorded.

In the substantive test, the test article was administered 10 min before injection of the barbiturate. The number of animals and the duration of the righting reflex were then compared with the effect of barbiturate alone. Like the circle test, this test revealed the general depressant effect or potential metabolic interference with pentobarbitone. Diazepam, administered orally at a sub-threshold dose of 12.5 mg/kg, was used as the reference substance for the depressant effect.

The number of mice showing hypnosis was counted and the potential effect of diazepam and Gadoterate meglumine was studied using the χ^2 test with YATES' correction and compared with results of the pentobarbitone alone. YATES' correction is used when testing for independence in a contingency Table.

Animals: 40 mice weighing 18-22 g.

Test article: Gadoterate meglumine (G 449.06; Batch No. 202) diluted 1:5 with isotonic saline ((b) (4) Batch No. 81 625)

Reference compounds: The sedative and anxiolytic compound – diazepam (Valium, 12.5 mg/kg, orally equivalent to 2.5 mL/kg of the commercial solution containing 10 mg diazepam/2mL) was used as a reference compound.

Test articles: 1) Pentobarbitone (20 mg/kg, equivalent to 16.5 mL/kg of the diluted solution) and 2) Gadoterate meglumine (G 449.06; 1 mmol/kg).

Table 10: Study groups and doses

Study groups	No. of mice	Dose
I. Diazepam (oral)	10	12.5 mg/kg
II. Pentobarbitone (intraperitoneal)	10	20 mg/kg
III. Diazepam (as in I) + Pentobarbitone (10 min later)	10	
IV. Gadoterate meglumine + Pentobarbitone (10 min later)	10	1 mmol/kg

Reviewer's Table based on sponsor's data

Results: The number of animals not righting themselves was recorded at 15, 30, 60 and 120 min post-injection as shown in Table 11:

Table 11: Barbiturate induced hypnosis: Number of mice not righting their position

	15 min	30 min	60 min	120 min
Diazepam alone	0/10	0/10	0/10	0/10
Pentobarbitone alone	0/10	0/10	0/10	0/10
Diazepam + Pentobarbitone	8/10 $p \leq 0.001$	9/10 $p \leq 0.001$	3/10 NS	0/10 NS

Gadoterate meglumine + Pentobarbitone	0/10 NS	0/10 NS	0/10 NS	0/10 NS
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Reviewer's Table adapted from sponsor's Table on p.26 of 135; NS, not significant

Conclusion: Hypnosis were observed in mice which receiving both diazepam and pentobarbitone. The model demonstrated a significant sedative of diazepam for 30 min. Hypnosis was not observed in animals pretreated with gadoterate meglumine.

Reviewer's comment: I agree.

Study A4: Study of Thermoregulation in the mouse after intravenous administration of Test article (Gadoterate meglumine)

Objective: The purpose of this test was to study the effect of intravenous injection of Gadoterate meglumine on body temperature in the mouse.

Methods:

Principle and value of the test: Several substances and metabolic processes are capable of altering the core body temperature of an animal. A determination of the sigmoid temperature in the test animal (mouse) provided a measurement of change in temperature in response to administered substances or metabolic changes.

Test and control articles: Test article (Gadoterate meglumine, 1 mmol/kg); Control article (isotonic saline, 0.9% NaCl)

20 female mice weighing 17-21 g were used for the study.

Rectal body temperature was measured using an electronic thermometer with a digital display and a probe for small animals. The temperature was taken before the injection of Gadoterate meglumine and at 15, 30, 45 and 60 min after injection. The mean (\pm standard error) of the body temperature was determine in each study group for each given time point. Values obtained for Gadoterate meglumine-treated mice were compared with saline controls using the Student's t-test.

Table 12: Study design and dose groups

Study groups	No. of mice	Dose/Volume
I. 0.9% NaCl (isotonic saline)	10	10 mL/kg
II. Gadoterate meglumine (1 mmol/kg) IV	10	1 mmol/kg

Reviewer's Table based on sponsor's data; saline or test article (Gadoterate meglumine) were administered intravenously

Results: The results are shown in Table 13:

Table 13: Rectal temperature ($^{\circ}\text{C} \pm \text{SEM}$) before and after Gadoterate meglumine administration

Time (min)	Time zero (T_0)	15 min	30 min	45 min	60 min	120 min
Isotonic saline	37.2 \pm 0.14	37.7 \pm 0.14	37.7 \pm 0.09	37.5 \pm 0.11	37.3 \pm 0.10	36.9 \pm 0.13
Gadoterate meglumine	37.3 \pm 0.18	37.8 \pm 0.10	37.5 \pm 0.11	37.4 \pm 0.10	37.1 \pm 0.09	37.0 \pm 0.14
p-value	NS	NS	NS	NS	NS	NS

Reviewer's Table adapted from sponsor's data on p.30 of 135

Conclusion: There was no statistical difference in rectal temperature at all the time points tested in Gadoterate meglumine- and saline-treated (control) mice.

Reviewer's comment: I agree. The findings showed that Gadoterate meglumine when administered intravenously did not alter the body temperature of mice.

Study A5: Study of the antinociceptive effect in the Hot Plate Test in the mouse after intravenous administration of Gadoterate meglumine

Objective: The purpose of this test was to verify the absence of an analgesic effect of Gadoterate meglumine when injected intravenously in the mouse using a classical pharmacological screening test for pain (paw lick test)

Methods:

Principle and value of the test: This test method used in this study is an adaptation of the paw lick test (Hot plate test) in which the mouse is placed on the floor of a glass container partly immersed in a water bath whose temperature was kept constant at 65 $^{\circ}\text{C}$. In untreated animals, reactions – observed in chronological order – were 1) a simultaneous licking of the forelegs, 2) first jump and 3) an adjusted jump, which allowed the animal to escape from the container. In the absence of a negative reaction (a negative response was an absence of the above reaction(s)), the test duration would normally be set at 120 sec. The test is comparable to the tail squeeze test in that it assesses the effect of analgesics notably morphine which acts by inhibiting the responses described above. Some substances may have a partial effect by inhibiting only one or a number of the reactions. In this study, an alpha-adrenergic agonist (Xylazine, 5 mg/kg IV) and not morphine was used for its sedative and analgesic property.

Test and control articles: Gadoterate meglumine (G 449.06; 11 mmol/kg IV) diluted 1:5 with normal saline; 0.9% NaCl (saline) and Xylazine (2% injectable solution; batch no. 98.234 diluted 1:50 with distilled water) were used.

Animals: 30 naïve mice weighing 19-23 g were used for the study.

Study design and dose groups are shown in Table 14:

Table 14: Study design and dose groups

Study groups	No. of mice	Dose/Volume
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I. 0.9% NaCl (isotonic saline)	10	10 mL/kg
II. Xylazine, IV	10	5 mg/kg (or 12.5 mL/kg of Rompun (commercial Xylazine solution))
III. Gadoterate meglumine (G 449.06), IV	10	1 mmol/kg

Reviewer's Table based on sponsor's data; saline, Xylazine or test article (Gadoterate meglumine) were administered intravenously

Results: The mean (\pm SEM) of the times to onset of each of the reactions of mice described above was determined. The effect of Xylazine, Gadoterate meglumine and saline were compared with that of saline-treated mice using the Mann-Whitney Test. The results are shown in Table 15:

Table 15: Results of the Hot-plate test

Treatment groups	Response Time (sec \pm SEM)		
	Licking	1 st Jump	Adjusted Jump
Isotonic NaCl solution alone	7 \pm 0.9	42 \pm 1.9	50 \pm 3.1
Xylazine alone	21 \pm 4.3 ($p \leq 0.01$)	98 \pm 9.1 ($p \leq 0.01$)	106 \pm 7.6 ($p \leq 0.01$)
Gadoterate meglumine (G 449.06)	8 \pm 1.2 (NS)	35 \pm 3.3 (NS)	41 \pm 3.0 (NS)

Reviewer's Table adapted from sponsor's Table on p.34 of 135

Conclusion: All the saline-treated mice displayed the three reactions expected (licking their forelegs) within a period of 10 sec. Xylazine produced the expected analgesic effect by significantly increasing the time to onset of each of the responses (licking, 1st jump and adjusted jump). All the mice administered Gadoterate meglumine reacted as the saline-treated animals with a slight non-significant reduction in the time to onset of the jumps. Gadoterate meglumine, unlike the reference compound, Xylazine did not cause a significant analgesic effect on mice in the hot plate test.

Reviewer's comment: I agree.

Study A6: Study of the pro-convulsive stimulant effect by potentiation of Pentylenetetrazole-induced convulsions in the mouse after intravenous administration

Objective: The purpose of this study was to determine whether intravenous Gadoterate meglumine would potentiate the convulsive effect of a sub-threshold dose of pentylenetetrazole.

Methods:

Principle and value of test: The brain stem stimulant, Pentylenetetrazole (PTZ) when administered intravenously or intraperitoneally at a certain dose will provoke convulsions. Pre-treating an animal with a pro-convulsive agent followed by a sub-threshold dose of PTZ will be sufficient to trigger convulsions.

PTZ-induced convulsions are excitations of the mesencephalic reticular formation and the mode of action involves presynaptic inhibition and therefore a competition with gamma-amino-butyric acid (GABA). Drugs known to be convulsive at high doses reduce the brain concentration of

GABA. One of these – Isoniazide (a major anti-tuberculosis agent), was chosen as a reference compound. Isoniazide at a non-convulsive dose will potentiate PTZ. The isoniazide dose used (100 mg/kg IV) is equivalent to 2/3rd of its LD₅₀. In this study, Gadoterate meglumine was administered at 8mmol/kg – a dose representing the same proportion of the LD₅₀ (or 11.4mmol/kg).

Test, reference and control articles: Gadoterate meglumine (G 449.06) diluted 1:5 with isotonic saline (Pyrogen-free injectable isotonic NaCl solution, (b) (4) batch 81.633), pentylenetetrazole (Sigma, 20 mg in 20 mL distilled water) and Ramifin ((b) (4) batch F 002P containing 500 mg isoniazide diluted 1:25 times with distilled water) were used.

Animals: 50 mice weighing 21-25 g were used for the study.

Study design and dose groups are shown in Table 16:

Table 16: Study design and dose groups (sub-study 6; DGD-2-5-A)

Study groups	No. of mice	Dose (or volume administered)
I. Intravenous 0.9 % Saline + IP Pentylenetetrazole 30 min after	10	Saline (25 mL/kg) then PTZ (30 mg/kg or 30 mL/kg of solution)
II. Intravenous Isoniazide then PTZ 30 min later to a subset	20	All 20 mice: 100 mg/kg (or 25 mL/kg of diluted solution) then 30 min later, 30 mg/kg PTZ to 10/20 mice
III. Intravenous Gadoterate meglumine to all rats then Intraperitoneal PTZ (30 min) later to a subset	20	All 20 mice: Gadoterate meglumine (8mmol/kg) then 30 min later, 30 mg/kg PTZ to PTZ to 10/20 mice

Reviewer's Table based on sponsor's data

Scoring of reactions: Following an intraperitoneal injection of PTZ, mice (5 mice/cage) were observed for 1 hr and reactions scored as follows:

0 = No abnormal finding

1 = Shaking of the head or body

2 = Isolated convulsive attack (the convulsive attack was of the tonic-clonic type)

3 = 2 or more convulsive attacks

Results:

Table 17: Number of mice with convulsions

Score levels	Number of mice with scores				Total score	Mean score ± SEM)
	0	1	2	3		
I. Saline + PTZ	10	0	0	0	0	0
II. Isoniazide alone	9	0	0	1	3	0.3 ± 0.30

III. Isoniazide + PTZ	0	0	0	10	30	3.0 ± 0.0
IV. Gadoterate meglumine	10	0	0	0	0	0
V. Gadoterate meglumine + PTZ	10	0	0	0	0	0

Reviewer's Table based on sponsor's data; Mean time to onset of convulsions when isoniazide was administered alone was 23.00 min; In the presence of PTZ, the time to onset of convulsions was reduced to 6.30 ± 1.44 min.

No abnormal reaction was observed when PTZ was administered with saline (0/10 mice). A single mouse convulsed 23 min after Isoniazide injection. Following Gadoterate meglumine administration, 5 mice reacted significantly and showed signs of apnea at the end of the injection followed by polypnoea. However, no signs of convulsions were observed. When Gadoterate meglumine injection was followed with an injection of PTZ, 3 mice showed signs of apnea but no animals had convulsions.

Statistical comparisons were made to determine if there was a statistically significant difference in the frequency or intensity of reactions between (1) Isoniazide + PTZ versus PTZ alone, (2) Isoniazide alone versus Isoniazide + PTZ and (3) Gadoterate meglumine versus PTZ alone.

The frequency of the convulsions was compared using the chi² test with Yates correction and their intensity evaluated with the Mann-Whitney U test. The time to onset of the convulsive reaction was expressed as mean ± SEM.

Table 18: Statistical comparisons of frequency and intensity of reactions

	Frequency		Intensity of reactions	
	chi ² value	P-value	Mann-Whitney value	P-value
Isoniazide + PTZ vs. PTZ alone	16.20	p ≤ 0.001	0	p ≤ 0.01
Isoniazide alone vs. Isoniazide + PTZ	12.93	p ≤ 0.001	5	p ≤ 0.01
Gadoterate meglumine + PTZ vs. PTZ	0	NS	50	NS

Reviewer's Table based on sponsor's data

Conclusion: Given alone at the doses administered, Isoniazide (100 mg/kg IV) or Pentylentetrazole (30 mg/kg IP) were non-convulsive. When Isoniazide and PTZ were injected at a 30-minute interval in the mice, onset of convulsions was observed. Potentiation of the combined effects of PTZ and isoniazide was significant for frequency and intensity of convulsion. Gadoterate meglumine (8 mmol/kg dose) produced a strong reaction at the time of injection (reversible apnea) - attributable to its proximity to the LD₅₀ - but it was non-convulsive spontaneously and in the presence of PTZ.

Reviewer's comment: I agree with study design, results and conclusions. Isoniazide was an appropriate reference article in this test. Isoniazide is the most widely used anti-tuberculosis drug which if taken as a high dose, acutely causes toxicity - one sign of which is grand-mal seizures. Isoniazide-induced seizures are particularly resistant to anticonvulsants (Okutor et al., 2006). An

acute overdose of Isoniazide results in absolute pyridoxine deficiency. Pyridoxine is an essential cofactor in the synthesis of GABA – the major inhibitory neurotransmitter in the CNS.

Study A7: Study of the pro-convulsive stimulant effect by potentiation of Picrotoxin-induced convulsions in the mouse after intravenous administration

Objective: The purpose of the study was to determine whether intravenously administered Gadoterate meglumine would potentiate the effect of Picrotoxin - a convulsive agent with a specific medullary action.

Methods:

Principle and value of test: The action of the neurostimulant, picrotoxin is first manifested on the medulla oblongata and the mid-brain and then to a lesser degree on the cortex. At a high dose, its effect extends to convulsions. This convulsive effect has been shown to correspond to a presynaptic rather than a post-synaptic inhibition. Picrotoxin competes with GABA. When an animal is pretreated with a pro-convulsive agent, a sub-threshold dose of picrotoxin will be sufficient to trigger convulsive attacks.

Isoniazide at a non-convulsive dose will potentiate PTZ. The isoniazide dose used (50 mg/kg, IV) was equivalent to 1/3rd of its LD₅₀. In this study, Gadoterate meglumine was administered at 4mmol/kg – a dose representing the same proportion of the LD₅₀ (11.4mmol/kg) as well as the standard dose (1 mmol/kg) used in other tests.

Test, reference and control articles: Gadoterate meglumine (G 449.06) diluted 1:5 with isotonic saline (Pyrogen-free injectable isotonic NaCl solution, (b) (4) batch 81.633); picrotoxin ((b) (4) 20 mg in 200 mL distilled water) and Isoniazide ((b) (4) batch F 002P containing 500 mg isoniazide diluted 1:25 times with distilled water) were used.

Animals: 61 mice weighing 20-24 g were used for the study.

Study design and dose groups are shown in Table 19:

Table 19: Study design and dose groups (sub-study A7; DGD-2-5-A)

Study groups	No. of mice	Dose (or volume administered)
I. Intravenous 0.9 % Saline + IP Picrotoxin 30 min after	10	Saline (40 mL/kg) + Picrotoxin: 2 mg/kg (or 20 mL/kg of solution)
II. Intravenous Isoniazide (21 mice); 10 of which received Intravenous Isoniazide + IP Picrotoxin (30 min later)	21	All mice: 50 mg/kg (or 25 mL/kg of diluted solution) then 10 of which received 2 mg/kg Picrotoxin after 30 min
III. Intravenous Gadoterate meglumine (4mmol/kg; 20 mice) 10 of which received 2 mg/kg picrotoxin 30 min later	20	Gadoterate meglumine (4mmol/kg) then 10 of which received 2 mg/kg picrotoxin 30 min later

IV. Intravenous Gadoterate meglumine (1mmol/kg) + Picrotoxin (30 min later)	10	Gadoterate meglumine (1mmol/kg) + 2 mg/kg Picrotoxin 30 min later
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Reviewer's Table based on sponsor's data

Following an intraperitoneal injection of Picrotoxin, mice in cages of 5 mice per cage were observed and the number of animals which reacted in each group scored as follows:

0 = No abnormal finding

1 = Shaking of the head or body

2 = Isolated convulsive attack (the convulsive attack was of the tonic-clonic type)

3 = 2 or more convulsive attacks

The frequency of the convulsions was compared using the χ^2 test with Yates correction and their intensity evaluated with the Mann-Whitney U test. The time to onset of the convulsive reaction was expressed as mean \pm SEM.

Results:

Table 20: Number of mice with convulsions

Score levels	Number of mice with scores				Mean score \pm SEM)
	0	1	2	3	
I. Saline + Picrotoxin	9	0	0	1	0.3 \pm 0.30
II. Isoniazide alone	10	0	0	0	0
III. Isoniazide + Picrotoxin	3	0	2	5	1.9 \pm 0.43
IV. Gadoterate meglumine (4mmol/kg) alone	10	0	0	0	0
V. Gadoterate meglumine (4mmol/kg) + Picrotoxin	7	0	0	3	0.9 \pm 0.46
VI. Gadoterate meglumine (1 mmol/kg) + Picrotoxin	9	0	1	0	0.2 \pm 0.20

Reviewer's Table based on sponsor's data

Table 21: Time to onset of convulsions

	Average time (min)	No. of mice showing convulsions
Saline + picrotoxin	9.0	1
Isoniazide + Picrotoxin	21.43 \pm 4.29	7
Gadoterate meglumine (4mmol/kg) + Picrotoxin	22.20 \pm 2.11	3
Gadoterate meglumine (1mmol/kg) + Picrotoxin	18.00	1

Of the 10 mice that received Isoniazide alone, none (0/10) convulsed. In the Isoniazide and picrotoxin group, 7/10 mice convulsed within a mean time of 21 min and 43 sec after picrotoxin injection. When gadoterate meglumine was administered alone at 4mmol/kg, none of the 10 mice (0/10) convulsed. In the gadoterate meglumine (4mmol/kg) plus picrotoxin group, 3/10 mice convulsed 22 min and 20 sec after intraperitoneal injection of picrotoxin. A single mouse (1/10) convulsed in the gadoterate meglumine (1 mmol/kg) plus picrotoxin group.

Statistical comparisons were made between (1) isoniazide and picrotoxin vs. picrotoxin, (2) gadoterate meglumine (4mmol/kg) / picrotoxin vs. picrotoxin and (3) Gadoterate meglumine (1 mmol/kg) / picrotoxin vs. picrotoxin. The statistical analysis is shown below:

	Frequency		Intensity of reactions	
	chi ² value	P-value	Mann-Whitney value	P-value
Isoniazide + picrotoxin vs. picrotoxin	5.20	p ≤ 0.05	21	p ≤ 0.05
Gadoterate meglumine (4mmol/kg) + picrotoxin vs. picrotoxin	0.31	NS	40	NS
Gadoterate meglumine (1mmol/kg) + picrotoxin vs. picrotoxin	0	NS	49.5	NS

Reviewer's Table based on sponsor's data

Conclusions: The doses of picrotoxin (2 mg/ml, IP) and Isoniazide (50 mg/kg IV) used in this study were non-convulsive. When picrotoxin was injected 30 min after isoniazide, convulsions were observed in 7/10 mice. The potentiating effect of isoniazide was significant (p ≤ 0.05) in terms of the frequency and intensity of convulsions. Gadoterate meglumine administered alone at 4mmol/kg P 449 was not convulsive. However, when mice previously administered Gadoterate meglumine subsequently received picrotoxin, 3/10 mice had convulsions with a time to onset of approximately 22 min similar to the time to onset when picrotoxin was administered after isoniazide (approximately 21 min). The potentiating effect of Gadoterate meglumine (4mmol/kg) was however, not statistically significant. Gadoterate meglumine (1 mmol/kg) was inactive in this model because the number of mice that convulsed was equal to the number that convulsed in the picrotoxin control group.

Reviewer's comment: I agree. Gadoterate meglumine even at the higher dose (4mmol/kg) did not significantly potentiate the convulsive effect of picrotoxin.

Study B1: Study of the Extrapyramidal effect through the study of catalepsy in rats after intravenous administration

Objective: The purpose of this study was to determine if the intravenous injection of Gadoterate meglumine did not alter the spontaneous and voluntary motor activity in the rat.

Methods:

Principle and value of test: The test involved placing the head and forelegs of a rat on a rectangular support (dimensions: 80 mm x 55 mm x 30 mm) covered with a grippable material. Within 10 sec, the untreated rat responds by resuming its normal posture denoted as a positive response.

The test revealed catalepsy – a state defined by immobility of the animal, loss of spontaneous and voluntary motor activity, a persistence of balancing reflexes and an ability to retain an imposed or even an unaccustomed posture. Compounds known as neuroleptics demonstrate catalepsy due to

their extrapyramidal effect (an inability to move). Haloperidol – a neuroleptic compound, administered at a dose of 2 mg/kg was used a reference compound in this test.

Test, reference and control articles: Gadoterate meglumine (G 449.06, batch 202) diluted 1:5 with isotonic saline (Pyrogen-free injectable isotonic NaCl solution, (b)(4) batch 81.625), and Haldol ((b)(4) batch No. 2999 B containing 5 mg Haloperidol diluted 1:10 in distilled water.

Animals: 30 rats weighing 160 -198 g were used for the study.

Study design and dose groups are shown in Table 22:

Table 22: Study design and dose groups (sub-study B1; DGD-2-5-A)

Study groups	No. of mice	Dose (or volume administered)
I. Intravenous 0.9 % Saline	10	Saline (10 mL/kg)
II. Intravenous Haloperidol	10	2 mg/kg (equivalent to 4 mL/kg diluted Halidol solution)
III. Intravenous Gadoterate meglumine	10	Gadoterate meglumine (1 mmol/kg)

Reviewer’s Table based on sponsor’s data

Results: The findings of the study are described in Table 23:

Table 23: Results of the catalepsy test in the rat (sub-study B1; DGD-2-5-A)

Study Groups	Test response at 30 min	
	Positive	Negative (Catalepsy)
I. Isotonic saline solution	10	0
II. Haloperidol	1	9
III. Gadoterate meglumine	10	0

Reviewer’s Table based on sponsor’s data

9 of 10 Haloperidol-treated rats showed catalepsy. Gadoterate meglumine- and saline-treated rats did not show any cataleptic reaction.

The chi² test with Yates’ correction was used to compare the number of catalepsies observed with Haloperidol or Gadoterate meglumine vs. saline control groups. Comparison of haloperidol vs. saline result was statistically significant (p ≤ 0.001). Gadoterate meglumine compared with saline was not significant.

Conclusion: The cataleptic effect of haloperidol was significant. The effect of Gadoterate meglumine was statistically not significant.

Reviewer’s comment: I agree.

Study B2: Study of the Allogenic effect in the rat after intra-arterial administration

Objective: The purpose of this study was to determine if Gadoterate meglumine had a potential to produce painful reactions when injected via the intra-arterial route in the rat. In this study, the influence of the hyperosmolality on the pain reaction was evaluated following an intra-arterial administration of Gadoterate meglumine.

Methods:

Principle and value of test: Based on its osmolality, an intra-arterial injection of a solution could produce painful reactions. It involved injecting the test product into the rat femoral artery in the direction of blood flow and then rating the resulting painful reaction using a scoring system. Gadoterate meglumine is a hyperosmolar solution and the test was used to evaluate the painful effect following its intra-arterial administration.

Test, reference and control articles: Gadoterate meglumine (G 449.06; Batch No. 202; Osmolality: 1381 mOsm/kg); 5% isotonic glucose solution ((b) (4) Batch No. 14.179; Osmolality: 280mOsm/L) and 30% isotonic glucose solution ((b) (4) Batch No. 14.280; Osmolality: 1666mOsm/L).

Animals: 30 male Sprague-Dawley rats weighing 138-151 g were used for the study. Rats were anesthetized with intravenous Pentothal and the femoral artery catheterized in the direction of blood flow. Animals were allowed to wake up before the injection of test and reference compounds. Three groups of animals were used. Rating (blinded) of the reactions was performed according to the scoring system described below.

Study design and dose groups are shown in Table 24:

Table 24: Study design (Sub-study B2; DGD-2-5-A)

Study groups	No. of mice	Dose (or volume administered)
I. Intravenous Isotonic Glucose	10	0.5 mL/kg
II. Intravenous Hypertonic Glucose	10	
III. Intravenous Gadoterate meglumine	10	

Reviewer's Table based on sponsor's data.

The degree of Allogenic reaction observed in each rat was rated as follows: **1** = no reaction; **2** = animal attempted to run without using the test paw; **3** = the rat lifted the test paw towards himself and stopped running; **4** = the rat licked or bit in the direction of the test paw; **5** = increased motor activity was observed (jumping or sudden recoiling movements); **6** = foot operated on went into spasm; **7** = presence of tears. Reaction scores less than 4 were classified as "good safety" and scores equal to or greater than 4 were classified as "poor safety". The average scores were calculated for each test group. The series of "good safety" and "poor safety" were compared using the χ^2 test.

Results:**Table 25: Results of the pain reaction test (Sub-study B2; DGD-2-5-A)**

	Totals

Score	'Good safety'			'Poor safety'				Well-tolerated
	1	2	3	4	5	6	7	
5% Glucose	3	5	2	0	0	0	0	10/10
30% Glucose	0	0	0	1	9	0	0	0/10
Gadoterate meglumine	2	3	0	0	0	0	0	5/10

Reviewer's Table based on sponsor's data (Page 66/135)

The results showed that the 10 rats injected tolerated the 5% Glucose solution (low osmolality of 280mOsm/kg) with all animals showing good safety scores. All 10 rats treated with the 30% Glucose solution (high osmolality of 1666 mOsm/kg) had poor safety scores and 0/10 rats had good safety scores. The test article, Gadoterate meglumine (intermediate osmolality of 1381mOsm/kg) showed a good safety score in 5/10 rats and poor safety in 5/10 animals.

Conclusion: Statistical analysis showed that the effect of hypertonic glucose - with high osmolality, poor safety scores and evidence of pain reaction - was highly significant ($p \leq 0.0001$) when compared to the isotonic glucose solution (low osmolality, good safety scores and little evidence of pain). The effect of Gadoterate meglumine solution (intermediate osmolality, intermediate safety scores and some evidence of pain reaction) was significantly greater than that of isotonic solution ($p \leq 0.05$). In summary, the effect observed increased with osmolality. 30% Glucose solution (high osmolality) was poorly tolerated while 5% Glucose (low osmolality) was well-tolerated. The effect of Gadoterate meglumine (intermediate osmolality) was well-tolerated in 50% of the animals and its tolerance for pain was equivalent to the level of its osmolality.

Reviewer's comment: I agree.

Study B3: Study of the pro-convulsive stimulant effect by potentiation of pentylenetetrazole-induced convulsions in the rat after intracisternal administration

Objective: The purpose of the test was to determine whether an intravenous injection of Gadoterate meglumine would potentiate the effect of convulsive pentylenetetrazole (PTZ).

Methods:

Principle of the test: The test was based on the observation that a dose of a test product administered intracisternally in rabbits, followed after 30 min by an intraperitoneal administration of a non-convulsive dose of PTZ, resulted in convulsions. Similarly, if the product was injected into the cerebrospinal fluid (CSF), CNS cells may be adversely affected and a subsequent injection of an inactive dose of the convulsive agent (PTZ) could trigger clonic or tonic convulsive attacks.

Value of test: The test was proposed as a means to assess the neurological safety of contrast agents as demonstrated by iopamidol, a non-ionic iodine-based contrast agent active in this test model. This model was applied to evaluate the neurological effects of Gadoterate meglumine-based MRI in indications in which existing pathology and damage to the blood-brain barrier allow the contrast medium to circulate. Iopamidol was used as a reference compound at an intracisternal

(IC) concentration of 370 mg iodine/mL. 211 mg/kg iopamidol was equivalent to 20% of its LD₅₀ by the IC route.

Test, reference and control articles: Gadoterate meglumine (Test article, Batch No. 202 diluted 1:20 with injectable normal saline solution) administered at 1 and 2 µmol/rat or 7.5 and 15%, respectively of the Gadoterate meglumine intracisternal LD₅₀; Pentylenetetrazole (PTZ; 300 mg in 25 mL distilled water; ^{(b)(4)}) and Iopamidol solution (containing 37% iodine, I₂).

Study design: The study was conducted as 2 studies each with three dose groups. A total of 104 rats weighing 160-194 g (Study 1) and 158-188 g (Study 2), were used. The study design and dose groups are shown in Table 26:

Table 26: Study design (Sub-study B3; DGD-2-5-A)

Group	No. of Rats	Route & Dose (or volume administered)
Study 1:		
I. Pentylenetetrazole (PTZ)	10	IP: 20mg/kg (or 1.7 mL/kg PTZ solution)
II. a) Iopamidol alone*	14	IC: 211 mg I ₂ /kg (or 0.57 mL/kg) Iopamidol
b) Iopamidol + PTZ	10	PTZ (20mg/kg) IP 30 min after Iopamidol
III. a) Gadoterate meglumine alone	10	IC: 2µmol/rat Gadoterate meglumine (or 0.08 mL of diluted solution)
b) Gadoterate meglumine + PTZ	10	PTZ (20mg/kg) IP 30 min after Gadoterate meglumine
	Total = 54	
Study 2:		
I. Pentylenetetrazole (PTZ)	10	IP: 20mg/kg (or 1.7 mL/kg PTZ solution)
II. a) Iopamidol alone	10	IC: 105mg I ₂ /kg (or 0.29 mL/kg) Iopamidol;
b) Iopamidol + PTZ	10	PTZ (20mg/kg) IP 30 min after Iopamidol
III. a) Gadoterate meglumine alone	10	IC: 1µmol/rat Gadoterate meglumine (or 0.04 mL of diluted solution)
b) Gadoterate meglumine + PTZ	10	PTZ (20mg/kg) IP 30 min after Gadoterate meglumine
	Total = 50	

Reviewer's Table based on sponsor's data on pages 56-57/135.

* = a high proportion of animals receiving iopamidol showed spontaneous convulsions within 30 min of iopamidol injection making administration of PTZ impossible leading to an increase in number of animals in order to obtain 10 rats in the iodopamidol+PTZ group.

Scoring of reactions: Following intraperitoneal injection of PTZ, rats (5/cage) were observed for 1 hr and reactions scored as follows:

0 = No abnormal finding

1 = Shaking of the head or body

2 = Convulsive attack* (*Convulsions were mostly clonic and sometimes of the clonic-tonic-clonic type)

3 = 2 or more convulsive attacks

*Convulsions were mostly clonic and sometimes of the clonic-tonic-clonic type

Statistic analysis: The results were processed per study. The number of animals with reacted was counted in components of each study, added, mean \pm SEM determined and frequency of occurrence of the convulsions compared with χ^2 test/Mann Whitney U test. The time to onset of convulsion was expressed as mean \pm SEM.

Results:

1). *Occurrence of pro-convulsive and convulsive effects:* The number of rats in the 2 studies showing or not showing convulsions with the different treatments is shown in Table 27:

Table 27: Number of animals showing convulsions (Sub-study B3; DGD-2-5-A)

Study/ Group	Treatment	No. of rats	Score				No. of rats with convulsions
			0	1	2	3	
Study 1							
Group I	PTZ alone	10	10	0	0	0	0
Group II	Iopamidol (211 mg I ₂ /kg)	14	8	0	1	5	6
	Iopamidol+PTZ	10	0	0	1	9	10
Group III	Gadoterate meglumine (2 μ mol/rat)	10	7	0	2	1	3
	Gadoterate meglumine +PTZ	10	4	0	5	1	6
Study 2							
Group I	PTZ	10	10	0	0	0	0
Group II	Iopamidol (105 mg I ₂ /kg)	10	9	0	0	1	1
	Iopamidol+PTZ	10	2	0	0	8	8
Group III	Gadoterate meglumine (1 μ mol/rat)	10	10	0	0	0	0
	Gadoterate meglumine +PTZ	10	6	0	2	2	4

Reviewer's Table based on sponsor's data on pages 58 & 60/135.

A non-convulsive dose of PTZ (20 mg/kg, IP) administered alone did not cause any convulsions (0/10) in rats in both studies.

In Study 1, when Iopamidol was injected alone intracisternally at a higher dose (211 mg I₂/kg) via the intracisternal route, it caused convulsions in 6/14 rats compared with 1/10 rats administered a lower dose (105 mg I₂/kg) of iopamidol in Study 2. When rats were administered Iopamidol (IC) followed by 20 mg/kg PTZ after 30 min, there were convulsions in 10/10 rats and 8/10 rats in Studies 1 and 2 respectively

Gadoterate meglumine administered alone intracisternally at 2 μ mol/rat caused convulsions in 3/10 rats (study 1). Gadoterate meglumine administered in study 2 at half the dose (1 μ mol/rat)

administered in study 1 did not cause convulsions. The ability of gadoterate meglumine to provoke convulsions in rats when administered alone by the intracisternal route was dose-dependent. Gadoterate meglumine, administered intracisternally and followed after 30 min by PTZ (20 mg/kg, IP) resulted in convulsions in rats in both studies. The number of convulsions appeared dose-dependent as more convulsions occurred study 1 (6/10 rats) when a higher dose of gadoterate meglumine (2 μ mol/rat) was injected compared to study 2 when a lower dose (1 μ mol/rat) resulted in convulsions in 4/10 rats. This finding indicated a pro-convulsive effect of gadoterate meglumine.

2). *Time to onset of convulsions*: The mean time to onset of convulsions was determined in studies 1 and 2 and the results described in Table 28:

Table 28: Time-to-onset of convulsions (Sub-study B3; DGD-2-5-A)

Study 1			Study 2		
Treatment	No. of animals convulsing	Mean time of onset of convulsion (min)	Treatment	No. of animals convulsing	Mean time of onset of convulsion (min)
Iopamidol (211 mg I ₂ /kg)	6	23.40 \pm 7.34	Iopamidol (105 mg I ₂ /kg)	1	61.00
Iopamidol (211 mg I ₂ /kg) + PTZ	10	14.00 \pm 3.50	Iopamidol (105 mg I ₂ /kg) + PTZ	8	13.45 \pm 3.09
Gadoterate meglumine (2 μ mol/rat)	3	34.20 \pm 8.34	Gadoterate meglumine (1 μ mol/rat)	0	0
Gadoterate meglumine (2 μ mol/rat) + PTZ	6	10.00 \pm 2.45	Gadoterate meglumine (1 μ mol/rat) + PTZ	4	15.30 \pm 8.44

Reviewer's Table based on sponsor's data on pages 59 & 61/135

The mean time (min) for onset of Iopamidol-induced convulsions when administered alone was shorter (approximately 24 min) with the higher dose (211 mg I₂/kg; study 1) than for the convulsion observed in 1/10 rats administered a lower dose of Iopamidol (105 mg I₂/kg; study 2).

When PTZ was administered 30 min after Iopamidol, the time to onset of convulsion in rats in both studies occurred at approximately the same time (~14 min).

The mean time to onset for convulsion induced by Gadoterate meglumine (2 μ mol/rat) in 3/10 rats was 34 min and longer than was obtained for iopamidol. A lower dose of Gadoterate meglumine (1 μ mol/rat), unlike a lower dose of iopamidol, did not induce convulsions.

Similar to Iopamidol, a subsequent injection of the convulsive agent provoked convulsions in Gadoterate meglumine -treated rats irrespective of dose. The time to onset for convulsion in rats receiving Gadoterate meglumine and PTZ in studies 1 and 2, did not appear significantly different.

3). *Evidence of potentiation of convulsions with IP PTZ using statistical analysis:*

In Study 1, Iopamidol or Gadoterate meglumine injected alone via the intracisternal route provoked convulsions in 6/14 or 3/10 rats, respectively. The frequency of convulsions due to Iopamidol (211 mg I₂/kg) alone versus Iopamidol (211 mg I₂/kg) in the presence of PTZ was designated as # (1) in the statistical comparisons. Similarly, Gadoterate meglumine (2 μmol/rat) alone versus Gadoterate meglumine (2 μmol/rat) in the presence of PTZ was designated as # (2) in Table 29.

In study 2, the doses of Iopamidol (105 mg I₂/kg) or Gadoterate meglumine (1 μmol/rat) each injected alone intracisternally caused only minimal convulsions in 1/10 rats (Iopamidol) or 0/10 rats (Gadoterate meglumine). The frequency of convulsions due to Iopamidol (105 mg I₂/kg) alone versus Iopamidol (105 mg I₂/kg) in the presence of PTZ was designated as # (1) in the statistical comparisons. Similarly, Gadoterate meglumine (1 μmol/rat) alone versus Gadoterate meglumine (1 μmol/rat) in the presence of PTZ was designated as # (2) in Table 29.

The intraperitoneal injection of PTZ caused a significant increase ($p \leq 0.02$ or $p \leq 0.01$ in study 1 or 2, respectively) in the number of convulsions with Iopamidol (211 mg or 105 mg I₂/kg) and not with Gadoterate meglumine (2 μmol or 1 μmol/rat).

Table 29: Potentiation of convulsions by PTZ: Statistical analysis (Sub study B3-DGD-2-5-A)

Comparison	Frequency		Intensity of Reaction		
	chi ²	p-value	Mann-Whitney U value	p-value	
Study 1	# (1)	6.06	$p \leq 0.02$	28	$p \leq 0.05$
	# (2)	0.81	NS	36	NS
Study 2	# (1)	7.27	$p \leq 0.01$	15	$p \leq 0.01$
	# (2)	2.81	NS	30	NS

Reviewer's Table based on sponsor's data on pages 62 & 63/135; NS, not significant

Conclusion: Based on the results of the study, the sponsor concluded in the first test (study 1) the doses of Iopamidol and Gadoterate meglumine were convulsive but the potentiation of PTZ-induced convulsion was significant only with iopamidol. In the second test (study 2), the doses of Iopamidol or Gadoterate meglumine were half as high as the doses used in study 1 and were less active (Iopamidol) or inactive (Gadoterate meglumine). The sponsor noted irrespective of the effect of the two doses of Gadoterate meglumine on PTZ was not significant; more convulsions were observed when PTZ was administered following the intracisternal administration of Gadoterate meglumine. This finding demonstrated that Gadoterate meglumine had a potentiating effect, albeit slight, in the model to describe pro-convulsive effect.

Reviewer's comment: I agree with the study design, results and conclusions.

Overall Conclusion (DGD-2-5-A):

The behavioral studies conducted in mice and rats were designed with the objective of revealing a potential neurotoxic effect of Gadoterate meglumine administered in most studies via the intravenous route intended for administering this product in humans.

The intracisternal route was used for studying the potential pro-convulsive effect of Gadoterate meglumine and the intra-arterial route was used to study the Algogenic effect of Gadoterate meglumine.

The dose of Gadoterate meglumine used was 1 mmol/kg or approximately 1/10th of the intravenous LD₅₀ in the mouse (i.e. 11 mmol/kg) which was equivalent to 10-fold the dose proposed for use in humans (0.1mmol/kg).

The following tests were performed:

Tests in mice -

- Effect on motility (circle test)
- General depressant effect in the traction, tail squeeze and righting reflex tests
- General depressant effect by potentiation of barbiturate-induced hypnosis
- Effect on thermoregulation
- Antinociceptive effect in the hot-plate test
- Pro-convulsive stimulant effect by potentiation of pentylenetetrazole-induced convulsions. Gadoterate meglumine was administered at 8 mmol/kg (or 6.5x the maximum human dose based on body surface area)
- Pro-convulsive stimulant by potentiation of picrotoxin-induced convulsions (Gadoterate meglumine was administered at 1 and 4 mmol/kg (or 0.8 and 3.2x the maximum human dose based on body surface area)

Tests in rats –

- Extrapyrmidal effect in the study of catalepsy
- Algogenic effect following intra-arterial injection of Gadoterate meglumine at 0.25 mmol/kg (or 0.41x the maximum human dose based on body surface area)
- Pro-convulsive stimulant effect by potentiation of pentylenetetrazole-induced convulsions in rats administered Gadoterate meglumine (1 or 2 µmol/rat) via the intra-cisternal route.

In behavioral tests in conscious animals, Gadoterate meglumine had no effect on motility; no central depressant effect; no extrapyramidal effect and no cataleptic effect. Gadoterate meglumine did not alter the body temperature following its intravenous administration.

Method-control (reference) substances were used to validate the various experiments. Reference substances produced the expected results. Gadoterate meglumine, in most of the studies, did not induce any effect. However, noteworthy findings included a painful intra-arterial administration in rats due to the hyperosmolarity of the injected Gadoterate meglumine solution (1350mOsm/kg) and a pro-convulsive effect following an intravenous injection of 4mmol/kg in picrotoxin-treated

mice and after an intra-cisternal administration of Gadoterate meglumine in pentylenetetrazole-treated rats.

Reviewer's comments (DGD-2-5-A):

I agree with the conclusions.

4.3.3 Cardiovascular System

4.3.3.1 Report No. 99.12.809: Evaluation of Effects on Blood Pressure, Heart Rate and Electrocardiogram after Single Intravenous Dosing in Conscious Dogs

Report location:	eCTD Module 4 §4.2.1.3.1
Conducting laboratory and location:	(b) (4)
Study #:	20000310P
Date of study initiation:	June 26, 2000
GLP compliance:	Yes (x), No (), page 13 of 142
QA report:	Yes (x), No (), page 10 of 142
Drug, lot #, and % purity:	(0.5mol/L); batch # 99M077, % purity – N/A
Control solution:	Hyperosmolar control solution and Negative control solution.
	<u>Hyperosmolar solution:</u>
	19.5% glucose solution with osmolality of 1371 and 1387 mOsm/kg
	<u>Isoosmolar:</u>
	5% glucose solution; Batch No. 95K23A2 (b) (4)
	Osmolality: 298mOsm/kg
Animal species/strain/sex per dose:	Dog/Beagle/ 3 per sex dosed sequentially; Source: (b) (4)
Age:	Adult (specific age N/A)
Weight:	11.5 - 14.8 kg (at the start of study)
Doses:	0.6, 2.4, 3.6 and 5.5mmol/kg
Duration/route:	Single / intravenous

Objective

The purpose of this study was to evaluate the effects of Gadoterate meglumine on blood pressure, heart rate and electrocardiogram after single intravenous dosing of Gadoterate meglumine in the conscious dog.

Key findings

At the lowest dose (0.6mmol/kg or 3.2 times the human dose), Gadoterate meglumine had no statistically significant effect on HR and arterial BP. ECG parameters (PR, QT interval or QRS complex) corrected for HR were not affected when compared with the negative control solution.

At 2.4 and 3.6 mmol/kg (or 13-20 times clinical dose) there was a transient, statistically insignificant increase in HR and arterial BP when compared to the negative control solution. The highest dose of 5.5mmol/kg induced a tachycardia significantly lower than observed with the 11mL/kg hypertonic control solution.

There was a slight, but significant reduction in QT interval 15 min after treatment at the high dose. Statistically significant increases in QT duration corrected for HR were observed 20h and 24h post-dose at 3.6 and 5.5mmol/kg Gadoterate meglumine, respectively.

There was no statistically significant effect of Gadoterate meglumine on PR interval and the QRS complex duration irrespective of Gadoterate meglumine dose when compared to control.

Lastly, there was no effect on 6-lead ECG, and T-wave was not affected irrespective of the dose of Gadoterate meglumine tested.

Methods

Telemetry: Telemetry transmitters was implanted in the peritoneal cavity of each dog at least 10 days prior to study, ECG electrodes were placed in lead II and the sensor catheter was placed in the femoral artery.

Dosing: Dogs were examined for 10 min before and after dosing. Gadoterate meglumine (0.1 mmol/kg clinical dose) was administered to dogs at 0.6, 2.4, 3.6 and 5.5mmol/kg or 3.2x, 13x, 20x and 30x the human dose adjusted for body surface area, respectively. The study involved 3 male and 3 female

Each animal was dosed as follows:

- Negative control solution (iso-osmolar 5% glucose solution) was administered in the same volume (1.2 mL/kg) as the lowest dose level of Gadoterate meglumine
- Hyperosmolar control solution: hyperosmolar 19.5% glucose solution administered under the same volume (11 mL/kg) as for 5.5 mmol/kg of Gadoterate meglumine,
- Gadoterate meglumine (0.6 mmol/kg)
- Gadoterate meglumine (2.4 mmol/kg)
- Gadoterate meglumine (3.6 mmol/kg)
- Gadoterate meglumine (5.5 mmol/kg)

All injections were done at 60 mL/min. Each treatment was performed after a washout period of at least 48h.

Parameters: Mean systolic and diastolic BP, HR, duration of PR and QT, duration of QRS complex was continuously for at least 24 h following dosing recorded. QT was corrected for heart rate variations using Fridericia's formula and Sarma equation.

Table 30: Study Design and Gadoterate meglumine Dose Groups (99.12.809)

Groups	Test or Control article	Administered Volume or Dose		Number of Dogs	
		Dose	Dose volume		

		(mmol/kg)	(mL/kg)	Males	Females
Control					
1	Negative control solution (5% glucose)	-	1.2	3	3
2	Hyperosmolar solution (19.5% Glucose)	-	4.8		
3	Hyperosmolar solution (19.5% Glucose)	-	11.0		
Test					
4	Gadoterate meglumine	0.6	1.2		
5		2.4	4.8		
6		3.6	7.2		
7		5.5	11.0		

Source: Reviewer's Table adapted from Sponsor's study design. Each animal was dosed in the following sequence: Negative control solution (isoosmolar 5% glucose), Hyperosmolar 19.5% glucose solution; each treatment was done after a minimum washout period of 48 h

Table 31: Gadoterate meglumine Human Dose multiples (99.12.809)

	Negative control	Gadoterate meglumine Dose levels			
		Level 1	Level 2	Level 3	Level 4
Dose (mmol/kg) in rats	0	0.6	2.4	3.6	5.5
Dose (mmol/m ²)	0	12	48	72	110
Dose multiples (based on BSA)	N/A	3.2x	12.97x (13x)	19.45x (20x)	29.7x (30x)

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult)

Observations and Results

Mortality

No deaths were reported

Clinical signs

No clinical signs were observed after the injection of the negative control (5% isoosmolar glucose solution) or the hyperosmolar glucose solution (4.8 mL/kg).

Excitation, vomiting and tremors were observed following the administration of the Hyperosmolar solution (11 mL/kg).

Gadoterate meglumine induced a dose-related occurrence of nausea, vomiting and tremors in treated dogs.

Cardiovascular effects

1. Pre-dose values: Predose values of mean, systolic and diastolic BP, heart rate, cardiac conduction times (PR, QT intervals and QRS complex) were within normal range.

2. Negative control solution: Based on the following findings, it appeared the negative control solution (5% isoosmolar solution/1.2mL/kg) had a short-lasting effect on BP and heart rate: 1)

The negative control solution (1.2 mL/kg) induced increases in blood pressure (Emax: +32%) and heart rate (Emax: +46%) shortly after injection, 2) there was no change in cardiac conduction times, and 3) ECG measurement by 6 leads (I, II, III, aVR, aVL and aVF) was normal and no change was seen in T-waves in the 6 dogs.

3. Hyperosmolar solution (4.8 mL/kg): Compared with the negative control values, the hyperosmolar solution had no effect on arterial blood pressure, PR interval and duration of the QRS complex. There was no effect on ECG in lead II at 24h post-dose and ECG by leads I, II, III, aVR and aVF and there was no change in the T-wave morphology in the 6 dogs.

4. Hyperosmolar solution (11 mL/kg): Compared with when dogs were dosed with the negative control solution, there was a marked and significant increase in HR (Emax: +179%) 5 and 15 min post-dose. This increase was associated with a decreased QT duration (Emax: -29%) 5 min post-dose. The 11 mL/kg hyperosmolar solution also induced statistically significant decreases in the mean arterial pressure (Emax: -8%, Tmax: 6 h post-dose). No statistically significant change was induced in systemic arterial BP, PR interval and QRS complex in the 6 dogs. There was no disturbance in ECG lead II 24 h post-dose and in the ECG by 6 leads. There was also no change in T-wave morphology. Hyperosmolarity (11 mL/kg) appeared to cause transient but marked increased HR and clinical signs in conscious dogs.

5. Gadoterate meglumine:

- Gadoterate meglumine at 0.6mmol/kg (or 3.2 times the human dose) did not have any statistically significant effect on HR and arterial BP. ECG parameters (PR, QT interval or QRS complex) corrected for HR were not affected when compared with the negative control solution
- At 2.4 and 3.6 mmol/kg (or 13-20 times clinical dose) there was a transient increase in HR (Emax: +95% and +115%, respectively) and arterial BP (Emax: +43% and +45%, respectively) in the minutes following administration. At 30 min to 2 hours, the heart rate was comparable to the negative control solution
- The highest dose of 5.5mmol/kg induced a tachycardia (+126%)
- There was a slight increase in arterial BP (Emax: +64%)
- There was a slight, but significant reduction in QT interval 15 min after treatment at the high dose that was attributed to HR changes since it was not confirmed by corrected QT (QTc). However, compared to pre-dose values, corrected interval QT (QTc) was slightly reduced (-2 msec or -0.9%) at 4 hr following the 3.6 mmol/kg dose and from -8 to -13 msec (or -2.3 to 6% reduction) between 1 and 4 hrs following the highest administered dose (5.5 mmol/kg). These effects were not significantly different from the negative controls in the same 4h time period.
- Statistically significant increases in QT duration corrected for HR were observed 20h and 24h post-dose at 3.6 and 5.5mmol/kg Gadoterate meglumine, respectively.
- There was no statistically significant effect of Gadoterate meglumine on PR interval and the QRS complex duration irrespective of Gadoterate meglumine dose when compared to control.
- There was no effect on 6-lead ECG and T-wave was not affected irrespective of the dose of Gadoterate meglumine tested.

Conclusions

Based on the results of this study the sponsor concluded as follows:

- That Gadoterate meglumine, administered by the intravenous route at doses of 0.6, 2.4, 3.6 and 5.5 mmol/kg induced only moderate cardiovascular effects in conscious dogs as transient tachycardia and increase in blood pressure that are attributable to the very large injected volume and/or the hyperosmolarity of the solution as these effects were also seen with control solutions.
- That for dose levels of Gadoterate meglumine up to 3.6 mmol/kg, heart rate returned more rapidly to predose values than with hyperosmolar control solutions
- The QT was slightly reduced (-2 msec or -0.9%) at 4 hr following the 3.6 mmol/kg dose and from -8 to -13 msec (or -2.3 to 6% reduction) between 1 and 4 hrs following the highest administered dose (5.5 mmol/kg). However, the effect was slight in severity when compared to predose values and no difference could be seen at all lower doses with the negative control solution for QTc. That the no-effect dose-level (NOEL) of Gadoterate meglumine on cardiovascular parameters in conscious dogs corresponded to 0.6 mmol/kg.

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

4.3.3.2 Report No. DGD-33-005 Evaluation of proarrhythmic effects of Gadoterate meglumine after intravenous administration in methoxamine pretreated anesthetized rabbits

Report location:	eCTD Module 4 §4.2.1.3.1
Conducting laboratory and location:	(b) (4)
Report #:	DGD-33-005 (Study No. 200220337PCL)
Date of study initiation:	June 17, 2002
GLP compliance:	Yes (x), No (), page 12 of 151
QA report:	Yes (x), No (), page 7 of 151
Drug, lot #, and % purity:	Gadoteric acid (Gd-DOTA) (batch # 99M077); % purity – N/A; Magnevist (Gd-DTPA) (batch 13495C & 13495K. purity- N/A
Animal species/strain/sex per dose:	Rabbit/New Zealand White (NZW)/ (b) (4)
	3/sex/group
Age:	N/A
Weight:	2.35 - 3.04 kg (at study initiation)
Doses:	1, 2 and 4 mmol/kg Gadoterate meglumine or Magnevist)
Duration/route:	Single / intravenous

Objective

The purpose of this study was to determine any potential proarrhythmic effects of Gadoterate meglumine following its intravenous administration in rabbits pretreated with methoxamine and anesthetized with α -chloralose.

Key findings

Gadoterate meglumine intravenously administered at 1, 2 and 4 mmol/kg to methoxamine-treated and alpha chloralose-anesthetized rabbits did not induce any alteration in the electrocardiogram or in cardiac conduction. Changes to QT and corrected QT (QTc) were not significant. Gadoterate meglumine at 2 and 4 mmol/kg induced a non-significant increase in heart rate. There was also a transient non significant decrease in arterial blood pressure at 4mmol/kg. Administration of the comparator compound, Magnevist at 1, 2 and 4mmol/kg to methoxamine-treated and anesthetized rabbits did not induce any alteration in the electrocardiogram or in cardiac conduction. Magnevist at 4mmol/kg did induce a statistically significant decrease in arterial blood pressure at the end of infusion. The validity of the effects cardiovascular effects of Gadoterate meglumine and Magnevist was confirmed with the positive effects of Sotalol on QT interval.

Methods

Rabbits were anesthetized with ketamine (35 mg/kg, intraperitoneally) and Xylazine (5mg/kg, intramuscularly) followed by α -chloralose (80-90 mg/kg). After tracheotomy, rabbits were artificially ventilated with room air. Polyethylene catheters were implanted in the ear vein and the left femoral vein for intravenous infusion of methoxamine (70 nmol/kg/min) and test substances respectively. Alpha-chloralose anaesthetized rabbits infused with methoxamine (α_1 -adrenergic receptor agonist) show an increased incidence of class III agent-related torsades de pointe.

Three (3) study groups of 3 rabbits/sex/group received the negative control (0.9% NaCl) followed by Gadoterate meglumine (negative control at 8 mL/min then test substance; group 1), Magnevist (negative control at 8 mL/min then reference/comparator compound, Group 2) or a method-control substance (negative control 2 mL/kg then Sotalol).

In all groups, there was a 30 minute interval between each injection. The parameters (mean, systolic and diastolic arterial blood pressure, RR and QT intervals) were recorded for 30 min after starting each treatment and analyzed before and 1, 5, 10, 20 and 30 minutes after treatment. QT interval was corrected for heart rate variations using Bazett and Fridericia formulas.

The dose levels of Gadoterate meglumine and Magnevist represented, in rabbits, 3.2, 6.3 and 13 times the human dose adjusted for body surface area.

Study Design

Table 32: Study Design and Gadoterate meglumine Dose Groups (DGD-33-005)

Groups	Control followed by Test article	Number of Dogs	
		Males	Females
1	Negative control [@] + Gadoterate meglumine (1, 2 & 4 mmol/kg)	3	3
2	Negative control [@] + Magnevist (1, 2 & 4 mmol/kg)	3	3
3	Negative control [@] + Sotalol (1, 3 & 10 mg/kg)	3	3

Reviewer's Table; Sotalol (used to treat ventricular arrhythmias and atrial fibrillation and used in this study as a method-control article); Magnevist was used as a comparator substance for Gadoterate meglumine and the same doses as Gadoterate meglumine; @ Negative control is 0.9% NaCl.

Table 33: Gadoterate meglumine Human Dose multiples (DGD-33-005)

Administration	Vehicle	Doses of Gadoterate meglumine or Magnevist		
		Low Dose	Mid Dose	High Dose
Dose (mmol/kg) in rats	0	1	2	4
Dose (mmol/m ²)	0	12	24	48
Dose multiples (based on BSA)	N/A	3.24x (3.2x)	6.49x (6.5x)	12.97x (13x)

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult).

Electrodes were placed in Lead II to monitor and record ECG. ECGs were recorded before dosing, and 1, 5, 10, 20 and 30 min after starting the infusion. Parameters investigated include mean, systolic and diastolic arterial pressure (mmHg), RR interval (ms) and QT interval (ms). Parameters were recorded before and at 1, 5, 10, 20 and 30 min after starting the infusion.

Observations and Results

Results

Predose values of the evaluated parameters were within the normal range. The negative control (saline) did not cause any abnormal change in arterial blood pressure, RR and QT intervals

At 1 mmol/kg, Gadoterate meglumine did not have any statistically significant effect on arterial blood pressure, heart rate and the related RR interval duration. At 2 and 4 mmol/kg, Gadoterate meglumine caused a statistically non-significant but dose-dependent decrease in RR interval with an E_{max} of 21% (2mmol/kg) and 29% (4mmol/kg) (T_{max} was 30 min). Also at 4 mmol/kg (or 13 times MHD) there was a short-lived decrease (24%) in arterial blood pressure at the end of infusion. There was a 22% increase in arterial blood pressure at T_{max} (5 min). The changes described were not statistically significant and it did not appear that the test article had any remarkable effect on BP and HR. ECG (lead II) was normal and there was no abnormal change to the T-wave. Changes to QT and corrected QT (QTc) were not significant.

The comparator compound produced similar effects to Gadoterate meglumine.

Sotalol did not cause a statistically significant change in arterial blood pressure at any of the doses tested. RR duration was reduced at 5 - 30 min after the start of infusion at the top dose of 10 mg/kg (E_{max}: 50%; T_{max}: 20 minutes) suggesting occurrence of a tachycardia.

At 1, 3 and 10 mg/kg, Sotalol induced a dose-dependent increase in the duration of QT interval (E_{max}: +48%, T_{max}: 5 minutes at 10 mg/kg) and QT interval corrected for heart rate (Bazett's (E_{max}: +30%, T_{max}: 5 minutes at 10 mg/kg) and Fridericia's formula (E_{max}: +35%, T_{max}: 5min at 10 mg/kg).

Conclusions

Methods

The study involved the preparation of 6 Purkinje fibers acquired from 4 dogs. A maximum of 3 left ventricular Purkinje fibers were obtained from each dog.

On each preparation, the following were tested successively at 30 min intervals in the order shown: Tyrode solution (Sequence 1), Tyrode solution (Sequence 2), Gadoterate meglumine (2.5 mmol/l); Gadoterate meglumine (5mmol/L); Gadoterate meglumine (10 mmol/L); Tyrode solution (Sequence 3), and Cisapride (0.3 μ mol/L). The concentration of Cisapride should induced an increase in APD₉₀ (*i.e.*, action potential duration at 90% of repolarisation) of about 20-50 ms minimum under normal stimulation rate (60 pulse per minute *i.e.*, ppm)

Concentrations of Gadoterate meglumine and of the method-control substance are expressed as mmol/L and μ mol/l respectively of the final concentration in the perfusion fluid. Gadoterate meglumine was diluted in Tyrode to achieve the tested concentrations and perfused at 5 mL/min. Cisapride was dissolved in sterile water containing 0.06% acetic acid (300 μ M) diluted at 0.3 μ M in Tyrode and perfused at 5 mL/min. Solutions containing Gadoterate meglumine or Cisapride were perfused into the 5 mL organ bath at the rate of 5 mL/min.

Table 34: Methods (DGD-33-001)

Protocol	Method, frequency and/or objectives
Preparation of animals and preparation of Purkinje fibers	According to SOP
Parameters measured	Amplitude of the action potential (APA) Resting Potential (RP) Maximal rate of depolarization (Vmax) Action potential duration (APD) was evaluated at 50% of (APD ₅₀), 70% (APD ₇₀) or 90% (APD ₉₀) repolarisation Only values obtained at the end of each period of stimulation at 1 Hz (25 th min) and at 0.33 Hz (30 th min) were analyzed

Results

At the 3 concentrations of Gadoterate meglumine tested (2.5, 5 and 10 mmol/L), there was no statistically significant effect on the amplitude of action potential (APA), resting potential (RP), maximal rate of depolarization (Vmax) when the fibers were stimulated at 1 Hz or at 0.33 Hz. No early after depolarization was observed.

Cisapride, as expected increased APD₅₀ (+25 ms), APD₇₀ (+44 ms) and APD₉₀ (+55 ms) at 1 Hz. It also increased action potential at 0.33 Hz: APD₅₀ (+37 ms), APD₇₀ (+74 ms) and APD₉₀ (+103 ms). The increases were more pronounced at the 0.33 Hz compared to the increases at 1 Hz.

Conclusions

Gadoterate meglumine at the doses tested had no effect on action potential duration in Purkinje fibers. The validity of the results was confirmed by the increase in action potential induced by Cisapride, the method-control substance.

Reviewer's comments

I agree with the study conclusions.

4.3.4 Cardio-Respiratory System**4.3.4.1 Report No. DGD-2-12-A: Cardiovascular and respiratory safety of G449-06 administered IV to anesthetized dogs. Study of dose-dependent effects**

Report location:	eCTD Module 4 §4.2.1.3.1
Conducting laboratory and location:	Laboratoire GUERBET Pharmacodynamics Department, 16/24 rue Jean Chaptal, 93601 Aulnay-sous-Bois Cedex France
Study #:	87-09-105
Date of study initiation:	September 02, 1987
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gadoteric acid (Gd-DOTA; 1381 mOsm/kg) (batch # 202); % purity – N/A; Hypertonic 21.7% Glucose solution (1387 mOsm/kg)
Animal species/strain/sex per dose:	Dog/Mongrel; 6 females and 2 males
Age:	N/A
Weight:	14 - 22 kg
Doses:	G449.06 (Gadoterate meglumine): 0.1, 0.5 and 1.0 mmol/kg
Dose volume:	0.2, 1.0 and 2 mL/kg (for 0.1, 0.5 and 1.0 mmol/kg, respectively)
Duration/route:	Single / intravenous (Saphenous vein)

Objective

The purpose of this study was to evaluate the adverse effects of Gadoterate meglumine on cardiovascular and respiratory functions in anesthetized male and female mongrel dogs.

Key findings

Gadoterate meglumine caused an 11% decrease in blood pressure; there was no effect on HR at the low dose with the mid and high dose causing a decrease in rate. Minimal variations were observed in the LVP. There was a slight increase in pulmonary blood pressure at the mid and high dose (0.5 and 1.0 mmol/kg, respectively). There was an increase of 4-16% increase in respiratory rate at the high dose of Gadoterate meglumine. Lower doses (0.1 and 0.5 mmol/kg) did not elicit any pulmonary effects. Based on the results, the NOAEL was determined as 0.5mmol/kg (or 2.7x the human dose).

Methods

Animals were divided into study groups as shown in Table 35:

Table 35: Study Design and Gadoterate meglumine Dose Groups (DGD-2-12-A)

Groups	Test or Control article	Administered Vol. or Dose		No. of Dogs	
		Dose (mmol/kg)	Dose volume (mL/kg)	Males	Females
1	Isotonic solution (0.9% NaCl)	-	2.0	2	6
2	Hypertonic solution (21.7% Glucose)	-	2.0		
3	Gadoterate meglumine	0.1	0.2		
4		0.5	1.0		
5		1.0	2.0		

Source: Reviewer's Table adapted from Sponsor's study design. Each animal was dosed in randomized order with a 20 min interval between each injection.

Table 36: Gadoterate meglumine Human Dose multiples (DGD-2-12-A)

Administration	Doses of Gadoterate meglumine		
	Low Dose	Mid Dose	High Dose
Dose (mmol/kg) in dogs	0.1	0.5	1.0
Dose (mmol/m ²)	2.0	10.0	20.0
Dose multiples (based on BSA)	0.5x	2.7x	5.4x

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult).

Animals: Dogs were anesthetized with an IV injection of Acepromazine (0.25mg/kg) and sodium pentobarbital (30 mg/kg) followed by tracheal intubation. The saphenous vein was catheterized for injections and additional pentobarbital administrations. The study was an open-thorax determination of dose-dependent effect of Gadoterate meglumine on the cardio-pulmonary system. After thoracotomy, ECG (lead II), heart rate, myocardial contractility, coronary blood flow, aortic pressure and flow, and aortic resistance were investigated.

Parameters evaluated:

1. ECG: ECG was recorded using lead II
2. Blood pressure (BP): BP was recorded via femoral artery catheterization to determine diastolic and systolic blood pressure
3. Heart Rate (HR): HR was recorded from the BP signal
4. Left Ventricular Pressure (LVP): LVP was recorded via a catheter inserted via the left carotid artery into the left ventricle.
5. Left Ventricular Contractility (dP/dt): dP/dt was recorded by derivation of the LVP signal.
6. Pulmonary Blood Pressure: Pulmonary BP was recorded via a catheter inserted into the right jugular vein then into the right ventricle and on into the pulmonary artery.
7. Respiration: Respiration was recorded via a transducer attached to the chest and connected to blood pressure recording device.

Results

Gadoterate meglumine (1 mmol/kg or 5.4x MHD) caused 11% bradycardia, 17% decrease on myocardial contractility, 24% increase in coronary blood flow, and a 9% and 7% increase in systolic and diastolic aortic pressure, respectively. There was an increase of 26% in aortic flow and

a 17% decrease in aortic arterial resistance. There were statistically significant differences between saline and high dose (1.0 mmol/kg) Gadoterate meglumine treatment for heart rate, coronary flow and aortic resistance at the highest level. There were no effects on ECG at all the Gadoterate meglumine doses tested.

Summary of results:

Blood pressure: No remarkable test article related changes were observed

Heart rate (HR): Isotonic saline solution and Gadoterate meglumine at the low dose had no effect on the heart rate. The mid and high dose caused a decrease in heart rate while and hypertonic glucose caused an increase in HR.

LVP: Only small variations from the baseline were detected with the isotonic saline solution and the three Gadoterate meglumine doses tested. A slightly greater effect was observed with the hypertonic glucose solution.

Pulmonary blood pressure: There was a slight increase in systolic arterial pulmonary blood pressure at 0.5 and 1 mmol/kg Gadoterate meglumine levels. Compared to baseline, there was an increase in systolic pulmonary BP of 10.3%.

Respiration: There were no remarkable test-article related effects on respiration at the low dose (0.1mmol/kg) of Gadoterate meglumine. At 1 mmol/kg (or 5x the clinical dose), there was a 4-16% increase in respiratory rate.

Conclusions

Based on the above results, the sponsor concluded that Gadoterate meglumine did not cause a change in the ECG at any of the tested doses. At 0.1 mmol/kg (the intended clinical dose), Gadoterate meglumine did not produce any remarkable cardiovascular or respiratory effects. However, at the high dose (1mmol/kg or 5x MHD), there was a slight but significant increase in pulmonary arterial blood pressure and a 4-16% increase in the rate of respiration.

Reviewer's comments

The results are acceptable. Based on the findings, it did not appear that Gadoterate meglumine, at the doses evaluated had any remarkable effects cardiovascular or respiratory effects in the anesthetized mongrel dog. Due to slight cardio-respiratory effects, NOAEL was established as 0.1mmol/kg or 0.5x-MHD.

4.3.5 Renal System

4.3.5.1 Report No. DGD-33-006 Evaluation of effect on renal function in glycerol-induced renal failure model in the rat following single intravenous administration

Report location:

eCTD Module 4 §4.2.1.3.1

Conducting laboratory and location:

[REDACTED] (b) (4)

Report #: DGD-33-006 (Study No. 20020340PGR)
Date of study initiation: September 02, 2002
GLP compliance: Yes (x), No (), page 11 of 94
QA report: Yes (x), No (), pages 7-8 of 94
Drug, lot #, and % purity: Gadoteric acid (Gd-DOTA) (batch # 99M077); % purity – N/A; Magnevist (Gd-DTPA) (batch 13490C. purity- N/A
Animal species/strain/sex per dose: Rat/Wistar (b) (4)
4 groups of 8 male rat/group were used
Age: N/A
Weight: 303.2-349.4 g
Doses: 2mmol/kg (Gadoterate meglumine or Magnevist)
Duration/route: Single / intravenous

Objective

The objective of this study was to evaluate the effect of Gadoterate meglumine on renal function in glycerol-induced renal failure model in the rat following single intravenous administration.

Key findings

Intramuscular injection of glycerol caused a significant increase in urinary excretion of proteins, plasma creatinine, and urea. Intravenous administration of a single-dose (2 mmol/kg or 3.2-fold human dose) of Gadoterate meglumine (or its comparator Magnevist at the same dose) did not cause an exacerbation of the impairment of renal function induced by glycerol in rats.

Methods

Principle of test: In the rat model of Glycerol-induced nephropathy, an intramuscular injection of glycerol induces renal damage by causing muscular injury at the site of injection. The muscular injury is characterized by rhabdomyolysis - a condition in which the breakdown of damaged muscle cells including myoglobin, are released into the bloodstream. These muscle breakdown products are harmful to the kidneys resulting in acute kidney failure. Other substances known to increase in rhabdomyolysis include creatine kinase and potassium ions. Hemolysis of red blood cells also occurs. It has been reported that renal morphological changes in Glycerol-induced nephropathy were similar to human ischemic vasomotor nephropathy. The objective of this study was to evaluate the effect of Gadoterate meglumine or its comparator on renal function by determining if a single injection of the test article (Gadoterate meglumine) or Magnevist exacerbated the effects of Glycerol-induced kidney damage.

1. Study design: The study design is shown in Table 37:

Table 37: Study Design and Dose groups (DGD-33-006)

Group	Treatment	No. of rats
1	Negative (saline 0.9%) control + water for irrigation	8
2	Glycerol + control substance (saline)	8

3	Glycerol + Gadoterate meglumine (2 mmol/kg or 3.2x-MHD)	8
4	Glycerol + Magnevist (2 mmol/kg or 3.2x-MHD)	8

Reviewer's Table based on sponsor's data

2. Administration of control and test articles:

Gadoterate meglumine, Magnevist and the negative control were administered intravenously. Glycerol was administered intramuscularly.

3. Study Timeline:

- Day 1: Drinking water and food removed and Glycerol injected after 24 h
- Day 2: Collection of urine and treatment with the test substances or the negative control substance
- Day 3: Collection of urine and blood sampling (24 h time point)
- Day 4: Collection of urine and blood sampling (48 h time point)

4. Sacrifice: After the 48 h blood sampling, animals were sacrificed by euthanasia. The kidneys were removed and preserved in 10% neutral buffered formol.

5. Hematology and urinalysis:

The following urine and plasma parameters were assessed: Urinary pH, osmolarities of plasma and urine, urinary and plasma sodium, potassium, chloride, creatinine, bicarbonate, plasma urea calcium and urinary proteins.

Results

I. Glycerol (24 h after injection and before dosing with Gadoterate meglumine or Magnevist):

Findings in this group include a marked increase in urinary protein excretion, and an increase in plasma creatinine and urea levels. In animals administered glycerol, there was an increase in urinary flow. Hematuria, increases in urine pH and excretion of bicarbonate and calcium were prominent findings.

II. Glycerol (48 h after dosing with Gadoterate meglumine, Magnevist or the Negative control at 24 h and 48h):

There were no changes observed in animals dosed with the negative control/water group (absolute control)

a) Glycerol/negative control group: Changes in the parameters evaluated were characteristic of renal failure namely, increased plasma creatinine and urea levels, increased excretion of protein and increased Glomerular filtration rate (GFR). A comparison of 24h and 48h results showed significant increases in plasma creatinine (+142% vs. +84%) and urinary protein excretion (+533% vs. +292%). A statistically significant increase in plasma urea level was seen at 24h (+176%) and a marked tendency to an increase was seen at 48h (+142%). A decrease (-37% and -31%) was noted in the GFR at 24h and 48h, respectively.

b) Glycerol + Gadoterate meglumine: No statistically significant changes were noted in plasma creatinine and urea, urinary excretion and GFR when compared with the control group dosed with glycerol/negative control article.

c) Glycerol + Magnevist: Similar to Gadoterate meglumine, there were no statistically significant changes in plasma creatinine and urea, urinary excretion and GFR when compared with the control group dosed with glycerol/negative control article.

III. Histopathological findings in kidneys:

The histological changes observed in all examined animals were consistent with acute kidney disease due to the presence of Glycerol. There were similar lesions in rats in the control group that received Glycerol and negative control but to a lesser degree.

Conclusions

Administration of Gadoterate meglumine (or its comparator Magnevist), as a single dose (2mmol/kg or 3.2-fold the human dose) did not contribute to an exacerbation of the impairment of renal function induced by glycerol in rats.

Reviewer's comments

I agree with the principle underlying the design of the study. A useful urinary parameter commonly used to assess renal function and determine renal failure is urinary excretion of proteins. Since a marked increase of urinary protein was observed in animals treated with Glycerol, it was concluded that glycerol had caused an impairment of renal function. However, if the dose of Glycerol used to induce muscle damage (rhabdomyolysis) and consequently renal damage already produced the maximal kidney damage possible, it will be difficult to evaluate any potential harmful effects of the test article (Gadoterate meglumine) or its comparator (Magnevist) on the kidneys since there will be no room to demonstrate additional nephropathy. The reviewer therefore concludes that the study design should have included groups of animals administered lower dose(s) of glycerol with the potential of causing less than maximal kidney damage. Such groups can then be treated with the same doses of Gadoterate meglumine as were used in this study.

4.3.5.2 Report No. DGD-33-007: Evaluation of effect of Gadoterate meglumine on renal function in normal rats and L-NAME treated rats following repeated intravenous administration for 14 days

Report location:	eCTD Module 4 §4.2.1.3.1
Conducting laboratory and location:	 (b) (4)
Report #:	DGD-33-007 (Study No. 2002338PGR)
Date of study initiation:	September 25, 2002
GLP compliance:	Yes (x), No (), page 19 of 373; ICH S7A compliant study
QA report:	Yes (x), No (), pages 8-10 of 373

Drug, lot #, and % purity:

1. Gadoteric acid (Gd-DOTA) complex; Gadoterate meglumine or G 449.06, batch # 99M077; Guerbet), % purity: N/A; Osmolality: 1350mOsm/kg
2. Gd-DTPA meglumine salt (Magnevist; batch# 13490C); % purity – N/A; Osmolality: 1960 mOsm/kg
3. Negative control solution (Isotonic NaCl solution), Aguettant; batch #: 440375A01

Animal species/strain/sex per dose:

Rat/Wistar/Males only (b) (4)

Rats were divided into 6 groups of 12 animals per group

Age:

N/A

Weight:

167.8 - 200.0 g

Objective:

The purpose of this study was to evaluate any potential effect of Gadoterate meglumine on renal function in normal and L-NAME (N- ω -nitro-L-arginine methyl ester)-treated rats following repeated intravenous administration of Gadoterate meglumine for 14 days. Reversibility of any potential effects was studied over a 28-day period. Magnevist was used as comparator substance.

Key findings

Potential effects of Gadoterate meglumine on renal function was evaluated in normal rats and in L-NAME (Nitric oxide synthetase inhibitor; N- ω -nitro-L-arginine methyl ester)-treated rats following repeated intravenous administration of Gadoterate meglumine (2 mmol/kg or 3.2x the clinical dose adjusted for body surface area) for 14 days. L-NAME administered alone for 14 days did not cause a significant increase in kidney weight in rats when compared to controls that did not receive L-NAME. A minimal to mild nephropathy, albeit statistically non-significant, was also observed in L-NAME-treated rats.

In the absence of L-NAME, there was an increase in kidney weight that was reversible after the 28-day treatment-free period in Gadoterate meglumine -treated rats. Reversible renal tubular vacuolation and increased urinary excretion of calcium were also observed.

In rats pretreated with L-NAME followed by Gadoterate meglumine treatment, there was no increase in body weight. A statistically-significant increase in kidney weight was observed. The increase in kidney weight was reversible following a treatment-free period. Nephropathy was greater than observed when L-NAME was administered alone and tubular vacuolation and dilatation persisted into the treatment-free period.

In rats treated with L-NAME and Indomethacin (Agmon test) only, there was a marked increase in plasma creatinine (32%) and urea (300%). There was also a 46% decrease in GFR and 71% decrease in urine flow. Urine excretion of urea, Na⁺, K⁺, and Ca⁺⁺ were also observed. When Gadoterate meglumine (2 mmol/kg or 3.2x the clinical dose based on body surface area) was administered to L-NAME/Indomethacin-treated rats, there was no statistically significant differences in the measured parameters compared to the control group treated with L-NAME and Indomethacin alone.

Methods

1. Principle of test: Nitric oxide (NO; a cellular signaling molecule) and prostanoids (local hormones from arachidonic acid) are important mediators released from the endothelium. Pharmacological inhibition of these mediators can produce renal vascular effects that may include an increase in the nephrotoxicity of a test substance. Radiocontrast media are recognized to induce changes in renal hemodynamics comprising of an initial vasodilatation followed by pronounced vasoconstriction (Yao et al, 2001). Clinically, these effects manifest as decreased Glomerular filtration rate (GFR) and development of acute renal failure (Salomon, 1998). A principal mediator of radiocontrast-media nephropathy is adenosine. Activation of adenosine A₁- receptors was shown to be involved in the renal hemodynamic response to radiocontrast-media (Erley et al, 1997). Studies have also shown that the nitric oxide synthetase inhibitor, N- ω -nitro-L-arginine methyl ester (L-NAME), a selective adenosine A₁-receptor antagonist, has renal protective effects (Yao et al, 2001). This principle was adapted to evaluate the potential effect of Gadoterate meglumine on renal function.

Two methods of treatment with L-NAME were combined to produce a model of nephropathy in this study: (1) Animals were chronically depleted of nitric oxide by pretreatment with L-NAME administered in drinking water for 21 days (**Method of Erley**; Yao et al, 2001). (2) The animals were then dosed with indomethacin and L-NAME by the intravenous route (**Method of Agmon**; Agmon et al, 1994). Indomethacin is a nonselective cyclooxygenase (COX) inhibitor hence an inhibitor of prostaglandin synthesis. It causes an elevation in serum creatinine and may result in acute renal failure.

2. Study design: Rats were divided into 6 groups as shown in Table 38:

Table 38: Study Design and Dose groups (DGD-33-007)

Group*	Treatment	Dose multiples (x-MHD) for Gadoterate meglumine or Magnevist
1	Normal rats dosed with the negative control (saline)	
2	Normal rats dosed with Gadoterate meglumine (2 mmol/kg/day)	3.2x
3	Normal rats dosed with Magnevist (2 mmol/kg/day)	
4	L-NAME treated rats dosed with the negative control (saline)	
5	L-NAME treated rats dosed with Gadoterate meglumine at 2 mmol/kg/day	
6	L-NAME treated rats dosed with Magnevist at 2 mmol/kg/day	

Reviewer's Table based on sponsor's data (page 24/373; DGD-33-007); * = 12 rats/group.

3. Study Schedule: Testing procedures commenced 8 days before (D-8) the day of treatment (D1) and continued to day 42 (D42). The study schedule is shown in the following sponsor Table (Table 39):

Table 39: Study Schedule (DGD-33-007)

	Groups 1 to 3	Groups 4 to 6
D-8	Animals placed in diuresis cages without feed but with drinking water	
D-7	collection of 24h-urine and blood sampling oral saline overload and removal of drinking water collection of urine and blood sampling 5 hours after saline overload (1)	
D-1	Animals placed in diuresis cages without feed but with drinking water (containing L-NAME for groups 4 to 6)	
D1	collection of 24 h-urine and blood sampling treatment with DOTAREM [®] or MAGNEVIST [®] or negative control substance oral saline overload immediately after dosing and removal of drinking water collection of urine and blood samples 5 hours after saline overload	
D2 to D12	Daily treatment with DOTAREM [®] , MAGNEVIST [®] or negative control substance	
D13	treatment with DOTAREM [®] , MAGNEVIST [®] or negative control substance immediately afterwards, animals placed in diuresis cages without feed but with drinking water (containing L-NAME for groups 4 to 6)	
D14	collection of 24-hour urine and blood sampling treatment with DOTAREM [®] , MAGNEVIST [®] or negative control substance oral saline overload immediately after dosing and removal of drinking water collection of urine and blood sampling 5 hours after saline overload	
D15	Euthanasia (2) of 8 animals and removal of kidneys	last treatment with L-NAME in drinking water last treatment with L-NAME and indomethacin by intravenous route (Test of Agmon) last treatment with DOTAREM [®] , MAGNEVIST [®] and negative control substance placed in diuresis cage but feed and without drinking water
D16		collection of 24h-urine and blood sampling euthanasia of 8 animals and removal of kidneys
<i>Reversibility (4 animals per group)</i>		
D41	animals placed in diuresis cages without feed but with drinking water	
D42	collection of 24-hour urine and blood sampling oral saline overload and removal of drinking water collection of urine and blood sampling 5 hours after saline overload euthanasia of animals and removal of kidneys	

Sponsor Table (Study Schedule on page 26/373; Table 2).

4. Administration of control and test articles and other procedures:

- **Day -8:** 8 days before the start of treatment, all rats received drinking water and no feed.
- **Day -7:** 7 days before treatment, 24h urine and blood samples were collected from all rats; all animals were overloaded with oral saline and drinking water was removed. Urine and blood samples were collected 5h after the saline overload. Rats in groups 4-6 were started on drinking water containing L-NAME. L-NAME administration continued on test day 1 through day 14. L-NAME supplemented water was prepared fresh daily.

Body weight was measured on Day -7, Day 1 to 15 for groups 1-3 and up to Day 16 for groups 4-6.

- **Day 1:** 24h urine and blood samples were collected, and Gadoterate meglumine, Magnevist or negative control solution administered via the caudal tail vein. Immediately after dosing, animals were placed on saline overload, Drinking water removed and groups 4-6 received drinking water containing L-NAME.

- **Day 2 - 12:** Rats in all groups received daily treatment with Gadoterate meglumine, Magnevist or negative control solution.
- **Day 13:** After treatment with Gadoterate meglumine, Magnevist or negative control solution, rats were immediately placed in diuresis cages without feed. Drinking water containing L-NAME was given to animals in groups 4-6.
- **Day 14:** After 24h urine and blood sampling, all animals were treated with Gadoterate meglumine, Magnevist or negative control solution. Following treatment, animals were placed on saline overload followed by a 5h urine and blood sampling. Animals in groups 4-6 received drinking water containing L-NAME for the last time.
- **Day 15:** 8/12 rats in groups 1-3 were euthanized 1 day after the last treatment using pentobarbitone (60-75 mg/kg, IP) followed by exsanguination via the abdominal aorta. Animals were necropsied, kidneys were removed, dissected free of fat, were weighed together and fixed in 10% neutral buffered formalin. **Test of Agmon:** Animals in groups 4-6 received L-NAME drinking water and indomethacin intravenously and received the last treatment with Gadoterate meglumine, Magnevist or negative control after which they were placed in diuresis cages with feed but without drinking water.
- **Day 16:** 24h urine and blood samples were collected from group 4-6. Eight rats were euthanized and their kidneys removed.

Four rats/group were used for the reversibility study (Day 17 - Day 42)

- **Day 41:** All rats were placed in diuresis cages without feed but with drinking water.
- **Day 42:** 24h urine and blood samples were collected, animals were overloaded with oral saline and drinking water was removed. Urine and blood samples were collected 5h after the oral saline overload. All rats were then euthanized and kidneys removed.

4. Urine and plasma analysis: Urine and plasma osmolarities was performed using an osmometer within 3 days of sampling. Determination of urinary and plasma sodium, potassium, chloride, bicarbonate, urea, calcium and urinary protein as described in the protocol for samples collected on the days shown in the study schedule. Only urea and creatinine were determined in plasma samples collected on days D-7, D14 and D42. Osmolarity was not measured in urine samples collection after 24h.

5. Histology of the kidneys: After fixation, kidneys were dehydrated, embedded in paraffin wax and sections of each right or left kidney were stained with H&E stain.

6. Parameters determined: The following findings on renal function were determined and results expressed as means \pm SEM.:

- Urinary flow rate (ml/hr/100g body weight)
- Urinary pH
- Urinary excretion of Na^+ , K^+ , Cl^- , Ca^{++} , HCO_3^- , Urea, total proteins expressed as $\mu\text{moles}/24\text{h}$ or $5\text{h}/100\text{g}$ body weight
- Glomerular filtration rate, (GFR; measured by creatinine clearance) in ml/min/100g body weight
- The results are described according to treatment groups
- Percentage excretion of Na^+ , K^+ , Cl^- , Ca^{++} , HCO_3^- , Urea, and total proteins
- Free water clearance in ml/hr/100g body weight

7. Statistics: The effects of Gadoterate meglumine and Magnevist were compared with the negative control substance.

Results

Group 1: Baseline values (Day -7). The values of urinary parameters (flow rate, pH, HCO_3^- , Ca^{++} , Na^+ , Cl^- , K^+ , proteins and urea) and GFR were similar between the 6 groups at the 24h diuresis period. The values were also homogenous in all 6 groups following the 24h diuresis period induced by saline overload.

Groups 2 & 3: Gadoterate meglumine - or Magnevist-treated normal rats (without L-NAME) vs. control/normal treatment - No difference was observed in body weight between Gadoterate meglumine -treated rats and rats treated with saline. A decrease in weight was observed in the Magnevist group and persisted until 28 days post-treatment.

There was an increase in absolute and relative kidney weight in Gadoterate meglumine or Magnevist-treated rats compared with saline-treated animals. This finding was reversible on day 42 and no increase in organ weight was observed in Gadoterate meglumine or Magnevist-treated animals.

Minimal to marked renal proximal convoluted tubule vacuolation was observed in rats dosed with Gadoterate meglumine or Magnevist and sacrificed on day 15. The lesion was not observed in the saline-treated group. Vacuolation was present in 1 of 4 rats in the Gadoterate meglumine or Magnevist groups by the end of the 28-day treatment-free period.

On day 14, 24h after diuresis done under baseline conditions, there was a significant increase in urinary calcium excretion in the Gadoterate meglumine or Magnevist group when compared with saline-treated controls. This increase resolved by day 42. Following diuresis induced by saline overload, there was a significant increase in potassium excretion on day 14 in both Gadoterate meglumine and Magnevist-treated rats. The effects were no longer present on Day 42 after the recovery period.

Group 4: Effect of L-NAME treatment vs. saline controls. Three rats treated with L-NAME (Day -7 to Day 14) died on days 16, 18 and 23. There were no clinical signs before death. Histopathology report did not report any lesions associated with the deaths of these animals.

There was no statistically significant difference in kidney weight measured on day 15 between control animals administered water and control animals treated with L-NAME. On day 42, the weight of kidneys in animals treated with L-NAME was not different from that of control animals administered water.

Minimal to mild early lesions of nephropathy were observed in 4 of 8 L-NAME-treated rats sacrificed on Day 16. The incidence and severity was similar in the control group that received water. 5 of 8 animals dosed with L-NAME and euthanized on Day 16 had minimal to moderate

tubular dilatation. Tubular dilatation was no longer evident on day 42 or 27 days after the cessation of L-NAME treatment.

On Day 1, seven days after the start of L-NAME treatment, no differences were seen after 24h diuresis under baseline conditions between L-NAME-treated rats vs. animals given tap water. On day 14 (or 21 days after the start of L-NAME treatment), there was a 6% and 10% increase in plasma creatinine and urea, respectively compared to controls. There was no effect on urinary flow rate of GFR. These changes in plasma creatinine and urea were reversed on Day 42 (i.e. 27 days after the end of L-NAME treatment).

Groups 5 & 6: Effect of Gadoterate meglumine or Magnevist on L-NAME-treated rats - No mortality occurred in animals treated with Gadoterate meglumine. There was no difference in the body weight between Gadoterate meglumine -treated rats and those receiving the negative control isotonic saline. A non-significant decrease in weight gain was seen in Magnevist-treated animals.

On Day 16 (24h after the last treatment with Gadoterate meglumine, Magnevist or control saline), statistically significant increases were observed in kidney weight in animals administered Gadoterate meglumine (+22%), Magnevist (+18%) relative to body weight. On Day 42, the kidney weights were not different from the weights of rats in the control group.

In Gadoterate meglumine -treated rats, there was nephropathy in 2 of 8 Gadoterate meglumine -treated rats at the end of treatment on Day 16 and in 3 of 4 rats on Day 42 (end of treatment-free period). Tubular dilatation occurred in 7 of 8 rats on Day 16 and in 3 of 4 rats on Day 42. These incidences were greater, albeit non-significantly, compared to rats administered L-NAME only (Group 4).

Renal tubular vacuolation was observed in 8 of 8 animals (Gadoterate meglumine -treated) on Day 16 and in 3 of 4 animals necropsied on Day 42. Nephropathy, tubular dilatation and vacuolation were also observed in Magnevist-treated rats on Days 16 and 42 in similar frequency as in Gadoterate meglumine-treated rats.

Agmon test in rats treated with L-NAME/Indomethacin (Groups 4-6). Animals in the Agmon test were treated with L-NAME and Indomethacin and then received the negative saline solution (Group 4), Gadoterate meglumine (Group 5) or Magnevist (Group 6):

a). Control group (Group 4): There were marked increases in plasma creatinine (+32%) and urea (+300%) following L-NAME and Indomethacin injection. A decrease of 46% in GFR and 71% urinary flow were observed. A decrease in urinary excretion of urea, Na^+ , K^+ and Ca^{2+} was also reported.

b). Gadoterate meglumine group (Group 5): No statistical differences with rats were observed in Gadoterate meglumine-treated L-NAME/Indomethacin rats vs. the negative saline control group also treated with L-NAME/Indomethacin.

c). Magnevist group (Group 6): Statistically significant differences were observed between Magnevist-treated and negative control-saline group. There was an increase in plasma protein level (+15%), urinary flow (+50%), urinary excretion of Na^+ (+100%), Cl^- (+72%), total protein (+281%) and urea (+312%). There was also an increase in plasma creatinine (+28%).

Conclusions

Treatment with Gadoterate meglumine (2 mmol/kg/day or 3.2x the clinical dose adjusted for body surface area) for 14 days was well tolerated and caused no adverse effect on renal function. Renal kidney hypertrophy observed 24h following the last treatment was no longer evident after the 28-day treatment-free period. The proximal convoluted tubule vacuolation seen in all animals euthanized 24h after the last treatment with Gadoterate meglumine was also not evident after the recovery period. There was good renal tolerance of intravenous Gadoterate meglumine in normal and L-NAME-treated rats. By the end of the treatment-free (reversibility) period, all notable and significant changes in plasma and urinary parameters and histological findings were reversible.

Reviewer's comments

This is a well designed study to evaluate the potential effects Gadoterate meglumine on renal function in normal and L-NAME (N- ω -nitro-L-arginine methyl ester)-treated rats following repeated intravenous administration of Gadoterate meglumine for 14 days. I agree with the sponsor's conclusion that Gadoterate meglumine was well tolerated with or without L_NAME.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Summary of Gadoterate meglumine Pharmacokinetics (PK)

Studies were conducted to determine the PK profile of Gadoterate meglumine when administered in single- or repeat-dose intravenous administration to mice, rats, rabbits, dogs and goats. Gadolinium (Gd) retained in the body was determined in tissues and biological samples using the Atomic Emission Spectrophotometry (AES) method. The Gadoterate meglumine formulation used in animal PK studies was the same as the to-be-marketed formulation tested in clinical trials. In multiple studies, the PK of Gadoterate meglumine (Gd-DOTA) was compared to that of Magnevist (Gd-DTPA), the gadolinium-based contrast agent that was available at the time these PK studies were performed.

Single-dose PK: Results of nonclinical PK single-dose studies of Gadoterate meglumine in different animal species revealed: 1) a rapid distribution of Gd-DOTA in several organs, 2) the highest Gd concentrations were observed in the kidneys and bone, 3) half-life ($t_{1/2}$) across species was rapid (approximately 1 hr), 4) there was no protein binding or metabolism of Gadoterate meglumine, 5) there was a rapid urinary elimination and a low biliary excretion of Gd, 6) results indicated negligible excretion of Gadoterate meglumine in milk, 7) there was evidence of transplacental transfer of Gadoterate meglumine, 8) Gadoterate meglumine was poorly absorbed via the oral route.

Repeat-dose PK: Following repeated dose administration of Gadoterate meglumine at doses of 0.5, 0.7 and 1.5 mmol/kg in rats (or 0.8, 1.14 and 2.43-fold MHD) over a period of 28 days followed by a 28-day treatment-free period. Gadolinium was detected in the kidneys, liver and femur 1 day after the end of the 28-day treatment period.

Gd concentration was dose-dependent and highest amounts were obtained in the kidney. A linear relationship was obtained between Gd concentration in tissues and the dose administered. Gd

concentration was considerably decreased following the treatment-free period with slight amounts measurable in high dose animals. The low and mid dose groups were not evaluated for Gd content after the treatment-free period.

Gadoterate meglumine was administered to dogs in doses of 0.5, 0.7 and 1.5 mmol/kg (or 2.70, 3.78, and 8.11-fold MHD) over 28 days of treatment followed by a 28-day treatment-free period. Similar to the finding in rats, Gadolinium was detected in the kidneys, liver and femur. Samples were obtained 24 h following the first injection, after the last injection, and at the end of the 28-day treatment-free period.

As for rats, PK was linear. At the end of the treatment-free period, highest amounts of Gd were obtained in the kidneys. The repeat dose PK studies confirmed the findings of the single-dose PK studies. Of note, in the single dose studies, a small fraction of Gd was detected in the liver and bone.

Under conditions of repeated exposure, higher levels of tissue Gd were obtained with implications for a greater, long-lasting retention of Gadolinium in the body. It has been shown in the literature that bone tissue may serve as a site for Gd storage. Long-term persistence and slow release of Gd^{3+} from bone stores could therefore enhance Gd-associated toxicity.

It is also noteworthy that the skin was not evaluated for Gd content in view of the importance of the role of skin Gd content in the pathophysiology of the onset and propagation of NSF.

5.1.1.1 Introduction: Studies were conducted to determine the PK profile (absorption, distribution, metabolism and excretion) of Gadoterate meglumine when administered in single- or repeat-dose intravenous administration (preferred route of administration in humans) to mice, rats, rabbits and dogs. The extent of Gadolinium (Gd) retained in the body was also determined in tissues and biological samples using the Atomic Emission Spectrophotometry (AES) method. The Gadoterate meglumine formulation used in animal PK studies was the same as the to-be-marketed formulation tested in clinical trials. In multiple studies, the PK of Gadoterate meglumine (Gd-DOTA) was compared to that of Magnevist (Gd-DTPA), the gadolinium-based contrast agent that was available at the time these PK studies were performed. An overview of Gadoterate meglumine PK studies is shown in Table 40:

Table 40: Overview of Gadoterate meglumine Pharmacokinetic Studies

Type of PK Study	Species and Route*	Report No.	Dose (mmol/kg)	Reviewed (Yes/No)
Absorption Plasma profile, distribution, excretion ¹	Rat/Oral	DGD-0-8-A	2.0	No
Distribution				
<i>Single-dose plasma and/or tissue distribution</i>				
distribution	mice	DGD-0-15-A	0.5	Yes
Plasma profile, distribution	rat	DGD-0-2-A	0.1	Yes
distribution	rat	DGD-0-4-A	1.0	Yes
distribution	rabbit	DGD-0-5-A	0.1	Yes

Plasma profile, distribution and excretion	rabbit	DGD-0-1-A	0.5, 0.1	Yes
Plasma profile and excretion	dogs	DGD-0-3-A	0.1	Yes
Plasma profile and excretion in milk	goats	DGD-0-9-A	0.086	Yes
<i>Repeat-dose plasma and/or tissue distribution</i>				
distribution	rats	DGD-0-13-A	0, 0.3, 0.7, 1.5	Yes
distribution and excretion	dog	DGD-0-12-A	0, 0.3, 0.7, 1.5	Yes
<i>Plasma protein binding</i>				
	human serum albumin	DGD-0-6-A	10^{-2} , 10^{-3} , 10^{-4} M	Yes
<i>Placental transfer</i>				
	rats	DGD-0-14-A	0.5	Yes
Metabolism				
Metabolites in urine	rats, rabbits, dog	DGD-0-10-A	0.1	Yes
Excretion				
excretion	rats	DGD-0-7-A	1.0	No
<i>Plasma profile, distribution and excretion</i>				
	rabbit	DGD-0-1-A	0.1	Yes
<i>Plasma profile and excretion</i>				
	dog	DGD-0-3-A	0.1	Yes
<i>Plasma profile and excretion in milk</i>				
	goat	DGD-0-9-A	0.086	Yes
Other PK studies				
Plasma, urinary and biliary excretion profile in a model of renal impairment	Rats with renal failure	DGD-0-16-A	1.0	Yes

Reviewer's Table based on Sponsor's data (Modules 2.6.4 – PK Written summary and 2.6.4 – PK Tabulated summary); * Route of administration is intravenous unless stated otherwise; ¹ study involved the oral route. Since Gadoterate meglumine will be administered via the intravenous route, PK studies using the IV route were reviewed; ² in vitro study; PK = pharmacokinetics

5.1.2 Single-dose PK studies

5.1.2.1 Summary of findings: Results of single-dose, intravenous PK studies of GD-DOTA in different animal species revealed the following:

- 1) Rapid distribution of Gd-DOTA in several organs with highest concentrations of Gd retention in the kidneys and bone
- 2) Short half-life ($t_{1/2}$) across species (about 1 hr)
- 3) No protein binding or metabolism of the product
- 4) Rapid urinary elimination and appreciably low biliary excretion
- 5) Negligible excretion in milk or transplacental transfer
- 6) Poor oral absorption

Table 41: Summary of Single-Dose Cross-Species PK findings

Report #:	DGD-0-2-A	DGD-0-1-A	DGD-0-1-A	DGD-0-3-A	DGD-0-9-A
Species	Rat	Rabbit	Rabbit	Dog	Goat

Gender / No.	3F	3M + 3F	3M + 3F	3M + 3F	4F
Fasting (Yes/No)	No	Yes	Yes	Yes	No
Vehicle	Aqueous solution				
Mode of Administration	intravenous	intravenous	intravenous	intravenous	intravenous
Dose (mmol/kg)	0.1	0.1	0.5	0.1	0.085
Type of Sample	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	Total Gd ³⁺				
Assay	AES	AES	AES	AES	AES
PK parameters					
T1/2α (min)	NA	5.3	6.5	2	NA
T1/2β (min)	18	38	58	68	50
Vd	88	132	191	271	330
Cl_R (mL/kg/min)	NA	1.9	2.4	5.0	NA

Reviewer's Table, PK, Pharmacokinetic; AES, Atomic Emission Spectrophotometry; NA, not available

5.1.2.2 Report No. DGD-0-15-A: Whole body retention of Gd-DOTA in the hairless mouse over 21 days. A comparative study versus Gd-DTPA

Report location: eCTD Module 4 §4.2.2.3.1
 Conducting laboratory and location: Laboratoire Guerbet, Service de Pharmacocinetique
 16-24, rue Chaptal, 93601 Aulnay-sous-Bois Cedex
 France
 Study #: 88-03-2-03
 Date of study initiation: March, 1988
 GLP compliance: Yes (), No (x)
 QA report: Yes (), No (x)
 Drug, lot #, and % purity: Gd-DOTA complex (or G 449.06, batch # 151) Gd-DTPA meglumine salt (Magnevist; batch 439 RG 08); % purity – N/A (CoA in Appendix 1)
 Animal species/strain/sex per dose: Mice/ HRC/Orl.hr/22 per sex (b)(4)
 Age: 6 weeks
 Weight: Mean weight: 25±4 g
 Doses: Aqueous solutions of Gadoterate meglumine (Gd-DOTA) and Magnevist were administered at 0.5mmol/kg or 0.41-fold MHD (based on 0.1 mmol/kg administration in a 60 kg human adult);
 Vehicle: water
 Duration/route: Single / intravenous

Objective

The purpose of this study was to determine the distribution of Gadoterate meglumine in mice and compare it to Magnevist after a single dose intravenous administration.

Key findings

Approximately 1% of the injected dose was detected after 3 days with both test articles and the detectable amount decreased over time becoming undetectable after 10 and 21 days for Gadoterate meglumine and Magnevist, respectively.

Methods

Mice were divided into 3 main groups (control, Gadoterate meglumine- and Magnevist-treated). Each treatment group was subdivided into 5 groups of 2 mice/sex/sacrifice time point (days 3, 7, 10, 14 and 21 post-administration). Whole mice were mineralized in 120 mL of concentrated nitric acid (HNO₃) in a water bath at 90°C for 6 hours. Assay of whole body Gd content was performed by atomic emission spectrophotometry (at 324.247 nm). Percentage retention of Gd for the two substances at corresponding times was compared by the student's t-test.

Table 42: Study Design and Dose groups (DGD-0-15-A)

Groups	No. of mice/sex	Contrast agent	Day of sacrifice
1	2	none	0
2	2	Gd-DOTA ()	3
3	2		7
4	2		10
5	2		14
6	2		21
7	2	Gd-DTPA (Magnevist)	3
8	2		7
9	2		10
10	2		14
11	2		21

Results

The total gadolinium (Gd³⁺) levels in mice following the administration of 0.5 mmol/kg (or 0.41x-MHD) of Gd-DOTA (Gadoterate meglumine) or Gd-DTPA (Magnevist) is shown below. Only 1% of the administered dose of Gd-DOTA (1.031 μmol) and Gd-DTPA (1.166 μmol) were detected 3 days post-administration (Table 43). No Gd was detected 10 and 21 days following treatment with Gd-DOTA and Gd-DTPA, respectively.

Table 43: AES assay of Total Gd³⁺ levels in Gd-DOTA or Gd-DTPA treated mice

Sampling time (day)	Mean Gadolinium level (μmol/whole body)		% injected dose recovered	
	Gd-DOTA	Gd-DTPA	Gd-DOTA	Gd-DTPA
3	0.13	0.14	1.031	1.166
7	0.11	0.11	0.755	0.765
10	0.04*	0.06	0.307**	0.497
14	0.04*	0.06	0.366**	0.417
21	0.02*	0.04*	0.159**	0.264**

* : Not significant compared with controls.

** : Not significant as the signal did not differ from that of the controls.

AES = Atomic Emission Spectrophotometry

Conclusions: Based on the findings, the sponsor concluded that approximately 1% of the injected dose (0.5 mmol/kg or 0.41x-MHD, based on BSA) was detected after 3 days with both test articles and the detectable amount decreased over time becoming undetectable after 10 and 21 days for and Magnevist, respectively.

Reviewer's Comments

I agree with the sponsor's conclusion. Retention of gadolinium in biological tissues is currently considered an important factor in the development of nephrogenic systemic fibrosis (NSF). Current research has focused on the stability of the Gd-based contrast media and a possible release of the Gd³⁺ ion from the complex (Taupitz et al., 2013) and the risk of transmetallation of Gd with ions of iron (Telgmann et al, 2012), zinc or other metals. The recent study by Taupitz and others (2013) investigated the effectiveness of other complexing agents like Heparin – a glycosaminoglycan (GAG) compound, capable of formation of a macromolecular Gd-heparin complex. GAGs occur in the human extracellular matrix and are known to be strong chelators. The authors postulated that Gd³⁺ ion released from GBCAs might be complexed by GAGs in vivo, which would explain their retention in body tissues. Plasma GAGs are known to be elevated in end-stage renal disease. Telgmann et al (2012) postulated that the findings of this study might contribute to the elucidation of NSF. I agree.

5.1.2.3 Report No. DGD-0-2-A: Variation in the biodistribution of Gd-DOTA in the rat during the first hour after IV bolus injection

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	86.01.2.04
Date of study initiation:	December, 1985
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA (A.G.67.302; batch #: 21); % purity – N/A (CoA in Appendix 1)

Animal species/strain/sex per dose: Rats / (b) (4) / 15 females divided into 5 groups of 3 animals/group

Age: N/A

Weight: Mean weight: 177g (177-187 g), (b) (4)

Doses/Vehicle: 0.1 mmol/kg (or 0.08x-MHD) in 0.9% NaCl
(vehicle)

Duration/route:

Objective

The purpose of this study was to determine the variation in distribution of Gadoterate meglumine in rats 1 h after a single dose intravenous administration.

Key findings

Gd was detected, albeit in decreasing amounts, in all the organs sampled at all the sampling times in the descending order: kidneys > lungs > heart > liver > spleen. Bone was not assayed. Based on the findings, half-life ($t_{1/2\beta}$) was 18 minutes, volume of distribution (V_d) was 88 mL, and bioavailability extrapolated to infinity ($AUC_{0-\infty}$) was 5.42 $\mu\text{mol/g/min}$ and a total clearance of 3.32 g/min.

The pharmacokinetic data indicated a rapid distribution to organs with high blood perfusion (heart and lungs) and a slower distribution to the liver and spleen.

Methods

15 female rats were injected IV with 0.1 mmol/kg with 3 rats sacrificed at each sampling times of 1, 5, 15, 30 and 60 minutes post-treatment. Blood, liver, kidneys, heart, spleen and lungs were harvested to determine Gd concentration.

Results

The following Table shows the mean organ gadolinium concentration after injection with Gd-DOTA (Gadoterate meglumine) obtained at the various sampling times:

Table 44: Mean Gadolinium concentration (DGD-0-2-A)

Tissues	Mean Gadolinium concentration ($\mu\text{mol/g}$ tissue)				
	Sampling time after treatment (min)				
	1	5	15	30	60
Blood	0.439	0.196	0.100	0.052	0.018
Heart	0.149	0.085	0.051	0.020	0.011
Lungs	0.405	0.228	0.134	0.054	0.025
Liver	0.130	0.052	0.043	0.023	0.026
Spleen	0.122	0.047	0.043	0.018	0.021
Kidneys	1.080	0.758	0.398	0.318	0.225

Gd was detected, albeit in decreasing amounts, in all the organs sampled at all the sampling times in the descending order: kidneys > lungs > heart > liver > spleen. Based on the findings, half-life

($t_{1/2\beta}$) was 18 minutes, volume of distribution (V_d) was 88 mL, and bioavailability extrapolated to infinity ($AUC_{0-\infty}$) was 5.42 $\mu\text{mol/g/min}$ and a total clearance of 3.32 g/min.

Conclusions

The pharmacokinetic data for Gadoterate meglumine indicated a rapid distribution to organs with high blood perfusion (heart and lungs) and a slower distribution to the liver and spleen.

Reviewer's Comments

Overall, I agree with the sponsor's conclusions. However, it is remarkable that the bone was not assayed for Gd content. Bone tissue has been shown to serve as a site for Gd sequestration and long-term storage. The bone can therefore be a source of delayed Gd release with consequences for persistent Gd toxicity. The potential of this occurrence can be contributory to delayed Gd toxicity especially in instances of nephrogenic systemic fibrosis (NSF) that may be aggravated by multiple exposures to Gadolinium-based contrast agent-mediated MRI.

5.1.2.4 Report No. DGD-0-4-A: Comparative biodistribution of Gd-DOTA and Gd-DTPA in the conscious rat from 24 hours to 7 days after an intravenous bolus injection

Report location	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	86.05.2.01
Date of study initiation:	May, 1986
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA, batch no 21; Gd-DTPA, batch no 6; % purity - N/A
Animal species/strain/sex per dose:	Rats / Sprague-Dawley (b) (4) [REDACTED]
Age:	12/sex divided into 6 groups (2/sex/group)
Weight:	N/A
Doses/Vehicle:	Mean: 270 g, Range: 255-298 g Aqueous solutions of Gd-DOTA and comparator, Gd-DTPA administered at 1 mmol/kg (or 1.62x-MHD) based on BSA and adult human body weight of 60 kg) / Vehicle: Water
Duration/route:	Single / intravenous bolus

Objective

The purpose of this study was to determine the variation in distribution of Gadoterate meglumine in various rat tissues 24, 48 and 168 hours after a single dose intravenous administration.

Key findings

This study evaluated organ/tissue Gd content over 7 days following a single intravenous injection of Gadoterate meglumine at 1 mmol/kg or 1.62-fold the clinical dose. Magnevist (Gd-DTPA) was

studied as a comparator compound. Gd concentration was predominantly higher in the kidneys and lesser amounts were detected in other organs evaluated. A decrease in Gd concentration was observed in the various organs except the bone. IV injection of Gadoterate meglumine and Magnevist was followed by a rapid elimination from the plasma although Gd was still detectable in major organs after a total clearance from the plasma had occurred. Since the residual levels of Gd in the organs decrease with time except in the bone, its persistence of Gd in this tissue and the possibility of ionic interaction of Gd with calcium are noteworthy in view of the potential sequestration of Gadolinium in bone.

Methods

Six (6) rats per sex were injected IV with 1 mmol/kg: Gd-DOTA). 2 rats per sex were sacrificed at each sampling times of 24, 48 and 168 hours (or 7 days) post-treatment. Magnevist was administered in parallel and as a comparator under the same experimental paradigm. Blood, liver, kidneys, heart, spleen, lungs (without the trachea) and one femur were harvested from treated animals to determine Gd concentrations.

Results

Mean values of Gd concentrations obtained in blood and organs for Gd-DOTA and Gd-DTPA after 24, 48 and 168 hours post-treatment are shown in the Table below.

Tissues	Mean Gadolinium concentration ($\mu\text{mol/kg}$ tissue) or ($\mu\text{mol/L}$ plasma)					
	Gd-DOTA			Gd-DTPA		
	24 h	48 h	7 days	24 h	48 h	7 days
Plasma	2.9	0.6	0.0	0.6	0.3	0.0
Heart	6.7	4.1	2.0	6.1	6.8	2.1
Lungs	10.6	9.3	4.0	9.8	9.6	4.1
Liver	13.7	18.6	2.2	12.1	13.1	4.3
Spleen	13.3	15.2	4.8	16.0	14.6	6.6
Kidneys	518.0	509.0	291.0	531.0	418.0	210.0
Bone	20.9	15.4	24.9	33.3	33.5	36.8

At 24 hours, plasma Gd concentration was close to the limit of quantitation for the 2 test articles and decreasing to almost undetectable levels as from 48 hours post-injection. Gd was detected in all the organs sampled with the highest concentrations obtained in the kidneys and in bone. Lower quantities were detected in the liver, spleen, lungs and heart. Gd concentration decreased over time in all sampled organs except in bone.

Discussion

This study evaluated organ/tissue Gd content over 7 days following a single intravenous injection of Gadoterate meglumine at 1 mmol/kg or 1.62-fold the clinical dose. Magnevist (Gd-DTPA) was studied as a comparator compound. Gd concentration was predominantly higher in the kidneys and lesser amounts were detected in other organs evaluated. A decrease in Gd concentration was observed in the various organs except the bone. According to the sponsor, the persistence of Gd in the bone was consistent with the properties of rare earth metals interfering with calcium metabolism.

Conclusions

The sponsor concluded that the IV injection of Gadoterate meglumine and Magnevist was followed by a rapid elimination from the plasma although Gd was still detectable in major organs after a total clearance from the plasma had occurred. The residual levels of Gd in the organs decrease with time except in the bone.

Reviewer's Comments

I agree with the results and conclusions of this study. The study is a follow up to a previous study in which Gd concentrations were monitored in a similar set of organs over a 1 hour post-administration of Gadoterate meglumine and the comparator compound, Magnevist. The persistence of Gd in the bone and the possibility of ionic interaction of Gd with calcium are noteworthy in view of potential Gd sequestration in bone tissue which can result in a slow release over time. The potential for Gd sequestration and its slow release have the potential to worsening existing occurrences of nephrogenic systemic fibrosis (NSF) which is thought to result from the toxicity of free Gadolinium ions (Gd^{3+}).

5.1.2.5 Report No. DGD-0-5-A: Comparative biodistribution of Gd-DOTA and Gd-DTPA in the anesthetized rabbit 1 hour after intravenous injection

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	85.02.2.01
Date of study initiation:	February, 1985
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA, batch No. 4; Gd-DTPA, batch No. 6; % purity – N/A
Animal species/strain/sex per dose:	Rabbit / New Zealand (b) (4) / 2 groups of 6/sex
Age:	N/A
Weight:	2.45-2.76 kg
Doses/Vehicle:	0.1 mmol/kg aqueous solution (or 0.32-fold MHD based on BSA in a 60 kg human body weight) / Vehicle – not stated
Duration/route:	Single dose / intravenous

Objective

In this study, the distribution of gadolinium in different organs was evaluated in pentobarbital-anesthetized New Zealand rabbits 1 hour after an intravenous administration of a single dose of Gadoterate meglumine. Magnevist was administered at the same dose as Gadoterate meglumine and evaluated under the same experimental conditions.

Key findings

For both Gadoterate meglumine and Magnevist, low levels of Gd were measured in the organs with highest amounts found in the kidneys. The measured amounts though less than 1% of the injected dose, may still constitute a considerable amount. Gd did not concentrate in any of the organs evaluated. The amount of Gd recovered in the parathyroid glands, lungs and liver were lower than the levels in the kidneys. The Gd concentration in other tissues evaluated was closer to the limit of quantitation and lowest amounts were found in the brain. The tissue and organ distribution in the rabbit was similar for both Gadoterate meglumine and Magnevist; that Gadolinium did not concentrate in any organ and that the highest concentration of Gd was measured in the kidney.

Methods

12 New Zealand rabbits were divided into 2 groups of 3 rabbits/sex/group. Animals were anesthetized with 6% intravenous sodium pentobarbital. Gadoterate meglumine and Magnevist (comparator) were administered via the jugular vein at a dose of 0.1mmol/kg. The femoral vein was used for blood sampling 1 hour after dose administration. Heparinized blood samples were collected and centrifuged to collect plasma. Selected organs (brain, heart, parathyroid glands and liver). Spleen, lungs, kidneys, and specimens of pancreas, muscle, bone and bone marrow) were collected immediately following sacrifice. After washing the samples in isotonic saline, each organ or tissue was weighed. Mineralization by nitric acid was performed per 100 g of tissue. Gd assay was performed by atomic emission spectrophotometric analysis.

Results

Average values of Gd concentrations in the blood and organs are described in the following sponsor's Table (Table 45).

Table 45: Mean plasma and organ Gd concentrations and % injected dose recovered after IV Gadoterate meglumine and Magnevist (DGD-0-5-A)

Tissues	Mean Gd concentration ($\mu\text{mol/g}$ tissue) ($\mu\text{mol/mL}$ plasma)		% injected dose recovered in the organ	
	Gd-DOTA	Gd-DTPA	Gd-DOTA	Gd-DTPA
Plasma	0.086	0.086	NA	NA
Brain	0.000	0.002	0.0	0.006
Parathyroid glands	0.055	0.090	0.003	0.005
Heart	0.019	0.020	0.048	0.044
Lungs	0.013	0.042	0.130	0.171
Liver	0.011	0.014	0.403	0.453
Pancreas	0.006	0.009	ND	ND
Spleen	0.013	0.018	0.006	0.006
Kidneys	0.247	0.354	0.699	0.959
Muscle	0.008	0.013	ND	ND
Bone	0.012	0.008	ND	ND
Bone marrow	0.037	0.018	ND	ND

ND: Not Determined NA : Not Applicable

For both Gadoterate meglumine and Magnevist, low levels of Gd were measured in the organs with highest amounts found in the kidneys. The measured amounts were less than 1% of the injected dose. Gd did not concentrate in any of the organs evaluated. The amount of Gd recovered in the parathyroid glands, lungs and liver were lower than the levels in the kidneys. The Gd

concentration in other tissues evaluated was closer to the limit of quantitation and lowest amounts were found in the brain.

Conclusions

It was concluded from this study that the tissue and organ distribution in the rabbit was similar for both Gadoterate meglumine and Magnevist; that Gadolinium did not concentrate in any organ and that the highest concentration of Gd was measured in the kidney.

Reviewer's comments

I agree with the results and conclusions.

5.1.2.6 Report No. DGD-0-1-A: Plasma, urinary and biliary kinetics of Gd-DOTA and Gd-DTPA and distribution in the organism at 3 hours after intravenous administration in the anesthetized rabbit

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	84.11.2.01
Date of study initiation:	October, 1984
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA, batch No. 4; Gd-DTPA, batch No. 6; % purity – N/A
Animal species/strain/sex per dose:	Rabbit / New Zealand / 9 per sex (3 groups of 3/sex);  (b) (4)
Age:	N/A
Weight:	2.36 – 3.35 kg
Doses/Vehicle:	Gd-DOTA (0.5 and 0.1 mmol/kg); Gd-DTPA (0.5 mmol/kg)
Duration/route:	Single / intravenous

Objective

The purpose of this study was to determine the plasma, urinary and biliary pharmacokinetic profile of Gadolinium in pentobarbital-anesthetized rabbits following a single IV administration of 0.5 and 0.1 mmol/kg in comparison with Magnevist (Gd-DTPA) administered at 0.5mmol/kg.

Key findings

The kinetics of Gadoterate meglumine and Magnevist was linear. There was no difference in the PK profile when administered at 0.1mmol/kg versus 0.5mmol/kg. Gd concentration was highest in the kidneys and lowest in the bone and bone marrow. With the exception of the kidneys, a much lower concentration of Gd compared to plasma levels was obtained in most tissues after 3 hours. The study indicated a bi-exponential decrease of Gadoterate meglumine or Magnevist over the 3 hours period that is consistent with a 2-compartment model. The data indicated a linear PK, rapid distribution and a slower elimination phase. Gadoterate meglumine and Magnevist were rapidly

cleared from the plasma as indicated by $t_{1/2}$ of approximately 1 h. PK results for were similar for the two doses tested.

Methods

Catheterization: The following vessels were catheterized: Jugular vein (for infusion); femoral artery (blood sampling); ureters and the bile duct. The kinetics of Gadoterate meglumine was studied over a period of 3 hours following the injection of Gadoterate meglumine. Magnevist was evaluated as a comparator using the same experimental paradigm. Gadoterate meglumine or Magnevist was administered according to the study design in Table 46:

Table 46: Study Design and Dose groups (DGD-0-1-A)

Groups	No. of rabbits/sex	Dose (mmol/kg)	Contrast agent
1	3	0.5	Gd-DOTA (Gadoterate meglumine)
2	3	0.1	Gd-DOTA (Gadoterate meglumine)
3	3	0.5	Gd-DTPA (Magnevist)

Reviewer's Table based on sponsor's data.

Gadolinium was assayed in the test solutions, biological fluids and in tissues by atomic emission spectrophotometry (AES).

Heparinized blood was collected at time zero, 5, 10, 20 40, 120 and 180 minutes post-injection.

Urine and bile samples were collected 60 to 30 minutes prior to injection and at the following intervals post-injection: 0-30, 30-60, 60-90, 90-120, 120-150 and 150-180 minutes. Total amount of excreted bile was collected at 30 min intervals as for collection of the urine samples.

At 3 hours post-injection, animals were sacrificed and the following organs were collected: brain, heart, parathyroid glands, liver, spleen, lungs, kidneys, and specimens of the pancreas, muscle, bone and bone marrow. A sample of 1 ± 0.1 g was taken from each organ for tissue Gd assay. Whole organs were taken when the collected organs were less than 1 g. To dissolve tissue samples, mineralization using nitric acid (HNO_3 ; (b) (4)) was performed using 1 mL of nitric acid per 100 mg of tissue in a sealed glass tube. Prior to performing the assay, the mineralized organ samples were diluted to twice their volume in cesium chloride (CsCl) solution at 25 g/L. CsCl is used in analytical chemistry to detect inorganic ions via the color and morphology of the precipitates, usually in conjunction with inductively-coupled mass spectrometric (IC-MS) techniques. Gd was assayed by AES at 347.247 nm.

Plasma: Total plasma Gd was expressed as $\mu\text{mol/mL}$.

Urine: In the urine, total urinary Gd concentration ($\mu\text{mol/mL}$); the amount of Gd excreted (μmol Gd), and percentage Gd recovery (% of injected dose excreted in the urine) and the cumulative % of injected dose excreted in the urine.

Bile: Amount of Gd excreted in the bile ($\mu\text{mol/mL}$); percentage of injected dose excreted in the bile (% recovery) and cumulative percentage of injected dose excreted in the bile.

Organs: Gd concentration ($\mu\text{mol/g}$ of organ); amount of Gd expressed in μmol of Gd recovered per gram of organ when the latter could be removed completely; percent injected dose recovered in the organ which has been removed completely and the ratio of the Gd concentration of the organ at 3 h to plasma concentration at 3 h.

PK Parameters: The following PK parameters were determined from plasma concentration values and from amounts excreted in urine/bile: renal clearance (mL/min), Volume of distribution (V_d ; mL/kg), half-lives ($t_{1/2}$; min), renal clearance (mL/min) and biliary concentration ($\mu\text{mol}/\text{mL}$).

Results

The plasma vs. time concentration values and mean tissue Gd levels are shown in Tables 47 and 48:

Table 47: Plasma Gadolinium concentration vs. Time after IV administration of (DGD-0-1-A)

Time (min)	Mean (\pm SD) concentration of Gd^{3+} ($\mu\text{mol}/\text{mL}$)		
	(Gd-DOTA)		Magnevist (Gd-DTPA)
	0.1 mmol/kg	0.5 mmol/kg	0.5 mmol/kg
5	0.8 ± 0.2	3.7 ± 0.6	4.8 ± 2.2
10	0.5 ± 0.2	2.7 ± 0.5	2.9 ± 0.5
20	0.3 ± 0.2	1.7 ± 0.5	1.9 ± 0.7
40	0.2 ± 0.1	1.3 ± 0.4	1.2 ± 0.5
60	0.1 ± 0.1	0.9 ± 0.4	0.8 ± 0.3
120	0.07 ± 0.02	0.4 ± 0.3	0.4 ± 0.2
180	0.05 ± 0.02	0.2 ± 0.2	0.2 ± 0.1

Reviewer's Table based on sponsor data; Magnevist was used in the study as a comparator

Table 48: Mean total Gd levels and % injected dose in recovered in rabbits (DGD-0-1-A)

Tissues	Mean Gadolinium concentration ($\mu\text{mol}/\text{g}$ tissue)			% injected dose recovered in the organ		
	Gd-DOTA	Gd-DOTA	Gd-DTPA	Gd-DOTA	Gd-DOTA	Gd-DTPA
	0.1 mmol/kg	0.5 mmol/kg	0.5 mmol/kg	0.1 mmol/kg	0.5 mmol/kg	0.5 mmol/kg
Brain	0.000	0.005	0.008	0.001	0.003	0.002
Parathyroid glands	0.025	0.098	0.078	0.002	0.001	0.002
Heart	0.008	0.038	0.037	0.016	0.011	0.014
Lungs	0.016	0.066	0.070	0.076	0.050	0.047
Liver	0.006	0.036	0.042	0.250	0.248	0.276
Pancreas	0.002	0.025	0.026	ND	ND	ND
Spleen	0.012	0.052	0.053	0.005	0.004	0.008
Kidneys	0.299	1.437	0.912	1.007	0.918	0.786
Muscle	0.004	0.041	0.020	ND	ND	ND
Bone	0.003	0.015	0.031	ND	ND	ND
Bone marrow	0.022	0.079	0.055	ND	ND	ND

ND: Not Determined

The mean PK parameters evaluated are described below in Table 49. Plasma kinetics indicated a bi-exponential decrease over the 180-minute time period consistent with a 2-compartment model. There was a rapid distribution of the test articles followed by a slower elimination phase. Both test

articles were rapidly cleared from the plasma as indicated by $t_{1/2}$ of approximately 1 hour. PK results for Gadoterate meglumine were similar for the two doses tested. The volume of distribution was consistent with distribution within the extracellular compartment. Highest percentages of injected doses were observed in the kidneys, heart and lungs.

Table 49: Mean PK parameters of Gadoterate meglumine in rabbits (DGD-0-1-A)

	T1/2 distribution (min)	T1/2 elimination (min)	Volume of distribution (mL/kg)	Renal clearance (mL/min)
Gd-DOTA 0.1 mmol/kg	5.3 ± 2.4	38 ± 5	132 ± 42	4.8 ± 2.5 ^a
Gd-DOTA 0.5 mmol/kg	6.5 ± 4.8	58 ± 18	191 ± 38	5.9 ± 3.2 ^b
Gd-DTPA 0.5 mmol/kg	9.0 ± 5.9	75 ± 32	224 ± 120	6.8 ± 2.1 ^c

^a: Equal to 1.9 mL/min/kg (Mean body weight = 2.6 kg).

^b: Equal to 2.4 mL/min/kg (Mean body weight = 2.5 kg).

^c: Equal to 2.4 mL/min/kg (Mean body weight = 2.8 kg).

Magnevist was used as a comparator compound to Gadoterate meglumine

Excretion: The mean cumulative percentages of recovery of Gadoterate meglumine and Magnevist in urine and bile are shown in Table 50. From the results, excretion of Gd was rapid and greater than 50% of the injected dose was recovered in urine after 1 h and more than 75% was recovered 3h after treatment. It was not possible to ascertain the fate of the remaining 25% since sampling was truncated at 180min post-treatment. According to the sponsor, the Gd concentration found after 3 h was much lower than plasma Gd concentration except for the kidney, which possibly exhibited traces of urine containing Gd due to the assay methodology. Biliary excretion was slow for the two compounds and only 0.2% was recovered in bile 3h after treatment.

Table 50: Percentage of Gadoterate meglumine or Magnevist excreted after IV injection in Rabbits (DGD-0-1-A)

Dose levels (mmol/kg)	Dotarem®				Magnevist®	
	Urine		Bile		Urine	Bile
	0.1	0.5	0.1	0.5	0.5	0.5
-60 to -30 min	BLD	BLD	BLD	BLD	BLD	BLD
-30 to 0 min	BLD	BLD	BLD	BLD	BLD	BLD
0 to 30 min	45.7	34.3	0.1	0.1	39.7	0.1
0 to 60 min	62.9	51.8	0.1	0.1	57.8	0.1
0 to 90 min	70.2	60.3	0.1	0.1	66.4	0.1
0 to 120 min	74.4	66.7	0.2	0.2	74.1	0.2
0 to 150 min	77.3	71.0	0.2	0.2	78.3	0.2
0 to 180 min	79.0	73.8	0.2	0.2	81.4	0.2

BLD: Below the Limit of Detection

Discussion

Based on the results of this study, the kinetics of Gadoterate meglumine and Magnevist was linear. There was no difference in the Gadoterate meglumine PK profile when administered at 0.1 mmol/kg vs 0.5mmol/kg. Gd concentration was highest in the kidneys and lowest in the bone and bone marrow. With the exception of the kidneys, a much lower concentration of Gd compared to plasma levels was obtained in most tissues after 3 hours.

Conclusion

The study indicated a bi-exponential decrease of Gadoterate meglumine or Magnevist over the 3 hours consistent with a 2-compartment model. The data indicated a linear PK, rapid distribution and a slower elimination phase. Both test articles were rapidly cleared from the plasma as indicated by $t_{1/2}$ of approximately 1 hour. PK results for Gadoterate meglumine were similar for the two doses tested.

Reviewer's comments

Since it was not possible to ascertain the fate of the remaining 25% since sampling was truncated at 180min post-treatment and the sponsor did not indicate if there was a saturation of the excretory mechanisms involved in Gd elimination from the body, there remains a possibility of Gd sequestration in tissues with potential implications for cumulative exposure to Gd in patients undergoing multiple exposures to Gd-based contrast agents.

5.1.2.7 Report No. DGD-0-3-A: Plasma, urinary and biliary kinetics of Gd-DOTA after intravenous administration in the anesthetized dog

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	85.03.2.01
Date of study initiation:	March, 1988
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA, batch No. 4 /% purity – N/A
Animal species/strain/sex per dose:	Dog / Beagle ((b) (4)) / 3 per sex
Age:	N/A
Weight:	11-13 kg
Dose(s)/Vehicle:	0.1 mmol/kg / Vehicle: N/A
Duration/route:	Single / intravenous

Objective

The purpose of this study was to determine the plasma, urinary and biliary pharmacokinetic profile of Gadolinium in anesthetized dogs following a single IV administration at 0.1mmol/kg of Gadoterate meglumine.

Key findings

The plasma kinetics of gadolinium followed a bi-exponential decrease according to an open two-compartment model. The parameters indicated a rapid extracellular distribution phase and a rapid elimination. A brief summary of the results is shown in the following Table.

	T1/2 distribution (min)	T1/2 elimination (min)	Volume of distribution (mL/kg)	Renal clearance (mL/min)
Gd-DOTA 0.1 mmol/kg	2.0 ± 0.5	68 ± 18	271 ± 42	58.7 ± 15.2 ^a

^a: Equal to 5.0 mL/min/kg (Mean body weight = 11.7 kg).

Methods

Dogs were administered Gadoterate meglumine a single IV dose of Gadoterate meglumine (0.1 mmol/kg). Blood was collected as heparinized samples before and 1, 2, 3, 4, 5, 10, 30, 45, 60, 90, 120, 180, 240, and 300 min (or 5 days) after the injection. Urine and bile samples were also collected up to 5 h after drug administration to determine the excretion of Gadoterate meglumine. The following PK parameters were determined from plasma concentration values and from amounts excreted in urine/bile: renal clearance (mL/min), Volume of distribution (V_d ; mL/kg), half-lives ($t_{1/2}$; min), renal clearance (mL/min) and biliary concentration ($\mu\text{mol/mL}$).

Results

Plasma kinetics: There was a rapid diffusion of Gadoterate meglumine after injection and mean distribution half-life was approximately 2 minutes. Although there was a bi-exponential decrease in Gd concentration over time consistent with a 2-compartment kinetic model, the product rapidly cleared from plasma (mean clearance of 59 min) and mean elimination half-life was 68 minutes. The plasma Gd concentration measured over 5 days is shown in Table 51:

Table 51: Plasma Gd concentration vs. Time after IV injection of Gadoterate meglumine (DGD-0-3-A)

Time (min)	Mean (\pm SD) concentration of Gd ³⁺ ($\mu\text{mol/mL}$)
1	1.16 ± 0.27
2	0.87 ± 0.23
3	0.71 ± 0.17
4	0.58 ± 0.17
5	0.52 ± 0.16
10	0.30 ± 0.11
20	0.21 ± 0.07
30	0.18 ± 0.05
45	0.13 ± 0.04
60	0.09 ± 0.04
90	0.06 ± 0.03
120	0.03 ± 0.02
180	0.02 ± 0.01
240	0.01 ± 0.01
300	0.00 ± 0.00

Reviewer's Table based on sponsor data

Table 52: Selected pharmacokinetic parameters following a single-dose administration of Gadoterate meglumine in the dog (DGD-0-3-A)

PK parameter	Mean (\pm SD)
Renal clearance (mL/min)	58.65 \pm 15.27
Total volume of distribution (mL/kg)	271.07 \pm 41.73
Distribution half-life ($t_{1/2}$; min)	1.95 \pm 0.53
Elimination half-life ($t_{1/2}$; min)	68.05 \pm 0.8

Reviewer's Table based on sponsor data

Urinary excretion of Gd: After IV injection, the urinary concentration of Gd reached a peak at approximately 1 hour and thereafter decreased over time. Renal clearance remained constant at the various times evaluated.

Table 53: Urinary Gd concentration vs. Time after IV injection of Gadoterate meglumine (DGD-0-3-A)

Time (min)	Mean (\pm SD) concentration of Gd ³⁺ (μ mol/mL)
-60 to -30	-
-30 to 0	-
0 - 5	0 \pm 0
5 - 10	32.94 \pm 36.55
10 - 20	99.98 \pm 45.17
20 - 30	128.81 \pm 15.35
30 - 40	119.26 \pm 8.45
40 - 50	101.73 \pm 17.07
50 - 60	91.06 \pm 21.09
60 - 120	44.38 \pm 20.62
120 - 180	22.80 \pm 12.94
180 - 240	12.75 \pm 5.12
240 - 300	3.69 \pm 3.26

Reviewer's Table based on sponsor data

Biliary excretion: There was no change in biliary excretion (choleresis) after IV injection of Gadoterate meglumine at the dose administered. The biliary excretion of Gd was marginal (0.02%).

Table 54: Biliary Gd concentration vs. Time after IV injection of Gadoterate meglumine (DGD-0-3-A)

Time (hours)	Biliary Excretion (mL/hr)	% Excreted Dose
-1 - 0	2.7 \pm 1.7	-
0 - 1	2.0 \pm 0.6	0.010 \pm 0.005
1 - 2	2.0 \pm 0.6	0.016 \pm 0.005
2 - 3	2.2 \pm 0.6	0.018 \pm 0.005
3 - 4	2.3 \pm 0.7	0.019 \pm 0.005

4 – 5	2.5 ± 0.7	0.020 ± 0.005
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Reviewer's Table based on sponsor data

Conclusions

The plasma kinetics of gadolinium followed a bi-exponential decrease according to an open two-compartment model. The parameters indicated a rapid extracellular distribution phase and a rapid elimination.

Reviewer's comments

I agree with the findings and conclusions

5.1.2.8 Report No. DGD-0-9-A: Excretion in milk and plasma kinetics of gadolinium after intravenous injection of Gd-DOTA

Report location	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24 Rue Jean Chaptal, 93601, Aulnay-Sous-Bois, Cedex, France
Study #:	86.07.2.01
Date of study initiation:	October, 1985
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA, batch No. 4 /% purity – N/A
Animal species/strain/sex per dose:	Goats / (b) (4) / 4 lactating goats
Age:	N/A
Weight:	35 kg
Doses/Vehicle:	0.086 mmol/kg / Vehicle – N/A
Duration/route:	Single / intravenous (jugular v.)

Objective

The purpose of this study was to evaluate the excretion of gadolinium in milk after intravenous injection of Gadoterate meglumine in the lactating goat.

Key findings

There was a rapid clearance of Gadoterate meglumine from plasma ($t_{1/2} = 50$ min). The plasma PK however followed a one-compartment model unlike the finding in the rabbit and dog which were bi-exponential. Gd concentrations were measureable in plasma up to 4 h post-injection. Plasma kinetics followed a pattern of mono-exponential reduction of Gd content. There was a transient and very low excretion in milk (0.016% of injected dose). No Gadolinium was detectable in milk after 24 h post-administration of Gd-DOTA.

Methods

Each lactating goat was administered 6 mL Gd-DOTA or a dose of 0.086mmol/kg by intravenous bolus injection. Milk and blood samples were collected at time zero (t_0), 15 min, 30 min, and 1, 2, 4, 8, 24 and 48 hours. Blood samples were anticoagulated with heparin and centrifuged for the separation of plasma. Milk samples were collected at 24 and 48h. Plasma Gd levels were

measured up to 4 hours after treatment. PK parameters measured were plasma Gd (nmol/mL), Gd concentration (nmol/mL) and percentage of the injected Gd-DOTA recovered in milk. Other PK parameters were Volume of distribution, and elimination half life.

Results

Plasma Gd concentration: Plasma Gd concentration was evaluated only up to 4 h. Plasma Gd disappeared rapidly and the plasma disappearance curve versus time followed a mono-exponential pattern and therefore a one-compartment type.

Table 55: Mean Plasma Gd concentration vs. Time after intravenous Gadoterate meglumine (DGD-0-9-A)

Time (min)	Mean (\pm SD) concentration of Gd ³⁺ (nmol/mL)
0	0 \pm 0
5	299.8 \pm 24.7
15	229.3 \pm 13.1
30	188.5 \pm 15.3
Time (h)	
1	129.0 \pm 13.8
2	66.5 \pm 9.4
4	13.8 \pm 3.8
8	0 \pm 0
24	0 \pm 0
48	0 \pm 0

Reviewer's Table based on sponsor data

Table 56 summarizes Gadoterate meglumine plasma PK in the goat:

Table 56: Summary of Plasma PK values after intravenous Gadoterate meglumine injection (DGD-0-9-A)

	T1/2 elimination (min)	Volume of distribution (L)	Volume of distribution (L/kg)
Gd-DOTA 0.086 mmol/kg	50 \pm 8	9.9 \pm 0.9	0.33 \pm 0.07

Excretion of Gadolinium in milk: Within a period of 48h following IV injection of Gd-DOTA, Gd was detectable in milk in the first 24 h. Milk gadolinium content increased rapidly reaching a peak of 4.3 - 4.5 nmol/mL Gd between 2 and 4 h post-administration. The excretion time of Gd in milk was short since gadolinium was no longer detectable after 24 h post-injection. The total percentage of Gd-DOTA recovered in milk was 0.016% (Table 57).

Table 57: Excretion of Gd in milk after Gadoterate meglumine administration in lactating goats (DGD-0-9-A)

Time (hours)	Mean (\pm SD) concentration of Gd ³⁺ (nmol/mL)	% Excreted Dose ($\times 10^{-3}$)
0	0	-
0.25	0.3 \pm 0.3	0.2 \pm 0.2
0.50	0.8 \pm 0.5	0.7 \pm 0.5

1	2.0 ± 1.4	1.7 ± 1.0
2	4.3 ± 1.3	4.5 ± 1.8
4	4.5 ± 1.2	7.6 ± 2.6
8	2.5 ± 0.3	9.3 ± 2.6
24	0.8 ± 0.3	16.0 ± 4.4
48	0 ± 0	16.0 ± 4.4

Discussion

There was a rapid clearance of Gadoterate meglumine from plasma ($t_{1/2} = 50$ min). The plasma PK however followed a one-compartment model unlike the finding in the rabbit and dog which were bi-exponential.

Conclusions

From the findings of this study the sponsor concluded that Gd concentrations were measurable in plasma up to 4 h post-injection. Plasma kinetics followed a pattern of mono-exponential reduction of Gd content. There was a transient and very low excretion in milk (0.016% of injected dose). No Gd was detectable in milk after 24 h post-administration of Gd-DOTA.

Reviewer's Comments

The finding that Gadolinium (Gd) was transiently excreted into milk within 48h of intravenous administration of Gadoterate meglumine and rapidly attained a peak concentration of 4.3 - 4.5nmol/mL at 2-4 h following administration has labeling implications for the use of this product in nursing mothers notwithstanding the fact that the amount excreted represented 0.016% of the injected dose. In view of the fact that Gd was detected in milk in the first 24 h, a temporary cessation of breast feeding is recommended. The potential implication of this finding on breast feeding will be reflected in product labeling recommendations.

5.1.3 Repeat-dose PK studies

5.1.3.1 Summary of Repeat-Dose PK findings

Following repeated administration of 0.5, 0.7 and 1.5 mmol/kg Gadoterate meglumine in rats (or 0.5, 1.14 and 2.4-fold MHD) over a period of 28 days followed by a 28-day treatment-free period, Gadolinium was detected in the kidneys, liver and femur 1 day after the end of the 28-day treatment period.

Gd concentration was dose-dependent and highest amounts were obtained in the kidney. A linear relationship was also obtained between Gd concentration in tissues and the dose administered. Gd concentration was considerably decreased during the reversibility (treatment-free) period with slight amounts measurable in high dose animals. The low and mid dose groups were not evaluated for Gd content after the treatment-free period.

Similar to rats, Gadoterate meglumine was administered to dogs in doses of 0.5, 0.7 and 1.5 mmol/kg (or 2.70, 3.78, and 8.11-fold MHD) over 28 days of treatment followed by a 28-day treatment-free period. Similar to the finding in rats, Gadolinium was detected in the kidneys, liver and femur. Samples were obtained 24 h following the first injection in dogs, after the last injection, and at the end of the treatment-free period (day 56). Plasma and urine were evaluated

for Gd content in the dog. As for rats, PK was also linear. At the end of the reversibility (treatment-free) period, highest amounts of Gd were obtained in the kidneys. The repeat dose PK studies confirmed the findings of the single-dose PK studies. Of note, in the single dose studies, a small fraction of Gd was detected in the liver and bone.

Under conditions of repeated exposure over a long dosing period, higher levels of tissue Gd were obtained with implications for a greater, long-lasting retention of Gadolinium in the body. It has been shown that bone tissue serves as a site for Gd storage and long-term persistence and slow release of Gd^{3+} from bone stores could be a cause for concern of Gd-associated toxicity with long potency. This significance of bone Gd sequestration is important in instances of nephrogenic systemic fibrosis (NSF).

It is also noteworthy that the skin was not evaluated for Gd content in view of the importance of the role of skin Gd content in the pathophysiology of the onset and propagation of the NSF syndrome.

5.1.3.2 Report No. DGD-0-13-A: Distribution of G 449-06 in the body during intravenous subacute toxicity studies with a 28-day reversibility period in the rat

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	(b) (4) and Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600 Aulnay Sous Bois Cedex, France (Gadolinium Assay)
Study #:	87.11.203
Date of study initiation:	N/A
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	G 449.06 (batch No. 202) / % purity – N/A
Animal species/strain/sex per dose:	Rat / (b) (4) 60/sex
Age:	N/A
Weight:	N/A
Doses/Vehicle:	G 449.06 (0, 0.3, 0.7 and 1.5 mmol/kg) / Vehicle (0.9% NaCl)
Duration/route:	Repeat-dose / intravenous

Objective

The purpose of this study was to evaluate the biodistribution of Gadolinium in the rat after 28 days of repeated intravenous administration followed by a 28-day treatment-free period.

Key findings

Gadolinium was detectable in the kidneys, liver and bone (femur) 1 day following a 28-day repeat-dose treatment with Gadoterate meglumine. Gd concentration was dose-dependent, highest amounts were obtained in the kidney and a linear relationship was obtained between the Gd concentration in the tissues and the dose administered in the organs sampled. The Gd concentration was considerably decreased during the reversibility (treatment-free) period with

slight amounts measurable in high dose animals. The low and mid dose group were not evaluated for Gd content after the treatment-free period. Comparison of the Gd concentration in the different organs showed a low accumulation of Gd in body organ systems.

Methods

The rats were divided into 4 dose groups as shown in Table 58. Animals and samples were from the repeated dose toxicity study DGD-1-9-A.

Table 58: Study Design and Dose group (DGD-0-13-A)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose		Number of rats	
	(mmol/kg)	X-MHD	Males	Females
1 (vehicle; 0.9% NaCl)	0	0	20	20
Gadoterate meglumine 2 (LD)	0.3	0.5x	10	10
3 (MD)	0.7	1.14x	10	10
4 (HD)	1.5	2.4x	20	20

Source: Reviewer's Table adapted from Sponsor's Study Design on page 6 of 58; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine (G 449.06)

The rats received the same doses of Gadoterate meglumine IV daily for 28 days according to the dosing schedule. The treatment period was followed by a 28-day treatment-free period to evaluate the elimination of gadolinium. 24 h after the last injection, 10 rats/sex/group were sacrificed and the liver, femur and kidneys were collected and analyzed for Gd content. At the end of the treatment-free period, 10rats/sex from the control and high dose groups were sacrificed and the same organs removed to evaluate for Gd content.

Results

The table below (Table 59) describes the Gd concentration in the liver, femur, and kidneys at the end of the treatment period (day 28) and after the end of the treatment-free period (day 56). Data was pooled for males and females for Gd analysis. Gd concentration was below the level of detection in the saline-treated controls.

Table 59: Mean Gd concentration (mmol Gd/kg) in selected organs after daily intravenous Gd-DOTA injections (DGD-0-13-A)

	D28 (post-28 day treatment period)			D56 (post-28 day treatment-free period)		
	LD	MD	HD	LD	MD	HD
Kidney	0.816	2.381	4.090	-	-	0.808
Liver	0.014	0.045	0.120	-	-	0.003
Femur	0.013	0.030	0.062	-	-	0.023

Reviewer's Table based on sponsor's data; LD, MD, HD = Low, mid and high doses (or 0.3, 0.7 and 1.5 mmol/kg); D28 and D56 = 28 days after treatment started, 56 days (end of treatment-free period)

The results showed that 24 h following the 28-day treatment period, Gd was detectable in the kidneys, liver and bone (femur). Gd concentration was dose-dependent, highest amounts were

obtained in the kidney and a linear relationship was obtained between the Gd concentration in the tissues and the dose administered in the 3 organs sampled.

The Gd concentration was considerably decreased during the reversibility (treatment-free) period with slight amounts measurable in high dose animals. At the high dose, the amount of Gd remaining in the tissues after the treatment-free period expressed as percentage of Gd after the treatment period was 80.3, 97.5 and 62.9% for kidney, liver and femur, respectively indicating a lower excretion of Gd from bone. The low and mid dose group were not evaluated for Gd content after the treatment-free period.

Conclusions

It was concluded from this that gadolinium was recoverable in the liver, kidney and bone at the end of the 28-day treatment-free period, that a linear relationship existed between Gd levels in the organs evaluated and the dose administered. A comparison of the Gd concentration in the different organs showed a low accumulation of Gd in body organ systems.

Reviewer's comments

I agree with the results and conclusions. It is of note however, that a greater percentage of Gd content of the kidneys and liver following the treatment period was eliminated by the end of the treatment-free period when compared to the bone. It is plausible to argue that bone tissue might serve as a possible organ of Gd sequestration following repeated exposures to this product. This assertion is supported by Thakral et al (2007) who noted that bone tissue serves as a site for Gd storage hypothesized that long-term persistence and slow release of Gd³⁺ from bone stores could be a cause for concern of Gd-associated toxicity with long potency. This significance of bone Gd sequestration is especially of concern in instances of nephrogenic systemic fibrosis (NSF). It is also noteworthy that the skin was not evaluated for Gd content. This is considered an important omission in view that Gd content of the skin and attendant histopathological consequences are pivotal considerations in the onset and propagation of the NSF syndrome.

5.1.3.3 Report No. DGD-0-12-A: Distribution of G 449.06 in dogs during a subacute toxicity study by intravenous administration followed by a 28-day reversibility period

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	(b) (4) and Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600 Aulnay Sous Bois Cedex, France (Gadolinium Assay)
Study #:	88.11.201
Date of study initiation:	N/A
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	G 449.06 (batch No. 202) / % purity – N/A
Animal species/strain/sex per dose:	Dog / Beagle; 32 (16 per sex)
Age:	N/A
Weight:	N/A
Doses/Vehicle:	G 449.06 (0, 0.3, 0.7 and 1.5 mmol/kg) / Vehicle (0.9% NaCl)

Duration/route:

Repeat-dose / intravenous

Objective

The purpose of this study was to evaluate the biodistribution of Gadolinium in dogs after 28 days of repeated intravenous administration followed by a 28-day treatment-free period.

Key findings

Gadolinium was not detected in plasma 24 h after the injections, 27 days after dosing or following the treatment-free period. Gd was however found in the kidneys, liver and bone in a dose-dependent (linear) manner. Study of the treatment-free period indicated there was a gradual elimination of Gd over time from the different tissues sampled.

Methods

The rats were divided into 4 dose groups as shown in Table 60. Animals and samples were from the repeated dose toxicity study DGD-1-10-A.

Table 60: Study Design and Dose group (DGD-0-12-A)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose		Number of rats	
	(mmol/kg)	X-MHD	Males	Females
1 (vehicle; 0.9% NaCl)	0	0	5	5
2 (LD)	0.3	2.70x	3	3
3 (MD)	0.7	3.78x	3	3
4 (HD)	1.5	8.11x	5	5

Source: Reviewer's Table adapted from Sponsor's Study Design on page 8 of 62; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine (G 449.06)

The animals received the same doses of Gadoterate meglumine I/V daily for 28 days according to the dosing schedule. The treatment period was followed by a 28-day treatment-free period to evaluate the elimination of gadolinium. 24 h after the last injection, 3 dogs/sex/group were sacrificed and the liver, femur and kidneys were collected and analyzed for Gd content. At the end of the treatment-free period 3 dogs/sex in the control and high dose groups, were sacrificed and the same organs removed to evaluate for Gd content. Blood samples and overnight urine specimens were collected after the first and the 27th injections to determine Gd concentrations. Blood and urine samples were analyzed for Gd content 24 h after sampling.

Results

The mean gadolinium concentrations are shown in Table 61. The Gd levels were below the level of quantitation in control group animals.

Table 61: Mean Gd concentrations (mmol Gd/kg) in plasma, urine, kidney, liver and bone after 28 daily IV injections of Gadoterate meglumine (DGD-0-12-A)

Doses	Low dose			Mid dose			High dose		
	24h	D28	D56	24h	D28	D56	24h	D28	D56

Tissues/organs									
Plasma	LLOQ	LLOQ	-	LLOQ	LLOQ	-	LLOQ	LLOQ	LLOQ
Urine	0.913	1.330	-	8.509	2.398	-	10.382	9.737	LLOQ
Kidney	-	1.871	-	-	3.647	-	-	5.101	0.358
Liver	-	0.052	-	-	0.136	-	-	0.330	0.046
Femur	-	0.004	-	-	0.017	-	-	0.032	0.016

Reviewer's Table based on sponsor's data; Low, mid and high doses (or 0.3, 0.7 and 1.5 mmol/kg); D28 and D56 = end of treatment, and end of treatment-free period, respectively; LLOQ = Lower than limit of quantitation; - = not sampled

The results showed that following the 28-day treatment period at which time Gd was detectable in kidneys, liver, bone (femur) and urine. Gd concentration was dose-dependent, with highest amounts obtained in the kidney and urine and the lowest concentration in bone. On Day 56 urinary Gd was below detection in the high dose group but was not sampled at the low and mid doses.

At the three doses tested (Table 61) urinary Gd, detected at 24 h and at 28 days post-treatment, was no longer evident after the treatment-free period (D56). Analysis of the plasma showed no evidence of Gd at 24h, day 28 or at the end of the treatment-free period and it did not appear there was any accumulation of Gd in plasma. The low and mid dose group were not evaluated for Gd content after the treatment-free period. At the high dose, the amount of Gd remaining in the tissues after the treatment-free period expressed as percentage of Gd after the treatment period was 93.0, 95.2 and 50.0% for kidney, liver and femur, respectively indicating a lower excretion of Gd from the femur (bone). The low and mid dose group were not evaluated for Gd content after the treatment-free period.

When compared to the urinary Gd at 24h, Gd level at D28 increased by 46% for the low dose, but decreased by 72% and 6.2% for the mid and high doses respectively (Table 62; Reviewer's analysis of sponsor's data). This appeared to be an indication of a dose-related decrease in Gd excretion.

Table 62: Percentage difference in Gd concentration at 24h and 28-post treatment (DGD-0-12-A)

	Low dose			Mid dose			High dose		
	24h	D28	% diff. excreted ¹	24h	D28	% diff. excreted	24h	D28	% diff. excreted
Urine Gd (mmol Gd/kg)	0.913	1.330	45.7 ↑	8.509	2.398	71.8 ↓	10.382	9.737	6.2 ↓

Reviewer's Table based on sponsor's data; Low, mid and high doses (or 0.3, 0.7 and 1.5 mmol/kg); D28 = end of treatment started; LLOQ = Lower than limit of quantitation; % diff = percentage difference between Gd in urine at 24h and at D28; ↑, ↓ = increased or decreased excretion of Gd.

Discussion

At the end of the dosing period, Gadolinium was present in the kidney, liver, bone, and urine. A linear relationship was obtained between the Gd concentration in the tissues and the dose administered in the 3 organs sampled. There was no indication that Gd accumulate in plasma since samples were obtained 24 hours after the preceding administration. It was concluded that Gd did

not accumulate in plasma. The Gd concentration was considerably decreased during the reversibility (treatment-free) period with slight amounts measurable in high dose animals.

Conclusions

Gadolinium was not detected in plasma 24 h after the injections, 27 days after dosing or following the treatment-free period. Gd was however found in the kidneys, liver and bone in a dose-dependent (linear) manner. Study of the treatment-free period indicated there was a gradual elimination of Gd taken up in different tissues.

Reviewer's comments

I agree with the results and conclusions. It is of note however that a greater percentage of Gd present in the kidneys and liver following the treatment period was eliminated by the end of the treatment-free period when compared to the bone. While a reduced excretion of Gd from the bone cannot be construed as increased retention in this organ, it is plausible to argue that bone tissue might serve as a possible organ of Gd sequestration following repeated exposures to this product. This assertion is supported by Thakral et al (2007), who noted that bone tissue serves as a site for Gd storage hypothesized that long-term persistence and slow release of Gd^{3+} from bone stores could be a cause for concern of Gd-associated toxicity with long potency. This significance of bone Gd sequestration is especially of concern in instances of nephrogenic systemic fibrosis (NSF).

Secondly, the finding of a dose-related decrease in Gd excretion between Gd levels at 24h and after 28 days of repeated injection might indicate Gd retention in animals exposed to multiples exposures of Gadoterate meglumine. The basis of the observed phenomenon is not apparent to the reviewer at this time.

5.1.4 Plasma Protein Binding Study

5.1.4.1 Report No. DGD-0-6-A

Report location:	eCTD Module 4 §4.2.2.3.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600 Aulnay Sous Bois Cedex, France
Study #:	87.07.206 and 87.06.204
Date of study initiation:	May, 1986
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	G 449.06 (batch No. 202) / % purity – N/A
Animal species/strain/sex per dose:	N/A; In vitro study
Age:	N/A
Weight:	N/A
Doses/Vehicle:	Gd-DOTA (meglumine salt) batch No. 4 (for the enzyme assay; batch 202 (for the ultrafiltration test); Gd-DTPA (meglumine salt) batch 6 (for the enzyme assay); batch 467.10.RG test 2 (for the ultrafiltration tests)

Duration/route:

In vitro study

Objective

The purpose of this study was to determine the in vitro protein binding of Gd-DOTA (Gadoterate meglumine) to human serum albumin. Gd-DTPA (Magnevist) was used as a comparator Gd-based contrast agent under the same experimental conditions.

Key findings

Using the methods described, the Gadoterate meglumine or its comparator (Magnevist) was not bound to human serum albumin. There was also no difference between Gadoterate meglumine and Magnevist.

Methods

Briefly, protein binding to human serum albumin was evaluated by 2 in vitro methods – a direct assay of unbound Gd after ultrafiltration and Gd levels determined by atomic emission spectrophotometry (AES) and an indirect enzymatic assay of the extent of bilirubin displacement from its binding site on albumin by the test articles. In the ultrafiltration method, Gadoterate meglumine was tested at 10^{-2} , 10^{-3} and 10^{-4} M to determine the percentage binding of Gadoterate meglumine to albumin. In the enzymatic assay, based on a competition between the test substance and bilirubin for the binding site on human serum albumin, the bilirubin displaced was assayed enzymatically using the peroxidase/H₂O₂ system since the albumin-bound bilirubin is protected against this system.

Results

In the ultrafiltration assay, the level of Gd recovery in the test system containing Gd-DOTA (or Gd-DTPA) and albumin was similar to that obtained in the control (recovery solution with contrast agent alone). In the enzymatic assay, results showed that Gd-DOTA had no measurable binding affinity for the binding site of bilirubin on human serum albumin.

Conclusions

Using the methods described, the Gadoterate meglumine or its comparator (Magnevist) was not bound to human serum albumin and had no measurable affinity for albumin. There was also no difference between Gadoterate meglumine and Magnevist.

Reviewer's Comments

I agree with the results and conclusions of this study.

5.1.5 Transplacental Transfer of Gadoterate meglumine**5.1.5.1 Report No. DGD-0-14-A: Transplacental transfer of G 449.06 administered at 0.5mmol/kg to female rats after 18 days of gestation**

Report location:

eCTD Module 4 §4.2.2.3.1

Conducting laboratory and location:

Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600
Aulnay Sous Bois Cedex, France

Study #:

88.03.2.01

Date of study initiation:	March, 1988
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	449.06 (batch No. 202) / % purity – N/A
Animal species/strain/sex per dose:	Rat / 13 Sprague-Dawley at 18 th week of gestation
Age:	N/A
Weight:	300 -351 g
Doses/Vehicle:	0.5mmol/kg (aqueous solution)
Duration/route:	In vitro study

Objective

The purpose of this study was to determine the pharmacokinetics of Gadoterate meglumine in the fetus following its administration to gestating female rats.

Key findings

The results of this study showed that Gadoterate meglumine administered intravenously to gestating female rats at 0.5 mmol/kg crossed the placental barrier with Gd level attaining a maximum in the fetus after 30 minutes. The amount of Gd that crossed the placenta (maximum of 5% of maternal plasma concentration) in the short span of 30 minutes cannot be considered negligible as stated by the sponsor.

Methods

12 pregnant female rats (300 – 351 g) were administered 0.5mmol/kg Gadoterate meglumine on gestation day (GD) 18 via the caudal tail vein. Two females were sacrificed at each of 6 time points: 0.5, 1, 3, 6, 16 and 24 h. The single control animal did not receive Gadoterate meglumine and was sacrificed at time point 0. Rats were anesthetized with halothane (4% halothane; O₂, 800 mL/min and N₂O, 200 mL/min).

Maternal blood was collected in heparinized tubes via aortic puncture and centrifuged to obtain plasma.

Fetuses were removed by cesarean section, litter counted and weighed. The fetuses were digested in nitric acid and Gd concentration determined by atomic emission spectrophotometer. The Gd level in maternal plasma was determined as follows: Fetuses were digested in (b) (4) concentrated nitric acid (1 mL/g of tissue) in a glass tube. The tubes were placed in a water bath for 6 h at 60°C. Plasma samples were diluted 1:2 with water. Digest or diluted plasma was diluted by half in 25 g/L cesium chloride solution and Gd concentration determined by atomic absorption spectrophotometry at 342 and 247 nm. Measurements were performed in triplicate. The Gd concentration in maternal plasma was expressed in µmol/L and the Gd concentration in the fetuses as µmol/g. The percentage of Gd in the whole litter was calculated as % of injected dose.

Results

The mean maternal Gd concentration in plasma (µmol/mL) and fetuses (µmol/g) decreased over time reaching the limit of sensitivity after 6 h and 24 h in maternal blood and fetuses, respectively. At 30 min there was evidence of transplacental transfer of Gadoterate meglumine to the fetus at an amount equal to 5% maternal plasma Gd concentration.

Table 63: Mean Gd concentrations in maternal plasma, fetus and % injected dose in litter after IV Gd-DOTA (DGD-0-14-A)

Time point	Gd ³⁺ concentration in maternal plasma (μmol/mL)	Gd ³⁺ concentration in fetuses (μmol/g)	% of injected dose found in litter
0	BLD	BLD	BLD
0.5	0.301	0.014	0.071
1	0.139	0.009	0.063
3	0.003	0.008	0.044
6	0.001	0.006	0.045
16	BLD	0.002	0.018
24	BLD	0.001	0.010

BLD: Below the limit of detection

Discussion

According to the sponsor, Gd levels reached a maximum in the fetuses after 30 min indicating a rapid transfer to the compound. It then decreased in the fetuses indicating an elimination that appeared to be later than maternal elimination.

Conclusion

The results of this study showed that Gadoterate meglumine administered intravenously to gestating female rats at 0.5 mmol/kg crossed the placental barrier with Gd level attaining a maximum in the fetus after 30 minutes amounting to 5% of the maternal plasma concentration. According to the sponsor, the quantity of Gd found in the litter was very small and accounted for 0.07% of the injected quantity after 30 min and 0.01% of the injected amount after 24 h.

Reviewer's Comments

I do not agree with the sponsor that a transplacental transfer of Gd that reached a maximum of 5% of the maternal plasma concentration in the fetus after 30 min post-injection is negligible. Even though this amount of Gd in the fetus decreased with time, it is still noteworthy that Gd administered to the fetus crossed the placenta. Teratology studies in females rats (DGD-1-8-A) showed that at comparable doses, Gadoterate meglumine had no effect on both the course and results of gestation. This finding will be reflected in the product labeling.

5.1.6 Metabolism Studies**5.1.6.1 Report No. DGD-0-10-A: Detection of metabolites of Gd-DOTA after intravenous bolus in the rat, the dog and the rabbit**

Report location:	eCTD Module 4 §4.2.2.4.1
Conducting laboratory and location:	Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600 Aulnay Sous Bois Cedex, France
Study #:	87.05.2.01
Date of study initiation:	May, 1987
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)

Drug, lot #, and % purity:	Gd-DOTA (DOTA acid, Ca-DOTA, cyclone)
Animal species/strain/sex per dose:	rat/rabbit/dog (2 animals per species)
Age:	Provided in the referenced studies (DGD-0-7-A; DGD-01-A and DGD-0-3-A)
Weight:	Provided in the referenced studies (DGD-0-7-A; DGD-01-A and DGD-0-3-A)
Doses/Vehicle:	Rats (0.1 mmol/kg), rabbits (0.1 and 0.5 mmol/kg), dogs (0.1 mmol/kg) / Vehicle – Aqueous solution
Duration/route:	Intravenous in all species tested

Objective

The objective of this study was to determine the presence of possible metabolites of Gadoteric acid (DOTA acid and DOTA calcium and cyclone) in the urine of rats, rabbits and dogs receiving Gadoterate meglumine intravenously in doses between 0.1 and 1 mmol/kg using Thin Layer Chromatography (TLC).

Key findings

Following an intravenous bolus injection of Gadoterate meglumine in the rat, rabbit and the dog, Gd-DOTA was eliminated in its initial form. There was no evidence that the test compound was biotransformed into metabolites detectable in the urine.

Methods

The study was performed using urine samples collected from animals dosed as follows: rats (1 mmol/kg; Study DGD-0-7-A), rabbits (0.5 and 0.5 mmol/kg; Study DGD-0-1-A) and dogs (0.1 mmol/kg; Study DGD-0-3-A). Urine samples from rats were collected 3 days before and between 10-14 days after treatment. In rabbits, samples were collected 30 min before and between 150 and 180 min post-treatment. In dogs, urine samples were obtained 30 min before and between 20 and 30 min, and 240 and 300 min after-treatment in dogs. In each species, urine samples were pooled and analyzed using Thin Layer Chromatography to determine the presence, if any of metabolites, namely DOTA acid, calcium DOTA and cyclone.

Results

No products other than Gd-DOTA (Gadoteric acid) were detected in the urine samples analyzed for the different species evaluated.

Conclusions

The sponsor concluded that following an intravenous bolus injection of Gadoterate meglumine in the rat, rabbit and the dog, Gd-DOTA was eliminated in its initial form. There was no evidence that the test compound was biotransformed into metabolites detectable in the urine.

Reviewer's Comments

I agree with the findings and conclusions. Using TLC technique the sponsor showed that Gadoterate meglumine was excreted essentially as an intact and non-metabolized (non-decomplexed) macrocyclic Gd compound that was detectable in the urine. The study did not provide any evidence to exclude the presence of all potential metabolized forms of Gadoterate meglumine. The study has determined, by the analytical method used, that elements of the structural constitution of Gadoterate meglumine namely, DOTA, DOTA coupled to Calcium and

the cyclical ring structure (cyclone) were not identifiable in the urine. Consequently, the argument that Gadoterate meglumine was excreted intact and unmetabolized seemed plausible.

5.1.6 Excretion

5.1.6.1 Report No. DGD-0-16-A: Comparative study of the excretion of Gd-DOTA and Gd-DTPA administered intravenously in the anesthetized rat with renal failure

Report location:	eCTD Module 4 §4.2.2.7
Conducting laboratory and location:	Laboratoire Guerbet, 16-24, Rue Jean Chaptal, 93600 Aulnay Sous Bois Cedex, France
Study #:	88.06.2.01
Date of study initiation:	June, 1988
GLP compliance:	Yes (), No (x)
QA report:	Yes (), No (x)
Drug, lot #, and % purity:	Gd-DOTA (G 449.06, batch 152), % purity – N/A; Gd-DTPA (439 RG 08, trial 7), % purity – N/A
Animal species/strain/sex per dose:	Rat / Sprague-Dawley/ 12 females, 3 groups of 4 rats each
Age:	N/A
Weight:	234 -278 g (b) (4)
Doses/Vehicle:	1 mmol/kg / Vehicle -
Duration/route:	Single dose / intravenous

Objective

The purpose of this study was to evaluate plasma kinetics and excretion of Gadoterate meglumine intravenously administered in rats by using a model of renal failure.

Key findings

This study demonstrated that compared to normal rats, in animals with renal failure, a higher plasma Gd levels was accompanied by lower plasma clearance in addition to an increased biliary excretion. There was no significant difference between the results in Gadoterate meglumine and Magnevist-treated rats.

Methods

Rats were divided into 3 groups (2 rats per group): a control group consisting of normal rats; a second group of renally-impaired animals and a third group of renally-impaired rats with peritoneal dialysis. Gd-DOTA was used as a comparator compound (Table 64).

Table 64: Study Design (DGD-0-16-A)

Study Groups	1	2	3
	Control	Renal failure	Renal Failure + Peritoneal Dialysis
No. of rats	2	2	2
Gd-DOTA or Gd-DTPA (1 mmol/kg)**	✓	✓	✓

Renal Failure	No	✓	✓
Peritoneal Dialysis	No	No	✓

Reviewer's Table based on sponsor's data; ✓ = Yes; ** = the administered intravenous (jugular v.) dose selected (1 mmol/kg) was higher than the clinical dose in order to sensitize the renal-impairment model.

The jugular vein and the caudal artery were catheterized for injection of the test article and blood sample collection, respectively. Urine was collected via catheterization of the urinary tract. The bile duct was catheterized for collecting bile and renal failure induced by ligations of the renal vein and artery. Peritoneal dialysis was via placement of an infusion catheter under the intestines in the bladder and continuous infusion performed at the rate of 0.2 mL/min. Blood was collected in heparinized tubes followed by centrifugation to obtain plasma. Bile and urine were collected at 30 min before test article injection, and at 30 min, 90 min, and then at 240 min after injection. Urine and bile samples were pooled for analysis. Gd level in dialysates and plasma was measured by atomic absorption spectrophotometry at 342 and 247 nm. Each measurement was carried out 3 times to obtain a mean value. Parameters evaluated were body weight, PCV, plasma and dialysate fluid Gd concentration, Gd excreted in urine or bile and percentage of the dose recovered in the urine and bile.

Results

The mean Gd concentrations in plasma and dialysate fluid and urinary excretion after intravenous Gd product administration are shown in the following sponsor table (Table 65):

Table 65: Gd concentrations in plasma, urine and dialysate fluid and urinary excretion of Gd after IV injection of Gd-DOTA and Gd-DTPA in rats with renal failure (DGD-0-16-A)

Group number		Gd-DOTA			Gd-DTPA		
		1: control	2: renal failure	3: renal failure + peritoneal dialysis	1: control	2: renal failure	3: renal failure + peritoneal dialysis
Gd ³⁺ plasma concentration (µmol/l)	0'	0	0	0	0	0	0
	30'	1890	6886	5322	1846	5702	5764
	90'	420	6397	4239	550	5276	4209
	240'	31	5682	2734	53	4765	2563
Urinary cumulative % excreted	0'	0.0			0.0		
	30'	20.1	NA	NA	30.2	NA	NA
	90'	59.8			63.2		
	240'	90.7			88.0		
Biliary cumulative % excreted	0'	0.0	0.0	0.0	0.0	0.0	0.0
	30'	0.1	0.2	0.2	0.1	0.2	0.1
	90'	0.4	0.8	0.9	0.3	0.8	0.5
	240'	0.4	2.7	2.2	0.4	2.2	1.4
Gd ³⁺ dialysate concentration (µmol/l)	0'			0			0
	30'			1940			1697
	90'	NA	NA	2160	NA	NA	1847
	240'			1587			1587

NA: Not Applicable

Expectedly, plasma Gd levels were higher in animals with renal failure compared to normal animals and a slower reduction in Gd concentrations was observed under conditions of renal impairment. Approximately 90% of the injected dose was excreted in the urine after 4h in normal rats. No data was provided for the renal failure models. However, given the high plasma levels in rats with renal failure, a concomitant low level of Gd would be expected over the same 4h period.

The percentage cumulative amount of Gd excreted over time by the biliary route was comparable to the amount excreted in the renal failure rats with concomitant peritoneal dialysis. The amounts excreted by the biliary route in the renal failure rats or the renal failure/peritoneal dialysis group were greater than was excreted by the renal route in normal rats.

Although Gd levels of Gd were increased in the plasma of renal failure groups, lower levels were detected in the plasma and dialysate fluid in Group 3 animals suggesting that dialysis could be an efficient means of removing Gadoterate meglumine in the event of total renal failure.

There was no significant difference in the evaluated PK values between Gadoterate meglumine and the comparator Magnevist.

Conclusion

This study was conducted to determine the plasma kinetics and excretion of Gd-DOTA (G 449.06 or Gadoterate meglumine) and Gd-DTPA (439 RG 08) in rats totally devoid of renal function. Based on the findings, the sponsor showed that in renal failure, biliary excretion of the two Gd complexes increased 5-fold and that the existence of another excretion route can be envisaged.

The sponsor also concluded that peritoneal dialysis appeared to be effective because under the conditions of this study, approximately 30% of the injected dose was excreted in 4 hours.

Reviewer's comments

Induction of renal impairment was induced by the ligation of the renal artery and vein. There is literature support for this method of renal failure induction. However, other methods such as the 5/6 nephrectomy technique have the advantage of inducing renal impairment through a combination of surgical removal of one kidney and a 2/3 ablation of the second in order to assure certainty of induced kidney damage through tissue loss. Fekete et al (1975) described a rodent model of impaired renal function after unilateral renal artery ligation. Permanent hypertension and marked changes in renal function were observed following renal artery ligation. Changes in renal function seen within 24 h following unilateral renal artery ligation included marked alterations in renal function parameters that became more prominent over time. The method of renal vascular ligation in this study appears to be a variation of this method.

5.2 Toxicokinetics

(If not included in toxicity studies)

6 General Toxicology

6.1 Single-Dose Toxicity

6.1.1 Overview

Table 66: List of Single-Dose Toxicity Studies

Species/ Strain	Report No.	Route of Administration	Dose (mmol/kg)	GLP (Yes/No)	Reviewed (Yes/No)
SD Rats*	99.12.807	IV	0, 7, 10.1, 14.5 (11-24 times MHD)	Yes	Yes
	99.12.810	IV	0, 8.33, 12.5, 15	Yes	No
	DGD-1-4-A	Gavage	0, 15.2	No	No
	DGD-1-7-A	Intracisternal	0, 05.2, 7.7, 10.3, 12.9, 15.5 μ mol/animal	No	No
Beagle Dogs*	99.12.808	IV	0, 2.5, 5, 7.5 (14-41 times MHD)	Yes	Yes
	99.12.811	IV	1.25, 2.5, 5, 7.5	Yes	No
OF1 Mice	DGD-1-2-A	IV	0, 8.29, 9.09, 9.96, 10.92, 11.98, 13.13	No	No
	DGD-1-3-A	Gavage	0, 25.3	No	No

Reviewer's Table based on Sponsor's data; * = Pivotal study

6.1.2 Summary of Single-Dose Toxicity Studies

Expanded single-dose toxicity studies were performed in rats and dogs.

In rats, Gadoterate meglumine was administered intravenously at dose levels of 7, 10.1, and 14.5mmol/kg (or 11x, 16x or 24x the human dose, respectively). There were no treatment-related mortalities. Treatment-related clinical signs included piloerection and half-closed eyes were observed in rats of both sexes; swollen muzzle was observed in 6/10 males and 6/10 females, decreased physical activity (3/10 males) and respiratory difficulties (2/10 males). Gadoterate meglumine did not cause remarkable findings in hematology, ophthalmoscopy, food consumption or body weight. Biliary proliferation was observed in animals administered the high dose (14.5 mmol/kg or 24x the human dose). Findings were reversible at post-recovery day 15 sacrifice. Based on these results, the NOAEL for the single-dose toxicity study in rats was established at 7mmol/kg (or 11x the clinical dose).

In dogs, Gadoterate meglumine was administered as a single intravenous dose at the dose levels of 2.5, 5 or 7.5 mmol/kg. There was no mortality. Treatment-related clinical signs of vomiting and urination were observed in males and female dogs following injection at all administered doses. The vomiting and urination were dose-related. According to the sponsor, the urination was due to

the high osmolarity of the test article. There were no remarkable effects on body weight, food consumption, ocular signs, hematology, and serum chemistry parameters. Urinalysis showed an increased urinary volume on day 2 in males administered the high dose (7.5mmol/kg). High urine volume was associated with reduced sodium and chloride excretion. No abnormalities were observed in urine volume, sodium, or chloride at the end of the observation period. There were also no treatment-related changes in organ weight. On day 2, vacuolation of renal tubules occurred at the mid and high dose levels and in hepatocytes at the high dose level. The vacuolation, which was no longer evident at day 15 in males and females, was not associated with degeneration or tubular necrosis. Based on the results, NOAEL for single-dose toxicity in dogs was established at 2.5 mmol/kg (or 14-fold MHD) since no renal tubular vacuolation was observed at this dose level.

6.1.3 Single-Dose Toxicity Reports in Rats

6.1.3.1 Report No. 99.12.807

Report Title: Single-Dose Toxicity Study By Intravenous Route in Rats	
Report No.:	99.12.807
Study report location:	eCTD Module 4 §4.2.3.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 1, 2000
GLP compliance:	Yes (x), No (), page 5 of 241
QA statement:	Yes (x), No (), page 8 of 241
Drug, lot #, and % purity:	Gadoterate meglumine, lot #: n/a; batch no. 99M064 (CoA page 115), purity: n/a

Source: Reviewer's Table. The report of this study was submitted in 2 volumes (vol. 1 of 2, pp. 1-241; vol. 2 of 2, pp. 242-482) and an Amendment to Report (5 pages)

Objective

To evaluate the toxicity of Gadoterate meglumine in Sprague-Dawley rats following a single intravenous (slow bolus) injection and a 2-week observation period

Key findings

In rats, Gadoterate meglumine was administered intravenously at dose levels of 7, 10.1, and 14.5mmol/kg (or 11x, 16x or 24x the human dose, respectively). There were no treatment-related mortalities. Treatment-related clinical signs included piloerection and half-closed eyes were observed in rats of both sexes treated at the high dose (14.5mmol/kg or 24x-MHD); swollen muzzle was observed in 6/10 males and 6/10 females and decreased physical activity (3/10 males) and respiratory difficulties (2/10 males). Gadoterate meglumine did not cause remarkable findings in hematology, ophthalmoscopy, food consumption or body weight.

While no changes were observed in plasma Zinc levels at any dose level in male and female rats at interim or terminal sacrifice, urinary zinc, calcium and magnesium levels increased in all treatment groups in rats of both sexes on days 2 and 15. This finding may have significance in relation to the increasingly important nephrogenic systemic fibrosis (NSF). Treatment-related vacuolation was reported in the kidney and liver. Biliary proliferation was observed in animals administered the high dose (14.5 mmol/kg or 24x the human dose). Findings were reversible at post-recovery day 15 sacrifice. Based on these results, the NOAEL for the single-dose toxicity study in rats was established at 7mmol/kg (or 11x the clinical dose).

Methods	
Doses:	0 (control), 7, 10.1, and 14.5 mmol/kg
Frequency of dosing:	Single dose
Route of administration:	Intravenous (tail vein) at approx. 2mL/min
Dose volume:	29, 14, 20.2 and 29 mL/kg for the respective doses indicated above
Formulation/Vehicle:	Gadoterate meglumine solution (0.5 mmol/mL / Sterile saline (0.9%) solution
Species/Strain:	Rat/Crl CD [®] (SD) IGS BR strain; Caesarian Obtained, Barrier Sustained-Virus Antibody Free (COBS-VAF [®]). (b) (4)
Number/Sex/Groups:	80/sex; 4 groups (control, low, mid and high dose): 10/sex/group
Age:	9 weeks on Day 1 of treatment
Weight:	Males: 321-428g; Females: 205-278 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minimal deviations; Not considered to comprise study validity and integrity

Study design

Table 67: Study Design and Dose groups (99.12.807)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg)	Number of rats	
		Males	Females
1 (veh)	0	10	10
2 (LD)	7	10	10
3 (MD)	10.1	10	10
4 (HD)	14.5	10	10

Source: Reviewer's Table adapted from Sponsor's Study Design on page 15 of 241; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine

Table 68: Gadoterate meglumine Human Dose multiples (99.12.807)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg) in rats	0	7	10.1	14.5
Dose (mmol/m ²)	0	42.0	60.6	87.0
Dose multiples (based on BSA)	N/A	11.4x (11x)	16.4x (16x)	23.6x (24x)

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult). Dose selection was based on the results of an acute toxicity study in rats (No. 99.12.810)

Table of methods:

Table 69: Summary of Methods (99.12.807)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside; Twice/day for signs of mortality and morbidity, general health and toxicity
Body weight	Once prior to allocation to study groups, before day 1 and on days 2, 4, 8 11 and 14
Food/water consumption	Ad lib access. Food consumption per animal was recorded 2x/week (over 3-4 day periods)
Ophthalmology	All animals (control and high dose groups); Examination was done prior treatment and once before necropsy on days 2 and 14.
Clinical Pathology (performed prior to treatment and before necropsy on days 2 and 14)	Blood samples were taken from the orbital sinus under isoflurane anesthesia <u>Hematology:</u> <i>With EDTA:</i> Erythrocytes (RBC), Hemoglobin (HB), Mean cell volume (MCV), Packed cell volume (PCV), Mean Cell Hemoglobin Concentration (MCHC), Thrombocytes (PLAT) and Leucocytes (WBC), Differential WBC with cell morphology, Reticulocytes (RETIC) <i>With Sodium Citrate:</i> Prothrombin time (PT), Activated partial prothrombin time (APTT) and Fibrinogen <i>Bone marrow:</i> Bone marrow differential cell count <u>Clinical Chemistry:</u> <i>With Lithium heparinate:</i> Na ⁺ , K ⁺ , Cl ⁻ , Ca ²⁺ , Inorganic phosphorus (I.PHOS), Iron, Zinc, Copper, Glucose, Urea, Creatinine, Total bilirubin, Total proteins, albumin, albumin/globulin (A/G) ratio, Cholesterol, Triglycerides, Alkaline phosphatase, Aspartate aminotransferase, and Alanine aminotransferase
Termination/ Gross pathology	Necropsy was performed on day 2 or after a 14-day period (day 15). Animals were sacrificed by CO ₂ asphyxiation and exsanguination
Histopathology	<u>Tissue preservation:</u> 10% buffered formalin except eyes (Davidson's fixative) and testes (Bouin's fluid) <u>Tissues:</u> Organs were taken for organ weight, preservation and/or microscopic examination as specified on page 23/241.
Urinalysis	Urine was collected from animals fasted for at least 14h Volume, pH, Specific gravity, Na ⁺ , K ⁺ , Cl ⁻ , Ca ⁺⁺ , Mg ⁺⁺ , Inorganic phosphorus, Iron, Zn, Cu, Proteins, Glucose, Ketones, Bilirubin, Nitrites, Blood, Urobilinogen, leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium oxalate crystals, and cells; appearance and color
Dose formulation analysis	Yes (x), No ()

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms. Zn & Cu levels were determined by Atomic Absorption Spectrophotometer.

Results

Mortality

A total of four (4) female rats died. Deaths occurred after treatment on day 1 or after blood sampling at terminal (day 2) or final (day 15) sacrifice. One mid dose (group 3) female died after treatment on day 1. Three females died after blood sampling: 1 in the saline control group on day 2 and 1 each in the low- and mid-dose groups on day 15. There were no unscheduled deaths in rats of either sex treated at the high dose (14.5mmol/kg). No unscheduled deaths were reported in males in any study group in the duration of the study. No clinical signs were observed in the single rat (mid-dose) that died following treatment. Macroscopic examination showed reddened ovaries, marked steatosis (fatty liver) of the liver and pale kidneys. The kidneys were not examined microscopically due to early autolysis. There were no clinical signs or macroscopic observations in the 3 rats that died after blood sampling.

Clinical signs

Injection site: A blackened tail was observed in 1/10 females administered the mid dose (10.1mmol/kg) and 1/10 females at the high dose (14.5mmol/kg) 2-7 days after administration. Cutaneous scabs were observed as from 7-8 days. Microscopic examination of the cutaneous lesions indicated the presence of subcutaneous inflammatory cells and hyperkeratosis/parakeratosis

General clinical signs: No clinical signs were observed in rats of both sexes in the control, low-dose (7 mmol/kg) and mid-dose (10.1 mmol/kg) groups. At the high dose (14.5 mmol/kg), clinical signs of piloerection and half-closed eyes were observed in unspecified numbers of rats of both sexes; swollen muzzle was observed in 6/10 males and 6/10 females and decreased physical activity (3/10 males) and respiratory difficulties (2/10 males). None of the females showed a decrease in physical activity.

Body weight

No significant test article-related change in mean body weight was observed in Gadoterate meglumine-treated rats when compared controls

Table 70: Summary of Mean body weight gain (g)

Summary of variations of mean body weight gain (g)				
Dose-level (mmol/kg)	0	7	10.1	14.5
<u>Males</u>				
. body weight on day 14	450	440 NS	443 NS	424 NS
. day -1 vs. day 14	+69	+61	+64	+56
<u>Females</u>				
. body weight on day 14	267	266 NS	253 NS	264 NS
. day -1 vs. day 14	+27	+26	+17	+20

NS: not statistically significant

Feed consumption

Compared to controls, slightly lower mean food consumption (g/animal/day), albeit non-significant, was observed in males administered the high dose (14.5 mmol/kg). In females, the difference in the average food consumption when compared to controls was statistically significant in rats treated at the mid-dose ($p < 0.05$) and high-dose ($p < 0.05$) levels. The significant differences in mean food consumption in females were noted in the 11-14 day observation period.

Ophthalmoscopy

There were no test article-related effects.

ECG

Not conducted.

Hematology

There were no test article-related effects.

Blood biochemistry

When compared to control mean values (Table 71), sodium and chloride and inorganic phosphate levels were generally increased across all treatment groups in both males and females on days 2 and 15. Sodium levels were significantly increased ($p < 0.05$) on day 2 in males administered the low dose and on day 15 in females at the high dose. The increase in chloride was significant ($p < 0.05$) only on day 15 in the high dose male group. The phosphate level increased significantly ($p < 0.05$) on day 2 in females treated at the low dose. A significant decrease in copper levels was observed in the high dose male group on day 2, low dose males on day 15. Plasma zinc levels were not significantly different from controls in both males and females at any dose level on days 2 and 15. There was a change in the mean total bilirubin, albumin, cholesterol or triglycerides compared to controls on days 2 and 15. The changes were in most cases minimal and not dose-related.

There were no remarkable changes in potassium, calcium, iron, glucose, urea, creatinine, protein, albumin/globulin (A/G) ratio, alkaline phosphatase, aspartate aminotransferase and Alanine aminotransferase levels.

Table 71: Blood biochemistry values (99.12.807)

Time	Day 2						Day 15					
	Males			Females			Males			Females		
Dose level	Low	Mid	High	Low	Mid	High	Low	Mid	High	Low	Mid	High
Na ⁺	↑*	↑	↑	↑	↓	↑	↑	↑	↑	↑	↑	↑*
K ⁺	↑	↓	↓	↓	↓	n/ch	↓	↑	↑	↑	↓	↑
Cl ⁻	↑	↑	↑	↑	↑	↑	↑	↑	↑*	↑	↑	↑
Ca ⁺⁺	n/ch	n/ch	n/ch	↑	↑	↑	↓	↑	↑	↓	↓	↑
I-PHOS	↑	↓	↓	↑*	↑	↑	↑	↑	n/ch	↑	↓	↑
Zn ⁺⁺	↑	↑	↓	↓	↑	↑	↓	↓	↓	↓	n/ch	n/ch

Cu ⁺⁺	↑	↑	↓*	↑	↑	↓	↓*	↑	↓	↑**	↑**	↑
Total BILI	n/ch	↓	n/ch	n/ch	n/ch	n/ch	↓	n/ch	↓	n/ch	↓	↓*
ALB	n/ch	↓	↓*	n/ch	n/ch	n/ch	n/ch	↑	n/ch	↓	↓	↓
CHOL	↓	n/ch	↑	↑	↑sl	↑sl	↓	↓	↓*	↓	n/ch	↓
TRIG	↑	↑	↑*	↓	↓	↓*	↓*	↓	↓*	↓	↓	↓

Reviewer's Table based on sponsor's data; Na⁺, K⁺, Cl⁻, Ca⁺⁺, I-PHOS, Zn⁺⁺, Cu⁺⁺, Total BILI, ALB, CHOL, TRIG (Sodium, Chloride, Inorganic phosphate, Copper, Total bilirubin, Albumin, Cholesterol, Triglycerides);

↑ = increased mean value compared to control mean; ↓ = decreased mean value compared to control mean; n/ch = no change in value compared to control mean; * (p < 0.05); ** (p < 0.01); Low, Mid and High (low, mid and high doses or 7, 10.1 and 14.5 mmol/kg, respectively)

Urinalysis

There was a reduction in urine volume on day 2 in both sexes and all treatment groups when compared to control values. The % reduction in urine volume (Table 72) in males for low, mid and high dose groups was 12, 35 and 59%, respectively; in females, the % reduction in urine volume was 27, 55 and 46% for corresponding dose groups. Urinary sodium and chloride levels were also reduced in treated animals at all dose levels in both sexes on day 2 in comparison to respective control values. The observed decreases in these Na⁺ and Cl⁻ ions were significant in males. There was no remarkable variation in specific gravity of urine. The changes in urine volume, and sodium/chloride ion levels were reversible and had returned to near control levels by day 15. Compared to respective controls, the levels of calcium, magnesium and zinc ions increased in all treatment groups in males and females on days 2 and 15 when compared to respective controls. As indicated in Table 72, the increased was significant on day 15 for Ca⁺⁺ and Mg⁺⁺ for males at the high dose. Zn⁺⁺ level was significant on day 2 in high dose males and mid dose females.

Table 72: Urine Chemistry values (99.12.807)

Time	Day 2						Day 15					
	Males			Females			Males			Females		
Sex	Low	Mid	High	Low	Mid	High	Low	Mid	High	Low	Mid	High
Dose level	Low	Mid	High	Low	Mid	High	Low	Mid	High	Low	Mid	High
Urine Vol.	↓	↓	↓	↓	↓*	↓	↓	↓	↓	↓	↓	↑
% chg (Vol.)	-12	-35	-59	-27	-55	-46	-22	-28	-44	-10	-30	+10
S/Gravity	↑sl	↑sl	↑	↑	↑	↑	↑	↑	↑	ns	ns	ns
pH	↑sl	↑sl	↑sl	↑sl	↓	↓	n/ch	n/ch	n/ch	n/ch	n/ch	n/ch
Na ⁺	↓**	↓**	↓**	↓	↓	↓	↑	↑	↑	↓	↑	↑
% chg (Na ⁺)	-68	-71	-76	-34	-37	-46	+57	+33	+59	-14	+15	+0.5
Cl ⁻	↓*	↓*	↓*	↓	↓	↓	↑	↑	↑	↓	↑	↓
% chg (Cl ⁻)	-54	-61	-54	-55	-47	-54	+12	+12	+20	-18	+6	-15
Ca ⁺⁺	↑	↑	↑↑	↑sl	↑sl	↑sl	↑↑	↑↑	↑↑↑**	↑sl	↑sl	↑sl
Mg ⁺⁺	↑	↑	↑↑↑	↑	↑↑	↑↑	↑	↑	↑↑↑**	↑sl	↑sl	↑sl
Zn	↑	↑	↑↑*	↑↑	↑↑*	↑↑	↑sl	↑	↑↑	↓	↑	↑sl

Reviewer's Table based on sponsor's data; Vol., S/Gravity, pH, Na⁺, Cl⁻, Ca⁺⁺, Mg⁺⁺, Zn (Volume [mL], specific gravity; sodium [mmol/l], chloride [mmol/l], calcium [mmol/l], magnesium [mmol/l], zinc [μ mol/l], respectively); \uparrow = increased mean value compared to control mean; $\uparrow\uparrow$, $\uparrow\uparrow\uparrow$ = 2-fold or 3-fold increase; \downarrow = decreased mean value compared to control mean; sl = slightly; ns = no significant change compared to control mean; n/ch = no change in value; * (p < 0.05); ** (p < 0.01); Low, Mid and High (low, mid and high doses or 7, 10.1 and 14.5 mmol/kg, respectively); % chg = percentage change from respective control.

Gross pathology

Organ weights (Tables 73 and 74):

On day 2 (see Table 73), the mean kidney weight increased in males following treatment at the low, mid and high dose levels when compared to the control. The increase in organ weight and % organ/body weight was significant (p < 0.01) at the high dose (14.5mmol/kg). The mean percentage organ/body weight ratio was significant at the mid dose (10.1mmol/kg). In females, although there was a moderate decrease in organ weight at all tested doses, there was an increase in the % organ to body weight ratio. This % ratio was significant (p < 0.05) at the high dose (14.5mmol/kg). The changes in mean liver weight and % organ to body weight ratio were not significantly different from controls in males. In females, the variation in these parameters showed an opposing trend at the low and mid doses although there was a significant increase in the % organ/body weight ratio. The variation in mean organ weight or % organ/body weight ratio in the brain, lungs (including bronchi), spleen, epididymides, and ovaries were not significantly different from their respective mean control values.

On day 15 (recovery; Table 74), the differences described above for day 2 were no longer present.

Table 73: Absolute and Relative Organ weight at Day 2 sacrifice (99.12.807)

Time	Day 2							
	Males				Females			
Sex								
Dose level	Control	Low	Mid	High	Control	Low	Mid	High
Mean body wt. (g)	319.2	\uparrow 334.1	\uparrow 339.1	\uparrow 338.1	221.1	\downarrow 202.9	\downarrow 196.5*	\downarrow 213.7
<i>Mean organ wt (g)</i>								
Brain	2.00	✓	✓	✓	1.93	✓	✓	✓
%Organ/Body	0.63	✓	✓	✓	0.88	✓	✓	✓
Epididymides	0.80	✓	✓	✓				
%Organ /Body	0.25	✓	✓	✓				
Kidneys	2.66	\uparrow 3.04	\uparrow 3.00	\uparrow 3.39** (27%)	1.78	\downarrow 1.68	\downarrow 1.64	\uparrow 1.92
%Organ/Body weight	0.83	\uparrow 0.91* (9.6%)	\uparrow 0.88	$\uparrow\uparrow$ 1.02** (23%)	0.81	\uparrow 0.83	\uparrow 0.83	\uparrow 0.897 *
Liver	10.1	✓	✓	✓	7.24	\downarrow 6.60	\downarrow 6.81	$\uparrow\uparrow$ 8.16
% Organ/Body	3.17	✓	✓	✓	3.27	\downarrow 3.25	\uparrow 3.46	$\uparrow\uparrow$ 3.82 *
Lung w/bronchi	1.54	✓	✓	✓	1.26	✓	✓	✓
%Organ/Body	0.48	✓	✓	✓	0.57	✓	✓	✓
Spleen	0.63	✓	✓	✓	0.41	✓	✓	✓

%Organ/Body	0.195	✓	✓	✓	0.18	✓	✓	✓
Testes	3.05	✓	✓	✓				
%Organ/Body	0.96	✓	✓	✓				
Ovaries					0.15	✓	✓	✓
%Organ/Body					0.07	✓	✓	✓

Reviewer's Table based on sponsor's data; ↑, ↑↑ = increased mean value compared to control mean; ↓ = decreased mean value compared to control mean; ✓ = no significant change compared to control mean; * (p < 0.05); ** (p < 0.01); Low, Mid and High (low, mid and high doses or 7, 10.1 and 14.5 mmol/kg, respectively); % Organ/Body = Mean % Organ weight to Body weight ratio.

Table 74: Absolute and Relative Organ weight at Day 15 sacrifice (99.12.807)

Time	Day 15							
	Males				Females			
Sex								
Dose level	Control	Low	Mid	High	Control	Low	Mid	High
Mean body wt. (g)	404.8	395.0	401.4	383.8	236.5	243.7	232.2	237.4
Mean organ wt (g)								
Brain	2.05	✓	✓	✓		✓	✓	✓
%Organ/Body	0.51	✓	✓	✓		✓	✓	✓
Epididymides	1.10	✓	✓	✓				
%Organ /Body	0.77	✓	✓	✓				
Kidneys	2.96	✓	✓	✓	1.79	✓	✓	✓
%Organ/Body	0.73	✓	✓	✓	0.76	✓	✓	✓
Liver	11.99	✓	✓	✓	7.25	✓	✓	✓
% Organ/Body	2.96	✓	✓	✓	3.06	✓	✓	✓
Lung w/bronchi	1.67	✓	✓	✓	1.35	✓	✓	✓
%Organ/Body	0.42	✓	✓	✓	0.57	✓	✓	✓
Spleen	0.67	✓	✓	✓	0.48	✓	✓	✓
%Organ/Body	0.17	✓	✓	✓	0.20	✓	✓	✓
Testes	3.36	✓	✓	✓				
%Organ/Body	0.83	✓	✓	✓				
Ovaries					0.13	✓	✓	✓
%Organ/Body					0.06	✓	✓	✓

Reviewer's Table based on sponsor's data; ✓ = no significant change compared to control mean; Low, mid and high (low, mid and high doses or 7, 10.1 and 14.5 mmol/kg, respectively); % Organ/Body = Mean % Organ weight to Body weight ratio.

The summary of organ weight differences in the kidneys on days 2 and 15 expressed as percentage is shown in the sponsor's table below (Table 75). In males on day 2, there was an increase in the absolute and relative kidney weight in the low (14%, 9%) and high (27%, 23%) doses. The pattern of variation in both parameters in females on day 2 was less apparent. On day 15, there was a lower increase in absolute or relative organ weight but a more inconsistent pattern of variation from controls in these parameters in comparison to males. What was apparent was that the effects seen on day 2 were reversed on day 15.

Table 75: Summary of organ weight difference (%) (No. 99.12.807)

Day	2						15					
	Males			Females			Males			Females		
Sex												
Dose-levels (mmol/kg)	7	10.1	14.5	7	10.1	14.5	7	10.1	14.5	7	10.1	14.5

Kidneys												
. absolute	+14	+13	+27	-6	-8	+8	-1	+0	+0	+2	-1	+0
. relative	+9	+6	+23	+2	+3	+11	+1	+1	+6	-2	+1	-1

Sponsor's Table (page 31/241; Vol. 1 of 2, Study No. 99.12.807)

Macroscopic findings: There were no remarkable macroscopic findings at day 2 or day 15 necropsy.

Adequate battery: Yes (x), No ()

As indicated in Table 76, the macroscopic findings identified on day 2 or day 15 were mostly described in typically 1/5 animals and were usually not dose-dependent except for a finding of dilated uterus on day 2 that was no longer present on day 15.

Table 76: Animals with Macroscopic findings at Day 2 sacrifice (99.12.807)

Time	Day 2							
	Males (n=5/dose group)				Females (n=5/dose group)			
Sex/No.	Control	Low	Mid	High	Control	Low	Mid	High
Dose level								
Adrenals (whitish)	-	-	-	-	1/5	-	-	-
Kidneys (pale)	-	-	-	-	-	-	1/5	-
Lungs/bronchi (reddish)	-	-	1/5	-	-	-	-	-
Mandibular Ln (reddish)	-	1/5	-	-	-	-	-	-
Ovaries (reddish)	-	-	-	-	-	-	1/5	-
Thymus (reddish foci)	-	1/5	-	-	-	-	-	-
Uterus (dilated/serous content)	-	-	-	-	-	-1/5	1/5	1/5
	Day 15							
Caudal v. inj. site	-	-	-	-	-	-	1/5	1/5
Caudal v. inj. site (scab/soreness)	-	-	-	-	-	-	-	1/5
Epididymides (yellow, reduced size)	-	-	1/5	-	-	-	-	-
Pancreatic Ln (enlarged/reddish)	-	1/5	-	-	-	-	-	-
Testes (soft, decreased size)	-	-	1/5	-	-	-	-	-
Uterus (enlarged/serous content)	-	-	1/5	-	-	-	-	-

Reviewer's Table based on sponsor's data; inj. = injection

Histopathology

Adequate battery: Yes (x), No ()

Peer review: Yes (), No (), Not determinable from submission (x)

Histopathological findings

Injection site: Following a microscopic examination of the injection site (caudal vein, distal caudal vein and proximal caudal vein) on days 2 and 15 there was evidence of fibroplasia, collagen degradation and presence of inflammatory cells in the vein. Hemorrhage, inflammatory cells, collagen degradation, mast cells, fibrosis and fibroplasia were also described in the perivenous area. The presence of subcutaneous inflammatory cells, collagen degradation, and fibroplasia were noted in addition to ulceration, hyperkeratosis and parakeratosis. These findings were neither sex- or dose-dependent and were comparable in both control and test dose groups on days 2 and 15 (Table 77).

Table 77: Injection site histopathology (99.12.807)

Sacrifice Dose-level (mmol/kg)	Day 2				Day 15			
	0		14.5		0		14.5	
Sex	M	F	M	F	M	F	M	F
Caudal vein dist. i.s.								
. total affected	5	5	5	4	5	5	4	4
. mean severity	1.2	1.4	1.2	1.3	1.0	1.2	1.0	1.0
Caudal vein i.s.								
. total affected	5	4	5	5	4	5	4	5
. mean severity	1.4	1.3	2.0	1.2	1.0	1.2	1.0	1.2
Caudal vein prox. i.s.								
. total affected	5	5	4	5	4	5	4	3
. mean severity	1.2	1.4	1.3	1.0	1.0	1.0	1.0	1.0

i.s.: injection site, prox.: proximal, dist.: distal.

Treatment-related findings identified in kidneys and liver on day 2: These include vacuolation of renal cortical tubular cells seen in all animals of both sexes. Treatment-related vacuolated hepatocytes were seen in the liver at the high dose (14.5mmol/kg) in males and females. Biliary proliferation was observed in all animals administered 14.5mmol/kg. When rats were examined on day 15 to determine if recovery from the effect of the test article had occurred (reversibility), vacuolated hepatocytes were still observed in 4/5 control males, 5/5 control females and all males and females administered the high dose (14.5 mmol/kg) but not in animals treated at the low and mid doses. Findings indicated continued presence of hepatic vacuolation in males and females administered the high dose. Biliary proliferation was seen in 1/5 animals in each sex at the high dose.

Table 78: Animals with Microscopic findings at Day 2 sacrifice (99.12.807)

Time	Day 2							
	Males (n=5/dose group)				Females (n=5/dose group)			
Sex/ No.								
Dose level	Control	Low	Mid	High	Control	Low	Mid	High
KIDNEYS								

Interstitial Mono. Cell Aggregation	1/5	-	-	1/5	-	-	-	-
Interstitial fibrosis	1/5	1/5	-	1/5	-	-	-	-
Tubular basophilia	1/5	1/5	1/5	2/5	-	1/5	-	-
Mineralization	-	-	-	-	1/5	-	-	-
Vacuolation of cortical tubular epithelium	-	5/5	5/5	5/5	-	5/5	4/5	5/5
Oil of Red (weak positive)	2/5	-	-	-	3/5	1/5	-	3/5
Oil of Red (v. weak positive)	-	-	-	2/5	2/5	4/5	4/5	1/5
Protein cast	-	-	-	1/5	-	-	-	-
Renal cyst	1/5	-	-	-	-	-	-	-
LIVER								
Mononuclear Cell Aggregation	4/5	-	-	5/5	3/5	-	1/5	3/5
Vacuolated hepatocytes	-	-	-	5/5	5/5	-	1/5	5/5
Oil of Red (weak positive)	1/5	-	-	3/5	1/5	-	-	4/5
Oil of Red (v. weak positive)	2/5	-	-	1/5	4/5	-	-	1/5
Extramedullary hematopoiesis	1/5	-	-	3/5	1/4	-	-	2/5
Tension lipidosis	1/5	-	-	-	-	-	-	-
Biliary proliferation	-	-	-	5/5	-	-	-	5/5

Reviewer's Table based on sponsor's data

Special evaluation

None

Toxicokinetics

No performed

Dosing solution analysis

Yes

Conclusions

The sponsor concluded that Gadoterate meglumine, administered as a single intravenous dose to rats, was well-tolerated at the injection site and showed little or no systemic toxicity. Based on histopathological findings of vacuolated hepatocytes and biliary proliferation occurring in rats of both sexes at the 14.5 mmol/kg dose a No Observed Effect Level (NOEL) was not established.

Reviewer's comments

According to the sponsor, in the absence of histopathological findings that may provide some explanation for the mortality seen in control and treated female rats, the reported deaths could be considered a consequence of the blood sampling technique. While it may be true that absence of histopathological findings can make it difficult to determine a cause(s) of death, this is a general statement that does not apply in all instances. Pooled together, the number of deaths was similar in

control (1 of 20) and treatment (3 of 40) groups. According to the sponsor, the kidneys of rats that died were not examined due to autolysis.

On the blackened tail and cutaneous scabs, I do not agree with the sponsor that these findings were due to the injection procedure and unrelated to treatment since these findings were not observed in controls and in animals administered the low dose. The reason for absence of injection site lesions in males and not in females is not readily apparent. On the clinical observations of piloerection, half-closed eyes, decreased physical activity and swollen muzzle, I agree with the sponsor that these signs were attributable to treatment at the high dose. Based on these observed clinical signs, this reviewer determined a NOAEL of 10.1 mmol/kg (or 16x MHD).

I agree with the sponsor's conclusion that the tendency to slightly lower average food consumption in high-dose group males and females treated at the mid and high doses was a consequence of treatment.

Plasma zinc levels were not significantly different from controls in both males and females at any dose level on days 2 and 15.

The sponsor's explanation that a decrease in urine volume on day 2 in rats of both sexes was a transient physiological response to the test article and changes in urinary Na^+ and Cl^- could be considered secondary to the observed changes in urine volume seemed plausible because there was substantial recovery in urine volume on day 15.

Results showed that when compared to respective controls, the levels of urinary calcium, magnesium and zinc ions generally increased in all treatment groups in males and females on days 2 and 15. The increase was significant on day 15 for Ca^{++} and Mg^{++} for males at the high dose. Zn^{++} level was significant on day 2 in high dose males and mid dose females.

With reference to zinc, the sponsor noted that the differences between treated and control animals in some parameters namely, elevated urinary zinc levels in males at 14.5 mmol/kg (HD) on day 2 and increased calcium and magnesium levels in males at 14.5 mmol/kg (HD) were not attributable to Gadoterate meglumine. The sponsor's argument was based on 1) that the reported values were slight; 2) increases were not observed females and 3) there were no corresponding changes in plasma Zn levels. I do not agree. Although it appeared that the reported values of these parameters were lower than the values in males, changes were undoubtedly present. The data indicated not only a treatment-related effect in both sexes but also that a significant and dose-dependent relationship persisted in males up to day 15 for calcium and magnesium. The level of Zn at the mid ($p < 0.05$) and high doses in males on day 15 remained elevated. In females, a less apparent and non-significant increase was observed for zinc, calcium and magnesium.

Physiologically, Zn absorption is concentration-dependent and under normal physiological conditions, transport processes of Zn uptake are not saturated. Zn is lost from the body through the kidney, skin and intestine. The body has no Zn stores and Zn concentration in plasma, blood cells, hair and urinary Zn excretion can be decreased in severe Zn deficiency. Of pertinence to this review is the level of zinc excretion following use of gadolinium-based MRI contrast agents. Kimura and others (2005) showed that differences in Zn excretion following GBCA use was

possibly related to in-vivo transmetallation of the Gd chelate complex, a process that correlated with the variable stability of the contrast agents. The authors concluded that the large amount of excess ligands contained in some MR contrast agents was also considered to be responsible for the increase in urinary Zn and Cu excretion (Kimura et al, 2005) in patients. In the cited study, Gd-DOTA (Gadoterate meglumine) did not cause a significant increase in Zn excretion in contrast to Gd-DTPA-BMA (Omniscan), or Gd-DTPA (Magnevist).. This finding may have significance in relation to the increasingly important nephrogenic systemic fibrosis (NSF).

As described in the results, only the kidneys and the liver showed findings with statistical significance. I agree with the sponsor's conclusion that the differences observed in other organs were minimal, not dose-related, and did not indicate similar trend in both males and females. These findings were therefore not considered to have a toxicological relevance. I agree that the predominant histopathology findings in this study were dose-related renal tubular vacuolation – a finding commonly observed with the gadolinium-based contrast agents – and occurrence of vacuolated hepatocytes and biliary proliferation at the highest dose level. There was a tendency towards partial reversibility of some of the hepatobiliary findings at 15 days post-treatment and there was no evidence of nuclear or cytoplasmic degeneration. Based on these results, the NOAEL for the single-dose toxicity study in rats was established at 7mmol/kg. This NOAEL however excludes vacuolation that was observed at all dose levels tested.

6.1.4 Single-Dose Toxicity Studies in Dogs

6.1.4.1. Report No. 99.12.808

Report Title: Single-Dose Toxicity Study By Intravenous Route in Beagle Dogs	
no.:	99.12.808
Study report location:	eCTD Module 4 §4.2.3.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 29, 2000
GLP compliance:	Yes (x), No (), page 5 of 273
QA statement:	Yes (x), No (), page 8 of 273
Drug, lot #, and % purity:	Gadoterate meglumine, lot #: n/a; batch no. 99M064 (CoA page 172 of 273), purity: n/a

Source: Reviewer's Table. The report of this study was submitted in 2 volumes (vol. 1 of 2, pp. 1-273; vol. 2 of 2, pp. 274-519 and an Amendment to Report (5 pages)

Objective

To evaluate the toxicity of Gadoterate meglumine in beagle dogs following a single intravenous (slow bolus) injection and a 2-week observation period

Key findings

Gadoterate meglumine, administered as a single intravenous dose to beagle dogs at the dose levels of 2.5, 5 or 7.5 mmol/kg was well tolerated at the site of injection. There was no mortality. Treatment-related clinical signs of vomiting and urination were observed in males and female dogs following injection at all administered doses. The vomiting and urination were dose-related. A large urine volume was voided in all males and females one day following the injection of the

high dose (7.5mmol/kg). There were no remarkable effects on body weight, food consumption, ocular signs, hematology, and serum chemistry parameters. Urinalysis showed an increased urinary volume on day 2 in males administered the high dose (7.5mmol/kg). High urine volume was associated with reduced sodium and chloride excretion. No abnormalities in urine volume, sodium or chloride at the end of the observation period. There were also no treatment-related changes in organ weight.

On day 2, vacuolation of renal tubules occurred at the mid and high dose levels and in hepatocytes at the high dose level. The vacuolation, which was no longer evident at day 15 in males and females, was not associated with degeneration or tubular necrosis. Based on the results, NOAEL for single-dose toxicity in dogs was established at 2.5 mmol/kg (or 14-fold MHD) since no renal tubular vacuolation was observed at this dose level.

Methods	
Doses:	0 (control), 2.5, 5, and 7.5 mmol/kg (dose levels were selected based on the results of a dose range finding study 99.12.811)
Frequency of dosing:	Single dose
Route of administration:	Intravenous (slow bolus) at approximately 6 mL/min
Dose volume:	15, 5, 10 and 15 mL/kg for the respective doses indicated above
Formulation/Vehicle:	Gadoterate meglumine solution (0.5 mmol/mL / Sterile saline (0.9%) solution
Species/Strain:	Dogs/Beagle; (b) (4)
Number/Sex/Groups:	50; 25/sex; 4 dose groups (control, low, mid and high dose): 6/sex/group
Age:	6 months
Weight:	Males: 6.2-7.4 kg; Females: 5.5-6.7 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minimal deviations (listed on page 29/273); Deviations were not considered to comprise study validity and integrity

Study design

Table 79: Study Design and Dose groups (99.12.808)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg)	Number of rats	
		Males	Females
1 (veh)	0	6	6
2 (LD)	2.5	6	6
3 (MD)	5	6	6
4 (HD)	7.5	6	6

Source: Reviewer’s Table adapted from Sponsor’s Study Design on page 16 of 273; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine

Table 80: Gadoterate meglumine Human Dose multiples (99.12.808)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg) in rats	0	2.5	5	7.5
Dose (mmol/m ²)	0	50	100	150
Dose multiples (based on BSA)	N/A	13.5x (14x)	27.0x (27x)	40.5x (41x)

Source: Reviewer’s Table constructed from sponsor’s data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult). Dose selection was based on the results of a dose range finding toxicity study in rats (No. 99.12.811)

Observations (summary of methods)

Table 81: Summary of Methods (99.12.808)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside; Twice/day for signs of mortality and morbidity, general health and toxicity
Body weight	Once prior to allocation to study groups, before day 1, and on days 3, 8 11 and 14
Food consumption	The amount of food consumed by each animal was estimated daily, for 7 days before the beginning of the treatment period and then each day throughout the study. Food consumption was expressed as a percentage of the quantity given
Electrocardiography	Electrocardiographic examinations were performed on all animals before the beginning of the treatment period, on the first day of treatment (at the end of injection, and 30 min, 1 h and 3 h after) and once before scheduled necropsy (day 2 or day 14).
Ophthalmology	Ophthalmological examinations were performed on all animals before treatment and once before scheduled necropsy (day 2 or day 13/14).
Blood pressure	Systolic and diastolic blood pressure (SBP and DBP) were measured on all animals before the beginning of the treatment period, on the first day of treatment (end of injection, 30 min, 1 h and 3 h post-dosing) and once before scheduled necropsy (day 2 or day 14)
Clinical Pathology	Anticoagulated (EDTA or Sodium citrate) blood samples were taken from the peripheral vein <u>Hematology:</u> <i>With EDTA:</i> Erythrocytes (RBC), Hemoglobin (HB), Mean cell volume (MCV), Packed cell volume (PCV), Mean Cell Hemoglobin Concentration (MCHC), Thrombocytes (PLAT) and Leucocytes (WBC), Differential WBC with cell morphology, Reticulocytes

	<p>(RETIC)</p> <p><i>With Sodium Citrate:</i> Prothrombin time (PT), Activated partial prothrombin time (APTT) and Fibrinogen (FIB)</p> <p><i>Bone marrow:</i> Bone marrow differential cell count</p> <p><u>Clinical Chemistry:</u></p> <p><i>With Lithium heparinate:</i> Na⁺, K⁺, Cl⁻, Ca²⁺, Inorganic phosphorus (I.PHOS), Iron, Zinc, Copper, Glucose, Urea, Creatinine, Total bilirubin, Total proteins, albumin, albumin/globulin (A/G) ratio, Cholesterol, Triglycerides, Alkaline phosphatase, Aspartate aminotransferase (ASAT), and Alanine aminotransferase (ALAT)</p>
Termination/ Gross pathology	<p>Necropsy was performed on day 2 or after a 14-day period (day 15). Animals were sacrificed by IV thiopental sodium and exsanguination</p> <p><u>Organ weights, macroscopic/microscopic examination</u></p>
Histopathology	<p><u>Tissue preservation:</u> 10% buffered formalin except eyes (Davidson's fixative) and testes (Bouin's fluid)</p> <p><u>Tissues:</u> Organs were taken for organ weight, preservation and/or microscopic examination as specified on page 24/273.</p>
Urinalysis	<p>Urine was collected from animals before treatment and before scheduled necropsy on day 2 or end of observation period on day 14.</p> <p><u>Quantitative parameters:</u> Volume, pH, Specific gravity, Na⁺, K⁺, Cl⁻, Ca⁺⁺, Mg⁺⁺, Inorganic phosphorus, Iron, Zn, and Cu.</p> <p><u>Semi-quantitative:</u> Proteins, Glucose, Ketones, Bilirubin, Nitrites, Blood, Urobilinogen, Cytology (leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals, cells)</p> <p><u>Qualitative:</u> Appearance and color</p>
Dose formulation analysis	Yes (x), No (); CoA page 172/273

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Results

Mortality

No deaths were reported

Clinical signs

Injection site: Gadoterate meglumine was well tolerated at the IV injection site except for a swollen injection site observed on day 1 in 1 dog administered the high dose (7.5mmol/kg)

General clinical signs: Vomiting and urination were observed after injection of Gadoterate meglumine on day 1 in males and females. Vomiting was observed as from 2.5mmol/kg, or 5mmol/kg in males or females, respectively. There were no other signs of systemic toxicity.

Vomiting was observed in 1/6 males at the mid- and high-dose levels or 27-41 times the clinical dose. In females, vomiting occurred in 1/6 (low dose), 2/6 (mid dose) and 1/6 (high dose) dogs or 14-41 times the clinical dose. A large volume of urine was voided at the end of injection in 6/6 males and 6/6 females administered the high dose (41x the clinical dose) but beginning at 5mmol/kg in 1/6 males and at 2.5 mmol/kg in 1/6 females.

Body weight

There were no treatment-related effects

Feed consumption

There were no treatment-related effects

Ophthalmoscopy

There were no treatment-related effects

ECG

There were no treatment-related effects

Blood Pressure

There were no treatment-related effects on blood pressure

Hematology

There were no treatment-related effects

Clinical chemistry

There were no treatment-related effects

Urinalysis

Compared to control values, an increased urinary volume was observed on day 2 in 2/6 high dose (7.5 mmol/kg) male dogs. High urine volume was associated with reduced sodium and chloride excretion. No abnormalities in urine volume, sodium or chloride at the end of the observation period.

Gross pathology

Organ weights: Differences in absolute and relative organ weight (kidneys, liver, spleen, epididymides, testes and ovaries) were observed in treated and control animals on day 2 and day 15. While the differences, albeit not significant, were notable for the epididymides and testes at the high dose (7.5 mmol/kg), the differences in other organs were relatively minor, tended to be of opposing trend, were not dose-dependent and appeared minor.

Macroscopic findings:

Adequate battery: Yes (x), No ()

There were no remarkable macroscopic findings at the injection site on day 2 or day 15.

Histopathology

Adequate battery: Yes (x), No ()

Peer review: Yes (), No (), Not determinable from submission (x)

Histopathological findings:

Injection site: Main findings on day 2 sacrifice included inflammation of the vein (phlebitis), degradation of venous wall collagen, perivenous hemorrhage, and presence of inflammatory cells. Folliculitis, epidermitis and parakeratosis were described in the injection site skin. The findings were observed in both controls and test-article treated animals. The findings were no longer present at day 15 sacrifice.

Systemic microscopic findings: On day 2, the prominent histopathological finding was the occurrence of slight to moderate renal cortical tubular vacuolation in dogs of both sexes treated at the 5 mmol/kg or 7.5 mmol/kg levels. Vacuolation was no longer present by day 15 sacrifice. Staining of vacuolated cells with Oil Red O in control and treated animals was negative for the presence of fat. A minimal to slight occurrence of hepatocyte vacuolation was observed in 2 male dogs at the high dose. All other microscopic findings on days 2 and 15 were not treatment-related.

Special evaluation

None

Toxicokinetics

No performed

Dosing solution analysis

Yes

Conclusions

The sponsor concluded that Gadoterate meglumine, when administered as a single intravenous dose to beagle dogs at the dose levels of 2.5, 5 or 7.5 mmol/kg was well tolerated at the site of injection.

No mortality occurred. Treatment-related clinical signs of vomiting and urination were observed in males and female dogs following injection at all administered doses of Gadoterate meglumine. The vomiting and urination were dose-related. A large urine volume was voided in all males and females one day following the injection of the high dose (7.5mmol/kg). According to the sponsor, the urination was due to the high osmolarity of the test article.

There were no remarkable effects on body weight, food consumption, ocular signs, hematology, and serum chemistry parameters.

Urinalysis showed an increased urinary volume on day 2 in males administered the high dose (7.5mmol/kg). High urine volume was associated with reduced sodium and chloride excretion. No abnormalities in urine volume, sodium or chloride at the end of the observation period.

There were also no treatment-related changes in organ weight.

On day 2, vacuolation of renal tubules occurred at the mid and high dose levels and in hepatocytes at the high dose level. The vacuolation, which was no longer evident at day 15 in males and females, was not associated with degeneration or tubular necrosis.

Based on the results, NOAEL for single-dose toxicity in dogs was established at 2.5 mmol/kg (or 14-fold MHD) since no renal tubular vacuolation was observed at this dose level.

Reviewer's comments

I agree with the findings of this study.

6.2 Repeat-Dose Toxicity

6.2.1 Overview

Table 82: List of Repeat-Dose Toxicity Studies

Species/ Strain	Report No.	Route of Administration	Dose (mmol/kg)	GLP (Yes/No)	Reviewed (Yes/No)
SD Rats*	99.12.806	IV	0, 2, 4, 8	Yes	Yes
SD Rats	99.12.805	IV	0, 2, 4, 8	Yes	No
CD Rats	DGD-1-9-A	IV	0, 0.3, 0.7, 1.5	Yes	No
Beagle Dogs*	DGD-1-10-A	IV	0, 0.3, 0.7, 1.5	Yes	Yes

Reviewer's Table based on Sponsor's data; * = Pivotal study

6.2.2 Summary of Repeat-Dose Toxicity Studies

Following repeated administration of Gadoterate meglumine in the rat at dose levels of 2, 4 and 8 mmol/kg (or 3.2x, 6.5x and 13x the human dose) over a 4-week period followed by a 13-week treatment-free period, no test article-induced mortality was reported. Findings, where present, were observed at the mid (4mmol/kg) and high dose (8mmol/kg) levels. The findings were mostly reversed after the 13-week post-treatment, treatment-free period. NOEL was not established since vacuolation was also observed at the low dose (2 mmol/kg/day or 3.2x MHD).

Similar to the rat, Gadoterate meglumine was administered in repeated doses (0.3, 0.7 and 1.5mmol/kg (or 1.6x, 3.8x and 8.1x the human dose) over a 4-week period followed by a 13-week treatment-free period in the dog. Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. There was also a dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility/recovery period. Based on the finding of renal tubular vacuolation in all treated animals, NOEL was not established in this study.

6.2.3 Repeat-Dose Toxicity Reports in Rats

6.2.3.1 Report No. 99.12.806

Study Title: 4-Week Toxicity Study By Intravenous Route In Rats Followed by a 13-Week Treatment-Free Period	
Study No.:	99.12.806
Study report location:	eCTD Module 4 §4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 05, 2000
GLP compliance:	Yes (x), No (), page 5 of 270
QA statement:	Yes (x), No (), page 8 of 270
Drug, lot #, and % purity:	Gadoterate meglumine, lot #: n/a; batch no. 99M064 (CoA page 135/270), purity: n/a (Appendix 1, p. 134/270)

Objective

To evaluate the potential toxicity of Gadoterate meglumine repeated (daily) IV administration to Sprague-Dawley rats for a period of 4 weeks followed by a 13-week treatment-free period recovery period for rats in the control and high dose groups.

Key findings

No test article-induced mortality was reported following the administration of Gadoterate meglumine in repeated doses over a 4-week period in rats. Most of the findings, where present, were observed at the mid and high dose levels. The findings were mostly reversed after the 13-week post-treatment, treatment-free period. NOEL was not established since vacuolation was also observed at the low (2 mmol/kg/day) dose.

Methods

Doses:	0 (saline control), 2, 4, and 8 mmol/kg
Frequency of dosing:	Repeated doses
Route of administration:	Intravenous (tail vein)
Dose volume:	16, 4, 8, 16 mL/kg/day for 0 (saline control), 2, 4, and 8 mmol/kg, respectively
Formulation/Vehicle:	Gadoterate meglumine (aqueous solution) / Saline (0.9% NaCl solution, Batch No. 2831, (b) (4))
Species/Strain:	Rat (b) (4)
Number/Sex/Group:	16/sex/group (Control and High dose groups); 10/sex/group (Low and Mid dose groups)
Age:	6 weeks (on Day 1)
Weight:	Males (191-226 g); Females (152-201 g)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minimal deviations; Not considered to comprise study validity and integrity

Study Design

The potential toxicity of Gadoterate meglumine was evaluated in Sprague-Dawley rats following daily IV injection at 0, 2, 4 and 8 mmol/kg/day (or 3.2, 6.5 and 13-fold the human dose based on body surface area) for 4 weeks. The dose levels were selected based on a preliminary GLP 7-day

toxicity study (No. 99.12.805). Groups of 16 rats per sex were administered saline control or the high dose level while 10 rats per sex received the low and mid dose levels over a treatment period of 28 days. After the end of the treatment period, 5 males and 6 females in the control group and 6 males and 3 females in the high dose group were retained for a 13-week recovery (reversibility) period.

Table 83: Study Design and Dose groups (99.12.806)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg)	Number of rats	
		Males	Females
1 (saline vehicle)	0	16	16
2 (LD)	2	10	10
3 (MD)	4	10	10
4 (HD)	8	16	16

Source: Reviewer's Table adapted from Sponsor's Study Design on page 17 of 270; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine.

Table 84: Gadoterate meglumine Human Dose multiples (99.12.806)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg)	0	2	4	8
Dose (mmol/m ²)	0	12	24	48
Dose multiples (based on BSA)	N/A	3.24x (3.2x)	6.49x (6.5x)	12.97x (13x)

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult). Dose selection was based on the results of an acute toxicity study in rats (No. 99.12.805)

Observations and Results

Summary of methods:

Table 85: Summary of Methods (99.12.806)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside; Twice/day for signs of mortality and morbidity, general health and toxicity. (Once/day during treatment-free period)
Body weight	Once prior to allocation to study groups, on day 1 and once/week thereafter.
Food consumption	Once/week over a 7-day period
Ophthalmology	Performed on all animals of the control and high-dose groups, once before the beginning of the treatment period and in week 4
Clinical Pathology	Blood samples from the abdominal vein at necropsy in week 5 and 18 and from the orbital sinus in week 5 in treatment-free animals only. Blood was collected from anesthetized animals into anticoagulant tubes for

	hematology and clinical chemistry analysis
Urinalysis	The following parameters were determined: Volume, pH, Specific gravity, Na ⁺ , K ⁺ , Cl ⁻ , Ca ⁺⁺ , Mg ⁺⁺ , Inorganic phosphorus, Iron, Zn, Cu, Proteins, Glucose, Ketones, Bilirubin, Nitrites, Blood, Urobilinogen, leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium oxalate crystals, and cells; appearance and color
Termination/ Gross pathology	On completion of the treatment and treatment-free periods, after at least 14 hours fasting, all surviving animals were anesthetized with isoflurane and blood was taken from the abdominal aorta for laboratory investigations. Any moribund animals were asphyxiated by carbon dioxide and killed by exsanguination.
Organ weights	The body weight of all animals killed at the end of the treatment and treatment-free periods was recorded before sacrifice, and the organs specified were weighed wet as soon as possible after dissection. The ratio of organ weight to body weight (recorded immediately before sacrifice) was calculated
Histopathology	<u>Tissue preservation</u> : 10% buffered formalin except eyes (Davidson's fixative) and testes and epididymides (Bouin's fluid). A bone marrow sample was taken from the femur of all animals killed on completion of the treatment and treatment-free periods
Dose formulation analysis	Yes (x), No ()

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Mortality

A total of 6 rats (1 male / 5 females) died or were sacrificed. Some deaths were seen in control group (1/32), at 4 (2/20) and at 8 (3/32) mmol/kg dose levels. No deaths occurred in either sex in the 2 mmol/kg (low dose) group. There was no death in males or females at the 2mmol/kg dose; 2/20 rats and 3/32 rats died in the mid dose (4mmol/kg) and high dose (8mmol/kg) groups, respectively. According to the sponsor, the deaths were not treatment-related. There was no indication in the report that post-mortem examination was performed on dead animals.

Clinical Signs

Injection site: At the injection site, clinical signs observed in males and females included presence of hematoma, blackish or damaged tail tissue. A dry tail was also observed in one male. (Table 86). These signs were observed in all dose groups except the low dose. The findings were more frequently observed in animals administered the high dose (3M/4F) than in the mid dose (2F) and control (2F) rats.

Table 86: Gross injection site findings (99.12.806)

Dose levels (mmol/kg/day)	Control (0)	Low dose (2)	Mid dose (4)	High dose (8)
	Males			

blackish tail	n/o	n/o	n/o	2
hematoma	n/o	n/o	n/o	1
damaged tail	n/o	n/o	n/o	1
skin lesions	n/o	n/o	n/o	1
dry tail	-	-	-	1
Females				
blackish tail	1	n/o	1	2
hematoma	1	n/o	1	3
damaged tail	n/o	n/o	n/o	1

Reviewer's Table based on sponsor's data; n/o = not observed

General clinical signs: No remarkable signs of systemic toxicity were observed

Body Weights

Compared to controls, the mean group body weight change was lower at the end of the 4 week treatment period in males that received the high dose (8mmol/kg/day). The reduction in mean body weight was less apparent in females. In both males and females, there was a complete recovery of body weight at the end of the 13-week treatment free (recovery) period (Table 87).

Table 87: Changes in Mean body weight after treatment or recovery (99.11.806)

Dose-levels (mmol/kg/day)	0	2	4	8
<u>Males</u>				
weeks 1 to 5 (g)	+157	+160	+163	+142
weeks 5 to 18 (g)	+194	-	-	+220
<u>Females</u>				
weeks 1 to 5 (g)	+67	+69	+68	+64
weeks 5 to 18 (g)	+90	-	-	+104

-: no animals assigned to the treatment-free period.

Feed Consumption

Compared to controls, slightly lower mean food consumption levels were observed treated at the high dose (8mmol/kg/day) in males and females. There was a complete recovery after the treatment-free period.

Ophthalmoscopy

There were no treatment-related findings

ECG

No remarkable findings were observed

Hematology

Significant differences were observed at the end of the treatment period in Hb, RBC and PCV values in male and female rats administered Gadoterate meglumine at the high dose level (8

mmol/kg/day) when compared to their respective controls. These differences were treatment related and were summarized in the following sponsor's Table (Table 88):

Table 88: Notable changes in hematological values post-treatment (99.12.806)

Dose-levels (mmol/kg/day)	0	8	historical data
Hb (g/dL)			
<u>Males</u>	14.4 ± 0.7	12.4 ** ± 0.6	[13.4-16.9]
. values outside range	1/15	15/16	
<u>Females</u>	13.4 ± 0.8	11.7 ** ± 0.6	[12.0-16.2]
. values outside range	1/15	9/13	
RBC (T/L)			
<u>Males</u>	7.49 ± 0.42	6.49 ** ± 0.27	[6.61-8.76]
. values outside range	0/15	11/16	
<u>Females</u>	6.90 ± 0.39	6.02 ** ± 0.35	[6.18-8.37]
. values outside range	1/15	8/13	
PCV (%)			
<u>Males</u>	43	37 ** ± 2	[40-50]
. values outside range	0/15	15/16	
<u>Females</u>	39 ± 2	35 ** ± 2	[30-50]
. values outside range	0/15	0/13	

** : p<0.01

Sp

ponsor's Table (page 32/270)

Changes in mean coagulation parameters were also noted between high dose (8mmol/kg/day) group females and their respective controls. These include lower prothrombin time (PT, 13.5 vs. 14.1 s, p < 0.05), lower activated partial prothrombin time (APTT, 13.9 vs. 16.6 s, p < 0.01) and lower fibrinogen concentration (FIB, ~1.9 g/L vs. 2.3 g/L, p < 0.01). The changes described in the above hematological and coagulation parameters were treatment-related and were reversed after the 13-week treatment. Other blood parameters evaluated did not show any defined trend in values in the pre- and post-treatment period. The changes were not considered of toxicological significance.

A tabulation of post-treatment (week 5) and post treatment-free (week 18) hematology data is shown in the Table below (Table 89). Except for a significant difference in fibrinogen (FIB) level in males administered the high dose and a significant difference in neutrophil count in females; the mean values of all other hematology parameters were not significantly different from their respective controls after the treatment-free period.

Table 89: Hematological values after a 4-week treatment period (99.12.806)

Time	Week 5 (post-treatment)							
	Males				Females			
Sex								
Dose level	Control	Low	Mid	High	Control	Low	Mid	High
WBC	6.82	↓	↓	↓	4.88	↓	↓	↓
RBC	7.49	↑7.53	↓7.18	↑6.49**	6.90	↑7.15	↓6.67	↓6.02*
HB	14.4	n/ch	↓13.6*	↓12.4**	13.4	↓13.5	↓12.9	↓11.7**

PCV	0.43	↑0.44	↓0.41*	↓0.37**	0.39	↓0.38	↑0.40	↓0.35**
MCV	57.5	ns	ns	ns	56.9	↓	↓	↓
MCH	19.2	ns	ns	ns	19.4	↑	↓	↓
MCHC	33.4	ns	ns	ns	34.2	↑	↓	↓
PLAT	1055	ns	ns	ns	1027	↓	↑	↑
Neutrophils	0.87	↑	↓	↓	0.55	↓	↓	↓
Eosinophils	0.09	↓0.06	↓0.05	↓0.06*	0.07	↓	↓	↓
Basophils	n/ch	n/ch	n/ch	n/ch	0.01	n/ch	n/ch	n/ch
Lymphocytes	5.70	↓	↑	↓	4.15	↓	↓	↑
Monocytes	0.15	↑	↑	n/ch	0.11	↑	↓	↑
PT	14.1	↓	↑	↓	14.1	↓13.7	↓13.7	↓13.5*
APTT	21.3	↓	↑	↓	16.6	↓15.7	↓14.9	↓13.9**
FIB	2.63	↓	↓	↓	2.25	↓1.91	↓1.92*	↓1.93**

Reviewer's Table based on sponsor's data; ↑ = increased mean value compared to control mean; ↓ = decreased mean value compared to control mean; ns = no significant change compared to control mean; * (p < 0.05); ** (p < 0.01); Low, Mid and High (low, mid and high doses or 2, 4 and 8 mmol/kg, respectively); n values and ±SD values are not shown

Table 90: Hematological values after a 13-week post-treatment period (99.12.806)

Time	Week 18 (post treatment-free period)			
	Males		Females	
	Control	High	Control	High
Dose level				
WBC, RBC, PCV, MCV, MCH, MCHC, PLAT, Eosinophils, Basophils, Lymphocytes, Monocytes, PT, APTT	ns		ns	
Neutrophils	1.11	↑1.18 ns	0.76	↓0.36*
FIB	2.34	↑2.78*	1.96	ns

Reviewer's Table based on sponsor's data; ↑ = increased mean value compared to control mean; ↓ = decreased mean value compared to control mean; ns = no significant change compared to control mean; * (p < 0.05); Low, Mid and High (low, mid and high doses or 2, 4 and 8 mmol/kg, respectively); n values and ±SD values are not shown

Clinical Chemistry

At the end of treatment period, significant differences were observed between treatment and respective control values in males and females triglyceride (TRIG) levels at the mid and high dose levels and in the Aspartate aminotransferase (ASAT) level in females at the high dose level, as shown in the sponsor's Table below (Table 91). The values of all other clinical chemistry parameters were in general not toxicologically significantly.

Table 91: Changes in Clinical Chemistry parameters after 4-week treatment (99.12.806)

Dose-levels (mmol/kg/day)	0	4	8	historical data
TRIG (mmol/L)				
<u>Males</u>	0.35 ± 0.15	0.23* ± 0.08	0.21** ± 0.05	[0.24-1.39]
. values outside range	4/15	5/10	11/16	
<u>Females</u>	0.25 ± 0.06	0.20* ± 0.05	0.17** ± 0.03	[0.22-0.80]
. values outside range	4/16	6/8	11/13	
ASAT (IU/L)				
<u>Females</u>	55 ± 10	/	83* ± 30	[41-85]
. values outside range	0/16	/	5/13	

*: p<0.05 ; **: p<0.01 ; /: no change

Sponsor's Table (page 33/270)

Urinalysis

After the 4-week treatment period (Table 92), a significantly lower sodium or chloride excretion was observed in males and females but there was no variation in urine volume. These differences were treatment-related. There was a dose-related and significantly different decrease in the mean urinary pH in males (all dose levels), but not in females, when compared to controls. In males but not females administered the low dose, significant differences were observed in inorganic phosphate, zinc and copper levels when compared to controls. Calcium and magnesium levels were also significantly different in males at both the low and mid dose. There was a complete reversibility in all the above urinary parameters after the 13-week treatment-free period.

Table 92: Post-treatment Urinalysis data (99.12.806)

Time	Week 5 (post-treatment)							
	Males				Females			
Sex								
Dose level	Control	Low	Mid	High	Control	Low	Mid	High
Urine volume	17	↓10*	↓15	↑18	11	↑	↑	↑
Specific Gravity	1018	↑	↑	↑	1023	↓	↓	↓
pH	7.3	↓6.8*	↓6.7*	↓6.3**	6.6	↓	↓	↓
Na ⁺	58.6	↑70.9	↓43.1	↓↓28.5**	76.7	↓50.0	↓32.5**	↓27.7**
K ⁺	75.3	↑125.1**	↑100.4	↑81.2	85.3	↓	↓	↓
Cl ⁻	43.6	↓42.6	↓28.7*	↓15.8**	56.6	↓33.8	↓24.6**	↓20.1**
Ca ⁺⁺	0.5	↑0.95**	↑0.9*	↑0.7	1.2	↑	↓	↓
Inorg Phosphate	28.3	↑44.5*	↑37.1	28.0 ns	43.8	↓	↓	↓
Mg ⁺⁺	4.0	↑9.1**	↑8.9**	↑6.6	7.7	↓	↑	n/ch
Iron	0.7	↑	↑	↑	0.8	↑	↑	↑
Zn	7.2	12.5**	↑11.0	↑8.5	11.7	↓	↓	↓
Cu	2.5	↑4.4*	↑3.0	↑2.6	1.8	↓	↓	↓

Reviewer's Table based on sponsor's data; ↑ = increased mean value compared to control mean; ↓ = decreased mean value compared to control mean; n/ch = no change from controls; * (p < 0.05); ** (p <

0.01); Low, Mid and High (low, mid and high doses of 2, 4 and 8 mmol/kg, respectively); n values and \pm SD values are not shown

Gross Pathology

Organ Weights

Following the 4-week treatment period and after the treatment-free period, there was dose-dependent increase in kidney weight (24% to 59%). Significant increases in liver weights (11% to 29%) were reported in high dose group animals. The differences in kidney and liver weight were no longer evident at the end of the treatment-free period and were considered as evidence of reversibility. The differences observed in other organs were minimal, not dose-related and were of opposing trend between tested dose levels. The differences were not considered toxicologically significant.

Injection site:

Systemic level:

Histopathology

Adequate Battery: Yes (x), No ()

Peer Review: (), No (), Not determinable from submission (x)

Histopathological Findings

Injection site: There was evidence of recent thrombus, organized thrombus and necrosis in the wall of the injected vein in control rats and in rats administered the high dose (8mmol/kg). Inflammatory cells, collagen degradation, signs of hemorrhage and fibroplasia were present in the perivenous tissue in control and treated animals. These injection site findings were no longer present after the treatment-free period.

Systemic microscopic findings: Treatment-related changes were noted in the kidneys, liver, urinary bladder and lymph nodes. Dose-related vacuolation of renal tubular epithelial cells was observed in all males and females and in all treatment groups and more prominently at the high dose (8mmol/kg/day). At the high dose, biliary proliferation was observed in 7/10 males and 10/13 females. After the treatment-free period, there was minimal renal tubular vacuolation in 5/6 males and in 2/3 females at the high dose. In the liver, vacuolated hepatocytes and in the lymph nodes (vacuolated histiocytes) were not longer evident.

Special Evaluation

None

Toxicokinetics

Not performed

Dosing Solution Analysis

Yes

Conclusions

No test article-induced mortality was reported following the administration of Gadoterate meglumine in repeated doses over a 4-week period in rats. Most of the findings, where present, were observed at the mid and high dose levels. The findings were mostly reversed after the 13-week post-treatment, treatment-free period. NOAEL was not established since vacuolation was also observed at the low (2 mmol/kg/day) dose.

Reviewer's Comments

The mortality reported in the mid and high dose groups was probably not related to treatment and that the hematoma and blackish tail were likely due to injection of high volume of saline (control) group or repeat dose administration with the test article in the treatment groups. Given that a similar incidence of hematoma/blackish tail in rats in the control and mid dose groups is further evidence that the observed clinical signs were not treatment-related. However, because the incidence of injection site findings was higher at the high dose (3M/4F) compared to the mid dose (2F) or control (2F) groups, I concur that the higher incidence of findings at the high dose may be due to treatment. However, since macro- and microscopic examination of the injection site did not reveal any difference between control and test groups, the injection site effects likely resulted from repeated administrations. Nevertheless, the significance injection site pathology should be considered. Proper technique of contrast media has been recommended in the manual of the American College of Radiology (ACR Manual on Contrast Media, Version 8, 2012).

Extravasations are known to occur during both hand or power injection of contrast media.

Complaints in most patients of initial swelling or tightness, and or/or burning pain at the site of extravasations. Such extravasations may be edematous, erythematous and tender (ACR Manual, 2012). Most extravasations are limited to immediately adjacent soft tissues, typically the skin and subcutaneous tissues. No permanent injury is involved. However, serious and major consequences have followed seemingly minor injection injuries (Dailiana et al., 2008).

Based on the above, the reviewer believes NOEL was 2mmol/kg or 3.2x MHD. The sponsor concluded that the changes observed in mean body weight correlated with the observed slight tendency to lower mean food consumption. For both sexes, a complete recovery in mean body weight and mean food consumption were apparent after the 13-week treatment-free recovery period.

6.2.4 Repeat-Dose Toxicity in Dogs

6.2.4.1 Report No. DGD-1-10-A

Study Title: Toxicity Study by Intravenous Administration to Dogs for 4 weeks followed by a 4-week Study By Intravenous Route In Rats Followed by a 4-Week Reversibility period	
Study no.:	DGD-1-10-A
Study report location:	eCTD Module 4 §4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1987
GLP compliance:	Yes (x), No (), page 2 of 252
QA statement:	Yes (x), No (), page 5 of 252
Drug, lot #, and % purity:	Gadoterate meglumine LAG/053/G 449.06, lot #: 152, purity: n/a (Appendix 1, CoA, p. 96/252)

Objective

The objective of this study was to determine the toxicity of repeated, daily intravenous injections of Gadoterate meglumine in beagle dogs over a 4-week period and the reversibility of any such effects 4 weeks after the end of treatment.

Key findings

Gadoterate meglumine was administered in repeated doses (0.3, 0.7 and 1.5 mmol/kg (or 1.6x, 3.8x and 8.1x the human dose) in the dog. Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. There was also a dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility/recovery period. Based on the finding of renal tubular vacuolation in all treated animals, NOEL was not established in this study.

Methods

Doses:	0 (saline control), 0.3, 0.7, and 1.5 mmol/kg
Frequency of dosing:	Repeated doses (4 weeks)
Route of administration:	Intravenous
Dose volume:	3.0, 0.6, 1.4 and 3.0 mL/kg/day for 0 (saline control), 0.3, 0.7, and 1.5 mmol/kg, respectively
Formulation/Vehicle:	Gadoterate meglumine (aqueous solution) / Saline (0.9% NaCl solution)
Species/Strain:	Dog/Beagle; (b) (4)
Number/Sex/Group:	5/sex/group (Control and High dose groups); 3/sex/group (Low and Mid dose groups)
Age:	16-19 weeks (on arrival)
Weight:	4.6 – 7.4 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minimal deviations; Not considered to comprise study validity and integrity

Study Design

The potential toxicity of Gadoterate meglumine was evaluated in beagle dogs following daily IV injection at 0, 0.3, 0.7 and 1.5 mmol/kg/day (or 1.6, 3.8 and 8.1-fold the human dose based on body surface area) for 4 weeks. Groups of 5 dogs per sex were administered saline control or the high dose level while 3 dogs per sex received the low and mid dose levels over a treatment period of 4 weeks. After the end of the treatment period, 2 dogs per sex in the control and high dose group were retained for a 4-week treatment-free recovery (reversibility) period.

Table 93: Study Design and Dose groups (DGD-1-10-A)

Groups (Gadoterate meglumine was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg)	Number of rats	
		Males	Females
1 (vehicle)	0	5	5
2 (LD)	0.3	3	3
3 (MD)	0.7	3	3
4 (HD)	1.5	5	5

Source: Reviewer's Table adapted from Sponsor's Study Design on page 17 of 252; veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gadoterate meglumine

Table 94: Gadoterate meglumine Human Dose multiples (DGD-1-10-A)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg) in rats	0	0.3	0.7	1.5
Dose (mmol/m ²)	0	6	14	30
Dose multiples (based on BSA)	N/A	1.62x (1.6x)	3.78x (3.8x)	8.11x (8.1x)

Source: Reviewer's Table constructed from sponsor's data; BSA = body surface area (proposed human dose = 0.1 mmol/kg or 3.7mmol/m² based on BSA, assuming a 60 kg adult).

Observations and Results

Table 95: Summary of Methods (DGD-1-10-A)

Protocol	Method, frequency and/or objectives
Clinical observations	Cageside; Once/day for signs of mortality and morbidity, general health and toxicity.
Body weight	Weekly prior to treatment, during treatment, before necropsy and in the post-treatment period
Food consumption	Daily to determine weekly consumption in the treatment and treatment-free periods
Ophthalmology	Performed before the commencement of study and on day 26
Clinical Pathology	Blood samples were taken via the jugular vein once prior to study, on day 23 before dosing and on day 22 of the post-treatment period. From each dog after overnight fasting <u>Hematology:</u> <i>With EDTA:</i> Erythrocytes (RBC), Hemoglobin (HB), Mean cell volume (MCV), Packed cell volume (PCV), Mean Corpuscular Hemoglobin (MCH), Mean Cell

	<p>Hemoglobin Concentration (MCHC), Thrombocytes (PLAT) and Leucocytes (WBC), Differential WBC with cell morphology, Reticulocytes (RETIC) <i>With Sodium Citrate:</i> Prothrombin time (PT), and Activated partial prothrombin time (APTT) <i>Bone marrow:</i> Bone marrow smears were obtained by biopsy of the iliac crest under local anesthesia on day 22 or during week 4 of the treatment-free period</p> <p><u>Clinical Chemistry:</u> <i>With Lithium heparinate:</i> Na⁺, K⁺, Cl⁻, Ca²⁺, Inorganic phosphorus (I.PHOS), Iron, Zinc, Copper, Glucose, Urea, Creatinine, Total bilirubin, Total proteins, albumin, albumin/globulin (A/G) ratio, Cholesterol, Triglycerides, Alkaline phosphatase, Aspartate aminotransferase, and Alanine aminotransferase. Additional samples were obtained using lithium heparinate and stored frozen for Mn, Fe, Cu, and Zn concentrations</p>
Urinalysis	The following parameters were determined: Volume, pH, Specific gravity, Na ⁺ , K ⁺ , Cl ⁻ , Ca ⁺⁺ , Mg ⁺⁺ , Inorganic phosphorus, Iron, Zn, Cu, Proteins, Glucose, Ketones, Bilirubin, Nitrites, Blood, Urobilinogen, leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium oxalate crystals, and cells; appearance and color
Termination/ Gross pathology	On completion of the treatment and treatment-free periods, dogs were anesthetized with IV sodium pentobarbitone and killed by rapid exsanguination
Organ weights	The body weight of all animals killed at the end of the treatment and treatment-free periods was recorded before sacrifice. The ratio of organ weight to body weight (recorded immediately before sacrifice) was calculated
Histopathology	SOP
Dose formulation analysis	Yes (x), No ()

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Mortality

There were no deaths related to treatment and all dogs survived to their scheduled sacrifice

Clinical Signs

No treatment-related clinical signs were described at the injection site. There were no signs of systemic toxicity at the doses of the test article evaluated

Body Weights

Body weight gain was not affected by treatment

Feed Consumption

There were no treatment-related differences when compared to controls

Ophthalmoscopy

Ophthalmoscopy did not reveal any treatment-related ocular effects

ECG

Not evaluated

Hematology

No changes were observed in the hematological parameters following the treatment period or after the treatment-free period. The bone marrow was also not affected by treatment

Clinical Chemistry

There were no treatment-related changes in the clinical chemistry parameters evaluated

Urinalysis

There were no treatment-related changes in urinary composition after the treatment period

Gross Pathology**Organ Weights**

No treatment-related changes were observed

Macroscopic pathology

There were no treatment-related adverse macroscopic findings

Histopathology

Adequate Battery: Yes (x), No ()

Peer Review: (), No (), Not determinable from submission (x)

Histopathological Findings

Injection site: There were no remarkable injection site findings

Systemic microscopic findings:

Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. This finding was also dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility/recovery period.

Special Evaluation

None

Toxicokinetics

Not conducted

Dosing Solution Analysis

Yes

Conclusions

Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. There was also a dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility / recovery period.

Reviewer's comments

I agree with the study results and conclusion.

7 Genetic Toxicology**7.0.0 Overview and Summary of Genotoxicity Studies****Overview**

Genotoxicity assessment of Gadoterate meglumine was performed using *in-vitro* (bacterial reverse mutation assay; chromosomal aberration study in Chinese hamster ovary (CHO) cells; and Gene mutation assay using CHO V79 cells) and *in-vivo* mammalian erythrocyte micronucleus test. Gadoteric acid was used in all the four studies as test article.

Summary

Gadoterate meglumine was not mutagenic (with or without metabolic activation) in the Ames test, Chromosomal aberration test on Chinese Hamster ovary cells, a gene mutation test in Chinese Hamster lung cells and in the *in vivo* micronucleus test in mice.

Table 96: List of *in-vitro* and *in-vivo* Genotoxicity studies

Type of study	Report No.	Test System	Dose of Gadoterate meglumine #	GLP (Yes/No)	Reviewed (Yes/No)
In-vitro	DGD-1-5-A	<i>Salmonella Typhimurium</i> strains (\pm S9 activation) TA1535, TA1537, TA1538, TA98, TA100	\pm S9 activation: 0 (saline), 0.1, 0.5, 2.5, 5, 50 mmol/plate	Yes	No
	DGD-33-004 [@]	<i>Salmonella Typhimurium</i> strains (\pm S9 activation) TA98, TA100, TA1535, TA1537, TA102	1. Plate incorporation \pm S9 activation: 0 (water), 52, 164, 512, 1600, 5000 μ g/plate	Yes	Yes
		<i>Salmonella Typhimurium</i> strains (\pm S9 activation) TA98, TA100, TA1535, TA1537, TA102	2. Pre-incubation \pm S9 activation: 0 (water), 492, 878, 1568, 2800, 5000 μ g/plate		
	DGD-1-12-A	<i>CHO Chinese hamster cells</i>	\pm S9 activation: 0 (distilled water), 3.33, 10.00, 16.66	Yes	Yes

			mmol/L		
	DGD-1-13-A	V79 Chinese hamster cells	± S9 activation: 0 (distilled water), 3.33, 10.00, 16.66 mmol/L	Yes	Yes
In-vivo*	DGD-1-11-A	Bone marrow micronuclei	0, 1.5, 2.5, 3.5 mmol/kg	Yes	Yes

Reviewer's Table based on Sponsor's data; * *In-vivo* Bone marrow micronuclei assay; # details of positive controls are described in the reviews; @ In report DGD-33-004, the in-vitro bacterial reverse mutation test (Ames test), *S. Typhimurium* strain TA 102 was included in accordance with current ICH Guidelines and Gadoteric acid was tested up to 5000 µg/plate using both the plate-incorporation and pre-incubation methods

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

7.1.1 Report No. DGD-33-004

Study Title: Bacterial reverse mutation test (Plate incorporation and Pre-incubation methods)

Report No.: DGD-33-004

Study report location: eCTD Module 4 §4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: 12 March 2002 (page 8 of 81)

GLP compliance: Yes (x), No (), page 4 of 81

QA statement: Yes (x), No (), page 5 of 81

Drug, lot #, and % purity: Gadoteric acid, Batch No. 99M077, % purity – assumed to be 100% for the dose calculation (CoA – page 81 of 81)

Objective

The purpose of this study is to evaluate the mutagenic potential of Gadoteric acid by measuring its ability to induce reverse mutation at selected loci of 5 histidine-dependent strains of *Salmonella typhimurium* in the presence and in the absence of S9-activation using both the plate incorporation method and the pre-incubation methods.

Key findings

In the *in vitro* bacterial reverse mutation assay (Ames test) with the direct plate incorporation and preincubation methods, Gadoterate meglumine was not mutagenic in the absence or presence of extrinsic metabolic (S9) activation. None of the five tester strains showed an increase in revertant mutant colonies. Growth inhibition of the background lawn was not observed and there were no precipitates in the agar. Under the conditions of this study, Gadoterate meglumine, tested at a concentration up to 5000 µg/plate, was negative in the bacterial reverse mutation assay.

Methods

Strains:	<i>Salmonella Typhimurium</i> mutant strains TA98, TA100, TA1535, TA1537, TA102 (b) (4)
Concentrations in definitive study:	<u>Plate incorporation method</u> : 0 (water), 52, 164, 512, 1600, 5000 µg/plate (half-log progression) in the presence and absence of metabolic activation <u>Pre-incubation method</u> : 0 (water), 492, 878, 1568, 2800, 5000 µg/plate (quarter-log progression) in the presence and absence of metabolic activation
Basis of concentration selection:	Selection of dose levels for the confirmatory mutagenicity assay was based on the toxicity and precipitation profile of the test article assessed in an initial toxicity-mutation assay using the plate incorporation method and one bacterial strain (TA100).

The highest dose for the confirmatory mutagenicity assay was selected to give some indication of toxicity without exceeding 5 mg/plate. At least 5 different dose levels were tested.

Each experiment was tested in triplicate. Concurrent (vehicle) controls and positive controls were included.

Negative control:	Sterile distilled water (for Test article)
¹ Positive controls (µg/plate):	9-aminoanthracene (5µg); sodium azide (10 µg), 9-aminoacridine (50 µg), t-butyl hydroperoxide (100 µg), and 2-nitrofluorene (5 µg)
Formulation/Vehicle:	Gadoteric acid / sterile distilled water
Incubation & sampling time:	- Incubation: 37 ± 2 ⁰ C for 48-72 hrs for both the direct plate incorporation and pre-incubation methods - 3 replicate plates ± S9-activation - 2 independent assays - Colonies were counted after incubation with test, negative and positive control articles

¹Concentration of positive controls provided as µg/plate in parenthesis

Negative control

Water

Positive controls

Positive controls in the absence and presence of metabolic activation are shown in the following sponsor Table (Table 97).

Table 97: Positive controls (DGD-33-004)

Strain	Chemical (Abbreviation)	S9 Mix	µg/plate
TA98	2-Nitrofluorene (2-NF)	Without	5
TA100	Sodium azide (NaA)	Without	10
TA1535	Sodium azide (NaA)	Without	10
TA1537	9-Aminoacridine (9-AA)	Without	50
TA102	<i>t</i> -Butyl hydroperoxide (t-BHP)	Without	100
All the strains	2-Aminoanthracene (2-A)	With	5

Preliminary experiment

A preliminary experiment was performed using the plate incorporation method with the strain TA100, both with and without metabolic activation at the dose levels of 52, 164, 512, and 1600 and 5000 µg/plate (half-log progression).

Confirmatory (Main) experiments

Independent experiments were conducted with Gadoterate meglumine to evaluate the mutagenicity of the test item using either the plate incorporation method or the preincubation method, with and without metabolic activation. Each experimental point in the study was tested in triplicate.

Study Validity

Selection of the bacterial tester strains was adequate based on the Guidance for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996, and OECD, 1998). Positive and negative controls produced expected responses. Dose selection for the plate incorporation method was adequate based on the use of the limit dose (5000 µg/plate).

Results

1. Preliminary experiment: The positive and negative controls gave the expected responses. No plates were lost through contamination or any other unforeseen events. All criteria for a valid experiment were met.

2. Plate incorporation method: The first assay using the plate incorporation method was performed based on the results of the preliminary experiment. The positive controls gave the expected responses, no plates were lost through contamination or unexpected responses, the experiment was considered valid and all data were accepted. No precipitation was observed and no signs of cytotoxicity were noted at any dose level, with or without activation. In the absence of or presence of activation no statistically significant increases in the number of revertants was noted at any dose level in any of the 5 tester strains when compared to the negative (vehicle) controls.

Table 98: Bacterial reverse mutation test - Plate incorporation assay (DGD-33-004)

Assay 1 plate incorporation: Revertant Colony Counts (Mean ± SD)							
Metabolic activation	Test Article	Dose Level (µg/plate)	TA98	TA100	TA1535	TA1537	TA102
Without Activation	H ₂ O	0	23 ± 6.6	114.7 ± 10.5	24.3 ± 9.8	14.7 ± 3.2	359.3 ± 14.5
	Gadoteric acid	52	34 ± 6.1	127.7 ± 13.6	15.7 ± 6.4	7.0 ± 3.0	311.3 ± 16.3
		164	29 ± 5.0	104.7 ± 18.2	24.7 ± 4.7	7.7 ± 1.5	340.7 ± 39.2
		512	24 ± 1.0	104.7 ± 7.1	21.7 ± 0.6	11.0 ± 6.6	327.0 ± 29.1
		1600	33 ± 1.4	97.7 ± 3.8	18.3 ± 3.1	10.7 ± 5.5	287.0 ± 11.8
		5000	32 ± 1.4	97.0 ± 8.5	19.3 ± 4.6	13.7 ± 7.0	253.0 ± 5.0
	Sodium azide	10		2213.7 ± 109.5	2341.0 ± 53.1		
		9-Aminoacridine	50			543.3 ± 36.8	
		t-butyl hydroperoxide	100				1769.3 ± 93.0
	2-Nitrofluorene	5	836 ± 36.4				
With Activation	H ₂ O	0	32.3 ± 6.1	99.7 ± 10.2	16.0 ± 6.2	16.0 ± 4.4	343.3 ± 13.0
	Gadoteric acid	52	32.3 ± 4.0	107.0 ± 15.1	18.3 ± 7.5	14.7 ± 4.9	256.3 ± 15.4
		164	33.7 ± 0.6	100.0 ± 8.7	21.7 ± 3.1	10.7 ± 3.1	294.3 ± 41.5
		512	33.0 ± 2.0	88.7 ± 9.5	19.7 ± 7.1	15.3 ± 2.3	320.3 ± 23.5
		1600	37.7 ± 10.7	107.3 ± 5.1	19.3 ± 2.1	13.3 ± 3.5	245.3 ± 7.6
		5000	38.3 ± 4.2	100.0 ± 4.6	23.0 ± 7.8	20.0 ± 4.4	250.3 ± 28.1
	9-Aminoanthracene	5	1440.7 ± 31.8	2184.3 ± 139.6	232.3 ± 27.4	250.7 ± 48.4	1022.3 ± 34.4

3. Preincubation method: The preincubation assay was performed based on the results of the plate incorporation method. The experiment was carried out on all strains at the doses (see methods) in the presence and absence of metabolic activation. The positive controls gave the expected responses, no plates were lost through contamination or unexpected responses, the experiment was considered valid and all data were accepted. No precipitation was observed and no signs of cytotoxicity were noted at any dose level, with or without activation. Results obtained in the absence of metabolic activation showed no significant increases in the number of revertants at any dose levels in any of the 5 tester strains when compared to the negative (vehicle) controls. In the presence of activation, weak and statistically ($p < 0.05$) increases were observed in strain TA1535 at 492 and 1568 µg/plate. However, no increases were noted in any other tester strains.

Table 99: Bacterial reverse mutation test -Pre-incubation assay (DGD-33-004)

Assay 2 Pre-incubation: Revertant Colony Counts (Mean ± SD)							
Metabolic activation	Test Article	Dose Level (µg/plate)	TA98	TA100	TA1535	TA1537	TA102
Without Activation	H ₂ O	0	19.0 ± 4.4	88.7 ± 6.4	17.3 ± 4.5	9.0 ± 2.0	348.3 ± 0.6
	Gadoteric acid	492	15.3 ± 2.5	86.3 ± 9.3	15.7 ± 6.4	10.7 ± 0.6	353.3 ± 27.2
		878	19.3 ± 9.7	82.7 ± 12.7	14.0 ± 1.0	8.3 ± 2.1	373.3 ± 9.1
		1568	17.7 ± 3.1	75.3 ± 5.5	11.7 ± 2.3	10.0 ± 2.7	352.7 ± 37.5
		2800	22.7 ± 4.9	96.0 ± 12.8	15.7 ± 4.0	6.0 ± 1.0	350.0 ± 37.3
		5000	23.3 ± 8.3	79.3 ± 20.7	13.7 ± 5.5	8.7 ± 3.8	331.0 ± 22.0
	Sodium azide	10		2649.7 ± 160.0	2042.3 ± 65.3		
	9-Aminoacridine t-butyl hydroperoxide	50				665.0 ± 78.3	
		100					2116.0 ± 117.2
	2-Nitrofluorene	5	721.3 ± 48.1				
	With Activation	H ₂ O	0	23.0 ± 6.0	85.3 ± 0.6	9.3 ± 1.2	8.0 ± 3.0
Gadoteric acid		492	27.0 ± 2.0	89.7 ± 7.5	16.3 ± 4.2*	14.0 ± 3.6	482.3 ± 24.0
		878	20.0 ± 3.5	96.7 ± 4.6	13.7 ± 2.5	11.3 ± 1.5	478.3 ± 42.7
		1568	20.3 ± 3.2	93.0 ± 14.7	16.7 ± 6.0*	8.7 ± 5.1	465.0 ± 39.2
		2800	25.3 ± 4.2	82.3 ± 3.8	12.7 ± 1.5	13.3 ± 0.6	475.7 ± 12.1
		5000	24.0 ± 8.7	91.7 ± 7.0	15.3 ± 4.0	14.0 ± 1.0	459.7 ± 15.1
9-Aminoanthracene		5	1283.7 ± 190.1	1384.7 ± 47.2	247.3 ± 13.3	301.3 ± 49.0	1028.0 ± 64.4

* p<0.05

Conclusion

When tested at the recommended maximum non-cytotoxic dose level of 5000 µg/plate in the presence and in the absence of metabolic activation and using either the plate incorporation or the pre-incubation methods, Gadoterate meglumine did not induce biologically significant increases in the number of revertants in the five *Salmonella typhimurium* strains used in this study. Therefore, under the conditions and criteria in which this test was performed, it was concluded that Gadoterate meglumine did not induce mutagenic effects in the Ames Bacteria mutation assay either in the presence or absence of metabolic activation.

Reviewer's comment

I agree with the sponsor's conclusion.

7.2 In Vitro Assays in Mammalian Cells**7.2.1 Report No. DGD-1-12-A**

Study Title: Chromosomal aberration study in CHO Chinese hamster cells

Report No.: DGD-1-12-A

Study report location: eCTD Module 4 §4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: May, 1988

GLP compliance: Yes (x), No (), page 3 of 23

QA statement: Yes (x), No (), page 4 of 23

Drug, lot #, and % purity: Gadoteric acid (*G 449.06), lot # 203, ~100%

*G 449.06 is a colorless pharmaceutical solution containing gadolinium (1,4,7,10 tetraazacyclododecane N, N', N'', N''' tetraacetic acid) at a concentration of 0.5 M.

Objective

This in vitro assay was performed to evaluate the ability of Gadoterate meglumine (G 449.06) to induce chromosomal aberrations in Chinese hamster ovary cells with and without metabolic activation.

Key findings

Under the conditions of this study, Gadoterate meglumine (Gadoteric acid; G 449-06) was considered negative for inducing chromosome aberrations in CHO cells with and without metabolic activation. A statistically significant ($p < 0.001$) and reproducible increase in the number of aberrant cells was observed in cells exposed to the positive controls (Methyl-methane-sulphonate and Cyclophosphamide) indicating the sensitivity of the system.

Methods

Cell line:	Chinese hamster ovary cells (b) (4) Designated as a CHO-K ₁ clone
Concentrations in definitive study:	0 (distilled water), 3.33, 10.00, and 16.66 mmol/L (corresponding to 1859, 5583 and 9300 mg/L of Gd-DOTA, respectively.
Basis of concentration selection:	Doses were selected based on a preliminary (range finding study) cytotoxicity study with and without metabolic activation
Negative control:	Only cells and either culture medium or S-9 mix
Positive control:	Mutagenic and chromosome-breaking agents <u>Without activation:</u> Methyl-methane-sulphonate (0.54 mmol/l dissolved in water, batch No. 93F 3669, (b) (4)) <u>With activation:</u> Cyclophosphamide (CPA; 0.12 mmol/L; batch No. 298, (b) (4) dissolved in water)
Formulation/Vehicle:	Distilled water (Test article and positive controls)
Incubation & sampling time:	Continuous treatment with test article for 4 hours at 37°C, with or without metabolic activation. The medium was then removed, fresh medium added to the cultures

Study Validity

The study appears to be valid for the following reasons: 1) the appropriate positive controls were employed according to the FDA/CFSAN Redbook Guidelines and produced expected results, 2) the appropriate number of cells was evaluated and 2 replicates of each test concentrations were tested in accordance with current practice, 3) metaphase cells were examined under high power. A minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The counting (scoring) method was in accordance with currently accepted methods and therefore considered valid, 4) according to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minor gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A

reproducibly statistically significant increase at the high concentration only or one other concentration was considered positive. The criteria for the evaluation of the positive results were considered valid, 5) the conditions were appropriate given the use of the limit dose for 4 hr incubations and toxicity measured in the 20 hr incubation (FDA/CFSAN Redbook guidelines). The dose selection based upon mitotic index was acceptable.

Results

The results of the study are summarized in the following sponsor's Table (Table 100).

Table 100: Results of the Chromosomal aberration assay in CHO cells (DGD-1-12-A)

Metabolic activation	Test Article	Concentration (mmol/L)	Total number of aberrations	Mean number of cells with aberrations	% cells with aberrations
Without Activation	Distilled water	0	19	19	9.5
	Gadoteric acid	3.33	14	12	6
		10.00	50 ^a	31	15.5
		16.66	16	12	6
		Methyl-methane-sulphonate	0.54	134	174***
With Activation	Distilled water	0	10	10	5
	Gadoteric acid	3.33	14	14	7
		10.00	7	6	3
		16.66	9	9	4.5
		Cyclophosphamide	0.12	130	98***

Chi² test *** p<0.001

^a: For the first culture, 11 aberrations came from 1 cell, for the second culture, 7 aberrations were observed which was within the range of historical values for negative controls. A significant increase of aberrant cells was obtained for the second culture only and not for pooled results. This effect was not reproducible and was not dose related.

3.33, 10.00, 16.66 mmol/ correspond to 1859, 5583 and 9300 mg of gadoteric acid/L, respectively (Mw=558.25).

Conclusions

Based upon the results of this study and under the described experimental conditions, Gadoterate meglumine did not induce significant chromosomal aberrations and was considered non-clastogenic in the cytogenetic test in CHO cells. A statistically significant ($p < 0.001$) and reproducible increase in the number of aberrant cells was observed in cells exposed to the positive controls (Methyl-methane-sulphonate and Cyclophosphamide) indicating the sensitivity of the system.

Reviewer's comment

I agree with the sponsor's conclusion.

7.2.2 Report No. DGD-1-13-A**Study Title:** Gene mutation assay in V79 Chinese hamster cells

Report No.: DGD-1-13-A

Study report location: eCTD Module 4 §4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: April 26, 1988

GLP compliance: Yes (x), No (), page 3 of 32

QA statement: Yes (x), No (), page 4 of 32

Drug, lot #, and % purity: Gadoteric acid (*G 449.06), lot # 203, ~100%

*G 449.06 is a colorless pharmaceutical solution containing gadolinium (1,4,7,10 tetraazacyclododecane N, N', N'', N''' tetraacetic acid) at a concentration of 0.5 M.

Objective

This in vitro assay was performed to evaluate the ability of Gadoterate meglumine (G 449.06) to induce gene mutation in V79 Chinese hamster cells with and without metabolic activation. The mutations were detected at the HPRT locus by 6-thioguanine selection.

Key findings

Under the conditions of this study, Gadoterate meglumine (Gadoteric acid; G 449-06) was considered negative for inducing mutagenic effects in V79/HPRT test system in the presence or in the absence of metabolic activation. No cytotoxicity occurred and the frequency of mutation was similar to what was observed in the negative controls in the absence or in the presence of activation. The positive controls produced expected results and a statistically significant ($p < 0.001$) and reproducible increase in the frequency of mutation was observed in cells exposed to positive controls. Based on the results, Gadoterate meglumine was considered negative for inducing mutagenic effects in V79/HPRT test system in the presence or in the absence of metabolic activation.

Methods

Cell line: Chinese hamster ovary cells (b) (4)

Concentrations in definitive study: 0 (distilled water), 0.33, 1.00, 3.33, 10.00, and 16.66 mmol/L (corresponding to 184, 558, 1859, 5583 and 9300 mg/L of Gd-DOTA, respectively).

Basis of concentration selection: Doses were selected based on a preliminary (range finding study) cytotoxicity study with and without metabolic activation

Negative control: Only cells and either culture medium or S-9 mix

Positive control: Mutagenic agents:

Without activation: N-Methyl-N-nitro-N-nitrosoguanidine (MNNG) (0.02 mmol/l dissolved in Dimethylsulfoxide, lot No. 123 300 59, (b) (4)

With activation: Dimethylnitrosamine (DMN; 23 mmol/L, lot No. 10427094, (b) (4)

Formulation/Vehicle: dissolved in water)
Distilled water (Test article), DMSO or distilled water and positive controls)

Incubation & sampling time: Cytotoxicity (preliminary assay) and mutagenicity tests:
Continuous treatment with test article (0.33 – 16.66 mmol/L for 2 hours at 37⁰C without metabolic activation and 4 hours with activation.

Cytotoxicity test

Cells were exposed at 37⁰C to half-log concentrations (0.33-16.66 mmol/L corresponding to 184, 558, 1859, 5583 and 9300 mg/L) of Gd-DOTA, respectively for 2 hours without activation and for 4 hours with activation. After the medium was removed and fresh medium added, cultures were re-incubated at 37⁰C. After 24 hours the cultures were examined for morphological alterations and confluence. 24 hours post-treatment, cultures were trypsinized and seeded for cloning efficiency (100 cells/dish and 6 dishes/concentration). After 7 days of growth at 37⁰C, clones were fixed in methanol and stained with Giemsa for counting.

Mutagenicity test

Cells were exposed at 37⁰C 0.33-16.66 mmol/L of Gd-DOTA for 2 hours without activation and for 4 hours with activation. Cells were subsequently treated under the same culture system as in the cytotoxicity test. Simultaneously, negative (untreated cultures) and positive (MNNG and DMN without and with activation, respectively) controls were made. In the 6 days of expression time, cultures were replated twice and then trypsinized and seeded: 100 cells/dish (6 dishes per concentration) to estimate the number of surviving cells. After 7 days of incubation at 37⁰C, the clones were fixed with methanol and stained with Giemsa for counting. 250,000 cells /dish (10 dishes/concentration) for mutant cell selection. The day after seeding, the cells were exposed to 6-thioguanine (5 µg/mL culture medium) during 10±1 days. The clones were fixed in methanol and stained with Giemsa for counting. 2 assays were carried out with and without activation on separate occasions.

Study Validity

For negative controls, the spontaneous mutation rate should range below 40 mutants per 10⁶ cells. Positive controls should cause an approximately 10-fold or greater increase in mutation frequency. The test substance was classified as mutagenic if it induced reproducibly at one of the test concentrations a mutation frequency 3-fold higher than the spontaneous mutant frequency in the experiment.

Results

The results of the study are summarized in the following sponsor's Tables (Tables 101 and 102).

Table 101: Gene mutation assay in V79 Chinese hamster cells – Assay 1 (DGD-1-13-A)

Assay 1						
Metabolic activation	Test Article	Concentration (mmol/L)	Cloning efficiency ^c	Number of clones/100 plated cells	SurvivorsX100	Mean TGR ^b clones/plate
Without Activation	H ₂ O	-	100/100 ^a	60/87 ^a	1500/2175 ^a	1.6/2.7 ^a
	DMSO	-	112/90 ^a	67/78 ^a	1675/1950 ^a	1.3/1.5 ^a
	Gadoteric acid	0.33	163	98	2450	0.8
		1.00	106	92	2300	0.2
		3.33	160	96	2400	2.3
		10.00	70	61	1525	2.3
		16.66	120	104	2600	0.0
	N-Methyl-N-nitro-N-nitrosoguanidine	0.02	66/88	44/69 ^a	1100/1725 ^a	141/2.5 ^a
With Activation	H ₂ O	-	100/100 ^a	100/94 ^a	2500/2350 ^a	0.5/0.1 ^a
	Gadoteric acid	0.33	88	82	2050	1.8
		1.00	70/93 ^a	70/87 ^a	1750/2175 ^a	1.8/1.1 ^a
		3.33	71/80 ^a	71/76 ^a	1775/1900 ^a	0.8/1.8 ^a
		10.00	55	51	1275	1.7
		16.66	58	58	1450	1.5
	Dimethylnitrosamine	23	36/69 ^a	36/65 ^a	900/1625 ^a	56.6/56 ^a

^a: Assay 1 with and without metabolic activation was conducted on two separate occasions, due to contamination during the 7 day-incubation period.

^b: TGR – 6-thioguanine resistant.

^c: Cloning efficiency – expressed as percentage of the cloning efficiency (percentage of viable clones among the 100 plated cells) in negative control. 0.33, 1.00, 3.33, 10.00, 16.66 mmol/L correspond to 184, 558, 1859, 5583 and 9300 mg of gadoteric acid/L, respectively (Mw=558.25).

Table 102: Gene mutation assay in V79 Chinese hamster cells – Assay 2 (DGD-1-13-A)

Assay 2						
Metabolic activation	Test Article	Concentration (mmol/L)	Cloning efficiency ^c	Number of clones/100 plated cells	SurvivorsX100	Mean TGR ^b clones/plate
Without Activation	H ₂ O	-	100	95	2375	0.4
	DMSO	-	106	101	2525	0.2
	Gadoteric acid	0.33	100	95	2375	1.5
		1.00	115	109	2725	1.3
		3.33	113	107	2675	0.4
		10.00	102	97	2425	0.9
		16.66	86	82	2050	0.8
	N-Methyl-N-nitro-N-nitrosoguanidine	0.02	56	57	1425	77.7
With Activation	H ₂ O	-	100	89	2225	0.6
	Gadoteric acid	0.33	64	57	1425	1.9
		1.00	76	67	1675	2.6
		3.33	131	116	2900	0.2
		10.00	91	81	2025	1.1
		16.66	98	87	2175	1.0
	Dimethylnitrosamine	23	56	50	1250	43.0

^b: TGR – 6-thioguanine resistant.

^c: Cloning efficiency – expressed as percentage of the cloning efficiency (percentage of viable clones among the 100 plated cells) in negative control.

0.33, 1.00, 3.33, 10.00, 16.66 mmol/L correspond to 184, 558, 1859, 5583 and 9300 mg of gadoteric acid/L, respectively (Mw=558.25).

Conclusion

No cytotoxicity occurred and the frequency of mutation was similar to what was observed in the negative controls in the absence or in the presence of activation. The positive controls produced expected results and a statistically significant ($p < 0.001$) and reproducible increase in the frequency of mutation was observed in cells exposed to positive controls. Based on the results, Gadoterate meglumine was considered negative for inducing mutagenic effects in V79/HPRT test system in the presence or in the absence of metabolic activation.

Reviewer's comment

I agree with the sponsor's conclusion.

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

7.3.1 Report No. DGD-1-11-A

Study Title: Micronucleus test in mice

Report No: DGD-1-11-A

Study report location: eCTD Module 4 §4.2.3.3.2.1

Conducting laboratory and location: (b) (4)

Date of study initiation: April 26, 1988

GLP compliance: Yes (x), No (), page 3 of 22

QA statement: Yes (x), No (), page 4 of 22

Drug, lot #, and % purity: Gadoteric acid (*G 449.06), lot # 203, ~100%

*G 449.06 is a colorless pharmaceutical solution containing gadolinium (1,4,7,10 tetraazacyclododecane N, N', N'', N''' tetraacetic acid) at a concentration of 0.5 M.

Objective

The *in vivo* micronucleus test was performed to evaluate potential clastogenic activity of Gadoterate meglumine (G 449.06) when administered as a single intravenous dose in CD-1 mice.

Key findings

Under the conditions of this study, Gadoterate meglumine did not induce an increase in the number of micronuclei and can therefore be considered as a non-clastogenic compound in the *in vivo* micronuclei test in mice.

Methods

Doses in definitive study: 0 (vehicle), 1.5, 2.5 and 3.5 mmol/kg (or 1.2, 2.0 and 2.8-fold-MHD)

Frequency of dosing: Single dose

Route of administration: Intravenous

Dose volume: 3, 5 and 7 mL/kg (corresponding to the dose levels 1.5, 2.5 and 3.5 mmol/kg), respectively

Formulation/Vehicle: Aqueous solution

Species/Strain: Mice / (b) (4)

Number/Sex/Group: 5/sex

Age: Initial age (5 weeks)

Satellite groups: none

Basis of dose selection: A preliminary study was performed using 7 mL/kg of the test article (G449.06, Gadoterate meglumine) corresponding to 3.5 mmol/kg. This was the maximum administration volume

Negative control: 0.9% NaCl

Positive control: Cyclophosphamide (50 mg/kg) (CPA; batch No. 298, (b) (4) dissolved in distilled water at a concentration of 7.14 mg/mL)

Study Validity

No statement was made on study validity. However, the study appeared valid for the following reasons: 1) there are reports of previous pharmacokinetic assessments of Gadoterate meglumine. 2) A preliminary study was performed using 7 mL/kg of the test article (G449.06, Gadoterate meglumine) corresponding to 3.5 mmol/kg and dosing at the levels indicated appeared adequate. 3) The preparation of the test substance was acceptable. 4) The species and number of animals/sex/group were acceptable. 5) the controls utilized in the study are valid and the positive control, Cyclophosphamide, exhibited appropriate responses. 6) Tissue sampling and analysis were acceptable.

Exposure conditions

Five mice per sex from each of the negative control and the Gadoterate meglumine (Gadoteric acid) groups were sacrificed at 24, 48 and 72h after treatment. Positive control animals (5/sex) were sacrificed 24h after treatment.

Analysis

Number of replicates: Not applicable

Sampling time: Bone marrow sampling took place at 24, 48 and 72 h after dosing

Cells evaluated: Polychromatic (PCE) and Normochromatic (NCE) erythrocytes in bone marrow. At each sampling time values from the treated group were compared to the simultaneous vehicle control group. Male and females values were pooled

Counting method: The bone marrow from the femur was flushed and smears were taken on slides. 2000 polychromatic erythrocytes (PCE) per animal were then evaluated for incidence of micronuclei and 1000 red blood cells per animal were counted for determination of the ratio of polychromatic to all erythrocytes.

Criteria for positive results: The following criteria were established for a positive response: a statistically significant result ($p < 0.05$) for which the median number of micronucleated polychromatic erythrocytes per 1000 cells evaluated should not be less than 5 or 3 for 24 hour and 48 hour sampling, respectively. For the solvent control group, the median number of micronucleated polychromatic erythrocytes per 2000 cells should not exceed 4 or 2 for 24 hour and 48 hour sampling, respectively.

Statistical evaluation: Statistical evaluation was conducted for proportion of micronucleated PCE or NCE and for PCE/NCE ratio. Statistical analysis of bone marrow cytotoxicity was made using the Student's t-test.

Results

A summary of bone marrow findings is given in the following sponsor's Table:

Test Article	Dose (nmol/kg)	No. of Animals/ sampling time	Mean % MN ^a ± SD			PCE/NCE ratio ^b		
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Vehicle	0	5M/5F	0.14 ± 0.12	0.13 ± 0.13	0.17 ± 0.16	1.5 ± 0.4	1.1 ± 0.3	1.1 ± 0.2
Gadoteric acid	1.5	5M/5F	0.20 ± 0.12	0.22 ± 0.12	-	0.8 ± 0.7	1.2 ± 0.3	-
	2.5	5M/5F	0.09 ± 0.09	0.12 ± 0.16	-	1.3 ± 0.3	1.0 ± 0.3	-
	3.5	5M/5F	0.12 ± 0.14	0.16 ± 0.07	0.11 ± 0.09	1.5 ± 0.6	1.1 ± 0.2	1.2 ± 0.2
Cyclophosphamide	Unknown	5M/5F	4.56 ± 2.51***	-	-	1.0 ± 0.2 ^{§§}	-	-

Kastenbaum and Bowman test: ***p<0.001 Student's t test: ^{§§}p<0.01

^a: Micronucleated polychromatic erythrocytes in 100 polychromatic erythrocytes.

^b: Polychromatic erythrocytes/normochromatic erythrocytes

Negative controls: In the negative control group, the mean values of micronucleated polychromatic erythrocytes (MPE) per 1000 polychromatic erythrocytes (PE), i.e. MPE / PE ratio were 1.4, 1.3 and 1.7 (or 0.14, 0.13 and 0.17 per 100 PEs), respectively at 24, 48 and 72 hours post-treatment

Cyclophosphamide positive control groups: At 24 hours post-treatment, a statistically significant increase ($p < 0.001$) in the number of micronuclei compared to the negative control groups was 45.6 ± 25.1 vs. 1.4 ± 1.2 per 1000 or 4.56 ± 2.51 (%) was observed. This was an indication of the sensitivity of the assay under the study conditions.

Gadoterate meglumine-treated groups: In all the test article treated groups, the MPE / PE values were similar to those of the respective negative controls at each sampling time and no differences were noted.

Ratio of polychromatic to normochromatic red blood cells (RBCs): The ratio of polychromatic to normochromatic RBCs significantly decreased ($p < 0.01$) in the positive control groups, an indication of the toxicity of Cyclophosphamide on bone marrow cells. However, in all treated groups, the ratio of polychromatic to normochromatic RBCs was not significantly different from that of the respective negative controls.

Conclusion

Under the conditions of this study, Gadoterate meglumine did not induce an increase in the number of micronuclei and can therefore be considered as a non-clastogenic compound in the in vivo micronuclei test in mice.

Reviewer's comment

I agree with the sponsor's conclusion.

7.4 Other Genetic Toxicity Studies

(SEE IMPURITIES)

8 Reproductive and Developmental Toxicology

Table 103: Overview of Reproductive and Developmental Toxicology

Type of Study / Report No.	Species	Route of Admin.	Dose(s) (mmol/kg)	GLP (Yes/No)	Reviewed (Yes/No)
Fertility & Early Embryonic Development					
99.12.803 (non-pivotal), DRF	Rat	IV	0, 2, 4, 8	Yes	No
99.12.804 (Segment I/II)	Rat	IV	0, 2, 4, 10	Yes	Yes
Embryo-fetal Development					
DGD-1-8-A (Teratology study - Segment II)	Rat	IV	0, 0.2, 0.4, 0.8	Yes	Yes
DGD-1-6-A (Embryotoxicity/Teratogenic effect)	Rabbit	IV	0, 0.2, 0.4, 0.8	Yes	Yes
99.12.802 (Embryotoxicity)	Rabbit	IV	0, 1, 3, 7	Yes	No
99.12.801 (Embryotoxicity (non-pivotal))	Rabbit	IV	0, 1, 3, 6	Yes	No
Pre- and Post-natal Development					
DGD-1-8-A (Teratology study - Segment II)	Rat	IV	0, 0.2, 0.4, 0.8	Yes	Yes

Reviewer's Table based on sponsor's data; IV = intravenous; DRF = Dose Range Finding study

8.0.0 Summary of Reproductive and Developmental Toxicology

i). Fertility and early embryonic development: In a study to evaluate the effect of Gadoterate meglumine on fertility and early embryonic development, Gadoterate meglumine was administered intravenously to rats at the doses of 2, 4 and 10 mmol/kg/day (or 3.2, 6.5 and 16-fold the clinical dose adjusted for body surface area, respectively). Males were treated for 63 days prior to mating and throughout mating while females were treated 2 weeks prior to mating and throughout mating until gestation day (GD) 17.

Renal tubular vacuolation was observed in rats in all the treatment groups. Pale and enlarged kidneys were observed in males and females administered the mid and high dose levels. There was a reduction in the rate of body weight gain during gestation in the 4 and 10 mmol/kg dose groups.

Based on these findings, the no-observed-adverse-effect-level (NOAEL) in this study was established as 2 mmol/kg (or 3.2-fold the human dose based on body surface area) for general toxicity in F₀ males and females and F₁ litters and ≥ 10 mmol/kg (or 16.2-fold human dose) for fertility and reproductive performance. Overall, there were no adverse effects on fertility or reproductive function/performance. There was no evidence of teratogenic effects following the daily intravenous administration of Gadoterate meglumine to male and female rat fetuses.

In rabbits, the NOAEL for F₀ females (dams) was 0.8 mmol/kg/day (or 2.6x-MHD) and for F₁ litters NOAEL was 0.8 mmol/kg/day (or 2.6x-MHD). There was no evidence of maternotoxicity, embryotoxicity or teratogenicity in rabbits.

ii). Pre- and postnatal development in the rat: Gadoterate meglumine did not appear to have any effect on breeding performance, fertility or reproductive performance in the F₁ litters. It did not demonstrate any adverse effects on the progress and outcome of pregnancy or on the development of the F₁ litters in the period of organogenesis. At the high dose of 0.8 mmol/kg (or 1.3-fold MHD) administered to F₀ dams, Gadoterate meglumine caused a slight toxic effect notably on body weight of the F₁ generation hence the NOAEL for F₀ dams was determined as 0.4 mmol/kg/day (or 0.7-fold MHD).

8.1 Fertility and Early Embryonic Development

8.1.1 Report No. 99.12.804

Study Title: Gadoterate meglumine (Dotarem) - Combined fertility and embryo toxicity study by the intravenous route in the rat (Segments I and II)

Study no.: 848.078-D

Study report location: eCTD Module 4 §4.2.3.5.2.1

Conducting laboratory and location:  (b) (4)

Date of study initiation: January 14, 2000

First dose: (males - April 17, 2000; females – June 5, 2000)

GLP compliance: Yes (x), No (), page 6 of 102

QA statement: Yes (x), No (), page 8-9 of 102

Drug, lot #, and % purity: Gadoterate meglumine (Dotarem), batch No. 00M018, 100%

Objective

The purpose of this study was to evaluate the effects of gadoterate meglumine Gd-DOTA) on gonadal function, mating behavior, reproductive performance and embryonic development in the rat when administered from before mating, during mating and until the end of organogenesis.

Key findings

Renal tubular vacuolation was observed in rats in all the treatment groups. Pale and enlarged kidneys were observed in males and females administered the mid and high dose levels. There was a reduction in the rate of body weight gain during gestation in the 4 and 10 mmol/kg dose groups. Based on these findings the NOAEL level in this study was established as 2 mmol/kg or 3.2-fold the human dose based on body surface area for general toxicity in F₀ males and females and F₁ litters and ≥ 10mmol/kg (or 16.2-fold human dose) for fertility and reproductive performance. Overall, there were no adverse effects on fertility or reproductive function/performance. There was also no evidence of teratogenic effects following the daily intravenous administration of Gadoterate meglumine to male and female rat fetuses.

Methods

Doses: 0, 2, 4 and 10 mmol/kg **

Frequency of dosing: Males (63 days prior to mating and throughout mating)
Females (2 weeks prior to mating, throughout mating until GD17)

Dose volume: 20 (control), 4, 8, and 20 mL/kg/day (for control, low, mid and high doses, respectively)

Route of administration: intravenous

Formulation/Vehicle: Aqueous solution of Gadoterate meglumine in sterile saline (0/9% NaCl), Vehicle supplied by (b) (4)

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

Number/Sex/Group: 25/sex/group
Males (6 weeks at the start of treatment: body weight range – 161 – 201 g)
Females (9 weeks at the start of treatment: 204 – 255 g)

Satellite groups: None

Study design: See Table 104 below

Deviation from study protocol: The study conformed to the original protocol no. 848/078-D and amendment nos. 1 and 2. Deviations were noted on page 29 of 102. The deviations were not considered to have affected study integrity or outcome

** The doses used in this study were selected based on the findings of a dose-range finding study (Report No. 99-12-803) in rats

Methods

Dose selection: The doses were selected based on the findings of a preliminary dose-range finding study (Report No. 99-12-803) in rats in which Gadoterate meglumine was administered intravenously to SD rats (8/sex) at the dose levels of 0, 2, 4 and 8 mmol/kg/day (or 3.2, 6.5 and 13-fold the clinical dose adjusted for body surface area, respectively). There was minimal toxicity in males namely, minor effects on body weight and food consumption. Based on a margin of safety (NOAEL) of 8 mmol/kg/day (males only), a high dose of 8 mmol/kg/day was or slightly higher for the main study was considered feasible.

Study Design

Gadoterate meglumine was administered intravenously to 25 rat/sex/group (Table 104) 63 and 14 days for males and females, respectively throughout the mating period and up to the day prior to necropsy for males and until gestation day 17 (GD17) for females.

Table 104: Study Design and Dose groups (99.12.804)

Groups (Gd-DOTA was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg/day)	Number of rats	
		Males	Females
1 (vehicle)	0	25	25
2 (LD)	2	25	25

3 (MD)	4	25	25
4 (HD)	10	25	25

Source: Reviewer's Table adapted from Sponsor's Study Design veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gd-DOTA (Gadoterate meglumine)

Table 105: Gadoterate meglumine Human Dose multiples (99.12.804)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg)	0	2	4	10
Dose (mmol/m ²)	0	12	24	60
Dose multiples (based on BSA)	N/A	3.2x	6.5x	16.2x

Parameters and endpoints evaluated: mortality, clinical signs, body weight, food consumption, gross pathology, reproductive organ weights and histopathology.

Males were necropsied after completion of the mating period, testes and epididymides weighed and used for sperm analysis. Histopathological examination was performed on kidneys with macroscopic lesions.

Female specific data includes estrus cycles, time to insemination, number of corpora lutea, implantations, live embryos, and resorptions. Caesarean section was performed in inseminated females on GD20, ovaries and gravid uteri weighed, and litter parameters recorded. Fetuses were examined for external, visceral and skeletal abnormalities.

Observations and Results

Observations

Survival, clinical signs, body weight and food consumption were recorded in all animals in the pre-mating, mating and postmating and gestation period (Table 106). Details are provided in the respective sections.

Table 106: Summary of Methods (99.12.804)

Protocol	Method, frequency and/or objectives
Clinical observations	Twice/day for signs of mortality and morbidity; Once/day for clinical signs to detect any abnormalities in behavior, appearance or toxicity due to treatment
Body weight	Twice/week (males); Females (twice/week in the pre-mating and mating periods (only pre-mating data are reported since the majority of rats mated within four days) and on days 0, 6, 11, 15, 18 and 20 of gestation
Food consumption	Males (weekly until mating); Females (weekly until mating then on days 0 to 6, 6 to 11, 11 to 15, 15 to 18 and 18 to 20 of gestation)
Mating	Rats were paired (1M/1F) from the same group for a maximum of 21 days. Vaginal smears were taken daily from the females during cohabitation and mating was

	confirmed by the presence of sperm in a vaginal smear or a vaginal plug. The presence of sperm was recorded and taken as day 0 of gestation (G0). Mated females were separated from the males once mating had been confirmed and smearing ceased
Necropsy	CO ₂ /exsanguination; Males were necropsied after a successful mating period. Females were sacrificed after caesarean section on GD20 and females with undetected mating were sacrificed 21 days after the first day of pairing Body weight was recorded before necropsy and animals macroscopically examined for structural or pathological changes. Ovaries and uterus were removed and examined including the placentae Other examinations were performed according to SOP
Organ weights	SOP
Histopathology	SOP
Dose formulation analysis	Yes (x), No (); CoA page 335 of 354 (Addendum 2)

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Animals were sacrificed by excess CO₂ inhalation and exsanguination (Details in Table 106 above). Males of all groups were sacrificed after completion of the mating period. Dams were sacrificed on gestation day 20. All animals were examined macroscopically for pathological changes. Parental necropsy observations were described as group incidences and group percentages. The number of corpora lutea, the number and uterine position of implantation sites, viable and dead fetuses and early and late resorptions were recorded.

Results

Mortality

Three males in the high dose group (10mmol/kg/day) died on days 70, 41 and 77. Deaths were attributed to convulsion after mating on day 70, and dosing trauma on day 41 and local injection site reactions on day 77, respectively. There were no deaths in the control, low and mid dose groups.

Clinical Signs

No test article-related clinical signs were observed in males. A moderate necrosis of the tail was observed in the high dose group.

Body Weight

Compared to controls, there was a significant ($p < 0.05$) decrease (-3.7 g) in the mean body weight of high dose males in the first 2 weeks of treatment. The low and mid dose groups were not significantly affected. There were no remarkable changes in the body weight of females on day 63.

Feed Consumption

There was a dose-related reduction in food consumption in males throughout the mating period and maternal food consumption was significantly reduced in the high dose group throughout the gestation period

Mating performance and fertility

There were no adverse effects of treatment on mating performance and fertility. All surviving animals of both sexes and in all groups mated within 4 days (or 1 normal estrus cycle). All females were pregnant with one exception each in the control, 2 and 10 mmol/kg groups.

Toxicokinetics

Not performed

Dosing Solution Analysis

Test solutions were used as provided to the conducting laboratory by the sponsor.

Necropsy

Pale and/or enlarged kidneys were observed in all males treated at the high dose level, in half of animals administered the mid dose and in 2 males administered the low dose. Two high dose females had pale kidneys.

Organ weights

The weight of the testes, epididymides and ovaries was unaffected by treatment. Gravid uterine weight was significantly reduced ($p < 0.01$) at the high dose. There were no other remarkable changes in organ weight.

Histopathology

Large vacuoles were present in the renal proximal tubular cells. There was a dose-related increase in severity of vacuolation. There were no test article-related adverse effects in the testes of males that did not induce a pregnancy.

Sperm analysis

There were no treatment-related findings. Sperm counts and motility were not adversely affected. No other treatment-related necropsy findings were reported

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Pre-implantation Loss, etc.)

The following reproductive indices were calculated as percentages: Female mating index (No. of inseminated females vs. No. of paired females), Male mating index (No. of males with copulation vs. No. of paired females), Female fertility index (No. of pregnant females vs. No. of inseminated females), Male fertility index (No. of males inducing pregnancy vs. No. of males with copulation), Pre-implantation loss (No. of corpora lutea minus No. of implantation vs. No. of corpora lutea) and Post-implantation loss (No. of implantations minus No. of viable fetuses vs. No. of implantations). Data for these indices are presented in Tabular form in the appropriate sections.

Mortality, clinical signs, body weight change, food consumption data, mating and sperm analysis data, Testes and epididymides weight and kidney vacuolation in males is summarized in the following sponsor data (see Table 107 below):

Table 107: Male Fertility and early embryonic development to implantation data (99.12.804)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	2 (LD)	4 (MD)	10 (HD)
Mortality				
No. evaluated	25	25	25	25
No. dead/moribund	0	0	0	3 (12%)
Clinical observations				
Necrosis of tail	✓	-	-	✓✓
Necropsy				
Pale/enlarge kidneys	-	✓	✓✓	✓✓✓
Body weight (%)				
Day 11	249.7 g [@]	0	-0.4 ↓	-.3.7* ↓
Day 63	431.0 g [@]	+1.4 ↑	0	-0.5 ↓
Food consumption (%) in wk 9	30.3g/animal/day	-2.0 ↓	-6.9 ↓	-12.2 ↓
Mean no. of days before mating	2.5	3.5	2.7	2.4
No. males mated	24 (96%)	24 (96%)	25 (100%)	25 (100%)
No. of fertile males	23 (92%)	23 (92%)	25 (100%)	24 (96%)
Sperm analysis, Testes weight, epididymides weight	-	-	-	-
Kidney vacuolation	-	✓✓	✓✓✓	✓✓✓

Reviewer's Table based on sponsor data (Table 2.6.7.12, Page 1 of 4, Tabulated summaries, Table 3_10_00102); ✓, ✓✓ or ✓✓✓ = mild, moderate or marked; - = No noteworthy findings; * = p < 0.05; @ = control group mean value; Statistical differences were based on actual data not on percentages; ↑, ↓ = increased or decreased

Mortality, clinical signs, body weight change, food consumption data, implantation data, ovary and gravid uterine weight is summarized in the following sponsor data (Table 108 below):

Table 108: Female Fertility & early embryonic development (99.12.804)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	2 (LD)	4 (MD)	10 (HD)
Mortality				
No. evaluated	25	25	25	25
No. dead/moribund	0	0	0	0
Clinical observations				
Subdued behavior	-	-	-	✓✓✓
Necropsy				
Pale/enlarge kidneys	-	-	-	✓
Body weight (%)				
Pre-mating body weight (%)	247.2g [@]	0	0	0
Gestation body weight (%)	411.6 g [@]	0	-1.0 ↓	-5.5** ↓
Food consumption				
Pre-mating food consumption (%)	21.6g/animal/day	+1.9 ↑	+1.4 ↑	-8.3 ↓

	@			
Food consumption for G0-G6	24.6g/animal/day @	0	-4.9 ↓	-17.1** ↓
Gestation food consumption (%)	30.2g/animal/day @	0	-1.0	-3.0 ↓
Fertility/Mating/post-mating data				
Mean No. of days before mating	2.5	3.5	2.7	2.4
No. of females sperm positive	24 (96%)	24 (96%)	25 (100%)	25 (100%)
No. of pregnant females	23 (92%)	23 (92%)	25 (100%)	24 (96%)
No. Aborted/No. of total litter resorptions	0	0	0	0
Avg. No. of corpora lutea	16.4	16.3	16.3	15.5
Avg. No. of implantations	15.8	15.5	15.5	14.2* ↓
Mean pre-implantation loss (%)	3.5	5.3	4.9	7.5
Mean No. live conceptions	15.1	14.6	14.7	13.3** ↓
Mean No. of resorptions	0.7	0.9	0.8	1.0
No. of dead fetuses	0	0	0	0
Mean post-implantation loss (%)	4.6	5.8	5.2	6.8
Ovary weight (g)	-	-	-	-
Weight of gravid uterus (g)	94.4	89.0	91.3	83.3** ↓
No. of litters	23	23	25	24
No. of live fetuses	347	335	367	318
No. of dead fetuses	0	0	0	0
Mean fetal body weight (g)	4.2	4.1	4.2	4.2
Fetal sex ratio (% males)	49.8	51.0	50.5	52.6

Reviewer's Table based on sponsor data (Table 2.6.7.12, Page 1 of 4, Tabulated summaries, Table 3_10_00102); ✓, ✓✓ or ✓✓✓ = mild, moderate or marked; - = No noteworthy findings; *, ** = $p < 0.05$; $p < 0.01$; @ = control group mean value; Statistical differences were based on actual data not on percentages; ↑, ↓ = increased or decreased

Pre-implantation: Mean number of pre-implantation sites was significantly ($p < 0.05$) lower in the high dose group compared to controls, according to the sponsor this value was greater than historical controls.

Post-implantation: The mean values of early resorptions and post-implantation loss were comparable across dose groups. Mean live litter size (live conceptions) was significantly lower ($p < 0.01$) in the high dose group compared to controls but greater than historical controls (sponsor information only; no historical data reviewed). There were no dead fetuses across the groups.

Fetal data: There were no remarkable effects on the fetal sex ratio due to treatment and the mean fetal weights were comparable across groups. There were no remarkable treatment-related gross or visceral fetal anomalies. Skeletal anomalies observed across dose groups included 1) parietal incomplete ossification of the cranium, 2) incomplete facial squamosal or zygomatic or mandibular ossification. Unossified or incomplete ossification of mandibular hyoid bone was also reported across dose groups.

Table 109: Fetal Skeletal Anomalies (99.12.804)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	2 (LD)	4 (MD)	10 (HD)
No. of litters evaluated	23	23	25	24
No. of live fetuses	347	335	367	318
No. of dead fetuses	0	0	0	0
Mean fetal body weight, g	4.2	4.1	4.1	4.1
Fetal sex ratios (% males)	49.8	51.0	50.5	52.6
Fetal Anomalies				
Gross external	-	-	-	-
Visceral Anomalies	-	-	-	-
Skeletal Anomalies				
Cranium: Parietal Incomplete Ossification				
No. fetuses (%)	2 (1.1)	3 (1.8)	8 (4.2)	12 (7.2)
No. of litters (%)	2 (8.7)	3 (13.0)	6 (24.0)	6 (25.0)
Facial: Squamosal Incomplete Ossification				
No. fetuses (%)	2 (1.1)	2 (1.2)	6 (3.2)	11 (6.6)
No. of litters (%)	2 (8.7)	1 (4.3)	5 (20.0)	7 (29.2)
Facial: Zygomatic Incomplete Ossification				
No. fetuses (%)	0 (0.0)	0 (0.0)	1 (0.5)	2 (1.2)
No. of litters (%)	0 (0.0)	0 (0.0)	1 (4.0)	2 (8.3)
Mandibular: Hyoid Unossified				
No. fetuses (%)	3 (1.7)	3 (1.8)	4 (2.1)	7 (4.2)
No. of litters (%)	2 (8.7)	3 (13.0)	3 (12.0)	4 (16.7)
Mandibular: Hyoid Incomplete Ossification				
No. fetuses (%)	1 (0.6)	4 (2.3)	5 (2.6)	0 (0.0)
No. of litters (%)	1 (4.3)	4 (17.4)	4 (16.0)	0 (0.0)

Reviewer's Table based on sponsor data (Table 2.6.7.12, Page 3 of 4, Tabulated summaries, Table 3_10 00102).

Discussion and Conclusions

Renal tubular vacuolation was observed in rats in all the treatment groups. Pale and enlarged kidneys were observed in males and females administered the mid and high dose levels. There was a reduction in the rate of body weight gain during gestation in the 4 and 10 mmol/kg dose groups.

Based on these findings the NOAEL level in this study was established as 2 mmol/kg or 3.2-fold the human dose based on body surface area for general toxicity in F₀ males and females and F₁ litters and ≥ 10mmol/kg (or 16.2-fold human dose) for fertility and reproductive performance.

Overall, there were no adverse effects on fertility or reproductive function/performance. There was also no evidence of teratogenic effects following the daily intravenous administration of Gadoterate meglumine to male and female rat fetuses.

Reviewer's comments

I agree with the study findings and conclusions.

8.2 Embryonic Fetal Development**8.2.1 Report No. DGD-1-8-A**

Study Title: Teratology study in the rat (Segment II)

Study no.: 88/LAG052/0037

Study report location: eCTD Module 4 §4.2.3.5.2.1

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: Date of first dose (April 15, 1987)

GLP compliance: Yes (x), No (); page 2 of 249

QA statement: Yes (x), No (); pages 4-5 of 249

Drug, lot #, and % purity: G 449.06 (Gadoterate meglumine drug substance),
batch No. 202, % purity – N/A

Objective

The purpose of this study was to evaluate the effects of intravenously administered Gadoterate meglumine on embryonic development during the organogenesis phase of pregnancy in the rat.

Key findings

Gadoterate meglumine did not appear to have had any effect on breeding fertility or reproductive performance in the F₁ litters. Gadoterate meglumine did not demonstrate any adverse effects on the progress and outcome of pregnancy or on the development of the F₁ litters in the period of organogenesis. At the high dose of 0.8 mmol/kg (or 1.3-fold MHD) administered to F₀ dams, Gadoterate meglumine caused a slight toxic effect notably on body weight of the F₁ generation hence the NOAEL for F₀ dams was determined as 0.4 mmol/kg/day (or 0.7-fold MHD).

Methods

Doses: 0 (control/saline vehicle), 0.2, 0.4 and 0.8 mmol/kg (i.e., 0.3, 0.7 and 1.3-fold the human dose adjusted for body surface area, respectively)

Frequency of dosing: Repeated doses (GD7 – GD17)

Dose volume: 1.6 (control/saline), 0.4, 0.8 and 1.6 (for low, mid and high doses levels, respectively)

Route of administration: Intravenous

Formulation/Vehicle: Aqueous solution

Species/Strain: Rat / CD of Sprague-Dawley origin [REDACTED] (b) (4)

Number/Sex/Group: F₀: 32 pregnant females/group were administered Gadoterate meglumine from GD7 - GD17 inclusive; 21 females/group were submitted for Caesarean section on GD20

Initial age: 10 weeks; Weight at Day 0: 208 – 246 g

Satellite groups: none

Study design: See Table 109 below

Deviation from study protocol: The study conformed to the original protocol. The 1st and

2nd amendments to the protocol and deviations were not considered to have affected study integrity or outcome

Methods

Dose selection

Basis of dose selection was not provided in the Report

Study Design

Table 110: Study Design and Dose groups (DGD-1-8-A)

Groups (G 449.06 was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg/day)	Number of rats
		Females only
1 (vehicle)	0	32
2 (LD)	0.2	32
3 (MD)	0.4	32
4 (HD)	0.8	32

Source: Reviewer's Table adapted from Sponsor's Study Design veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of G 449.06 (Gd-DOTA/ Gadoterate meglumine)

Table 111: Gadoterate meglumine human dose multiples (DGD-1-8-A)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg)	0	0.2	0.4	0.8
Dose (mmol/m ²)	0	1.2	2.4	4.8
Dose multiples (based on BSA)	N/A	0.32x (0.3x)	0.65x (0.7x)	1.297x (1.3x)

Reviewer's Table

The rats were dosed daily via the IV route from day 7 – day 17 (inclusive) of gestation (GD17). Control rats received sterile isotonic saline during the same treatment period. Animals in the F₁ generation were not dosed.

Observations and Results

Observations

Table 112: Summary of Methods – Segment II (DGD-8-A)

Protocol	Method, frequency and/or objectives
Pre-natal phase	
Clinical observations	Twice/day throughout the study for signs of reaction to treatment noting details of type, severity, time of onset and duration
Body weight	Maternal body weight was recorded on days 0, 3, 7-18 and 20 post-coitus
Food consumption	Food consumption was monitored regularly
Mating	Rats were paired 1F: 1M with males from the same strain.

	Each morning following pairing, the trays underneath the cages were checked for ejaculated copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which a sperm positive vaginal smear or at least 3 copulation plugs were found was designated Day 0 of gestation
Teratology phase	
Allocation	21/group were randomly selected for examination of uterine contents
Maternal examination	
Necropsy	<p>Animals were killed by inhaled CO₂ on day 20; Each animal was examined macroscopically for evidence of disease or adverse effects to treatment and specimen of tissues considered abnormal were retained in fixative</p> <p>The reproductive tract, complete with ovaries was dissected out to determine the number of corpora lutea, number of implantation sites, number of resorption sites (classified as early when there was no distinguishable fetus or late when a fetus could be recognized)</p> <p>Dissection performed according to SOP</p> <p>Body weight was recorded before necropsy and animals macroscopically examined for structural or pathological changes. Ovaries and uterus were removed and examined including the placentae</p>
Fetal examination	Other examinations were performed according to SOP
Post-natal (maternal phase)	Allocation, parturition and gestation, body weight record, maternal behavior, terminal examination of F ₀ females
Post-natal (Litter phase)	SOP (observations at day 1, clinical signs, mortality and litter size, adjustment for litter size, body weight, sex ratio, physical development. etc)
Dose formulation analysis	Yes (x), No (); subsection 3.8.2 page 231 of 249 Information on the homogeneity of mixing, stability and concentration of G 449 06 in the vehicle was determined by the Sponsor

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Results

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

F₀ generation

Mortality/Clinical Signs: No deaths or adverse clinical signs were reported.

Body Weight: No dose-related changes.

Feed Consumption: There was no increase in food consumption.

Toxicokinetics: Not performed

Necropsy: Necropsy of females on day 20 indicated no treatment-related macroscopic changes.

Litter responses: Pre/Post-implantation data including mean fetal and placental weights were not affected by treatment (see Table 113 below). There were no remarkable treatment-related fetal anomalies (F₁ litters from females sacrificed on GD20).

Table 113: The effect of Gadoterate meglumine on embryo-fetal development (DGD-1-8-A)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	0.2 (LD)	0.4 (MD)	0.8 (HD)
Dams (F₀)				
No. Pregnant	21	21	21	21
No. dead/sacrificed moribund	0	0	0	0
Clinical signs	-	-	-	-
Body weight (%)	332g	+3** ↑	+2.4* ↑	+2.1** ↑
Food consumption				
Food consumption (%)	-	-	-	-
Avg. No. of corpora lutea	16.0	16.8	16.2	16.9
Avg. No. of implantations	14.6	15.0	15.0	15.9
Mean pre-implantation loss (%)	9.5	10.2	7.9	6.2
F₁ litters (from females killed on GD20)				
No. of pregnant animals	21	21	21	21
Mean No. live conceptions	14.1	14.4	14.1	15.1
Mean No. of resorptions (early + late)	0.5	0.7	0.9	0.8
Mean post-implantation loss (%)	3.3	4.4	5.7	5.1
Mean fetal body weight (g)	3.31	3.34	3.31	3.26
Mean Placental weight (g)	0.50	0.50	0.51	0.51
Fetal sex ratio (% males)	53.0	45.7	42.2	55.8
Fetal anomalies				
Gross external, visceral and skeletal anomalies	-	-	-	-

Reviewer's Table based on sponsor data (Table 2.6.7.13A, Page 3 of 7, Tabulated summaries, Table 3_10_00103); - = no noteworthy findings; *, ** = p < 0.05; p < 0.01; ↑ = increase in value assessed

Offspring (Malformations, Variations, etc.)

Conclusions

At the high dose of 0.8 mmol/kg (or 1.3-fold MHD) administered to F₀ dams, Gadoterate meglumine caused a slight toxic effect notably on body weight of the F₁ generation hence the NOAEL was determined as 0.4 mmol/kg/day (or 0.7-fold MHD). In terms of its effect on F₀ females, Gadoterate meglumine did not appear to have had any effect on breeding performance, fertility or reproductive performance in the F₁ litters. The effect of Gadoterate meglumine on the F₁ generation was described under review of "Pre- and Postnatal Development Effects of Gadoterate meglumine" (Study DGD-1-8; Review Section 8.3 below).

Reviewer's comments

I agree with the findings

8.2.2 Report No. DGD-1-6-A

Study Title: G449-06 - Evaluation of the embryotoxic or possible teratogenic effects in the rabbit (intravenous route)

Report No.: DGD-1-6-A
 Study report location: eCTD Module 4 § 4.2.3.5.2.1
 Conducting laboratory and location: [REDACTED] (b) (4)
 Date of study initiation: November 10, 1986
 GLP compliance: Yes (x), No (); page 4 of 76
 QA statement: Yes (x), No (); page 5 of 76
 Drug, lot #, and % purity: G 449.06 (Gadoterate meglumine), batch No. 202, 100%

Objective

The purpose of this study was to evaluate the effects of Gadoterate meglumine on embryonic development during the organogenesis phase in rabbits

Key findings

This study evaluated the effect of repeated intravenous administrations of Gadoterate meglumine to pregnant rabbits on gestation day 6 through and including GD18 at three dose levels – 0.2, 0.4 and 0.8mmol/kg/day or (0.7, 1.3 and 2.6-fold MHD). There was no significant difference between the control group and the treated groups in terms of the number of corpora lutea and implantations, the number and mean weight of living fetuses, or the number of post-implantation losses and fetal abnormalities. NOAEL for F0 females (dams) was 0.8mmol/kg/day (or 2.6x-MHD) and for F1 litters NOAEL was also 0.8 mmol/kg/day (or 2.6 x-MHD). There was no evidence of maternotoxicity, embryotoxicity or teratogenicity.

Methods

Doses: 0 (control), 0.2, 0.4 and 0.8 mmol/kg (or 0.7, 1.3 and 2.7-fold of the human dose adjusted for BSA)
 Frequency of dosing: Repeated doses (GD6 – GD18 inclusive)
 Dose volume (mL/kg/day): Control (1.6), low dose (0.4), mid dose (0.8), high dose (1.6)
 Route of administration: Intravenous
 Formulation/Vehicle: Aqueous solution
 Species/Strain: Rabbit / New Zealand White/ [REDACTED] (b) (4)
 Number/Sex/Group: Initial age: 16-17 weeks
 Satellite groups: none
 Study design: See Table 114 below
 Deviation from study protocol: The study conformed to the original protocol. The amendment(s) to the protocol and deviations were not considered to have affected study integrity or outcome

Methods

Dose selection

Not provided

Study Design

Sixteen pregnant females per group received the test article (Gadoterate meglumine) daily for 7 days a week from gestation day (GD) 6 to GD18 inclusive (Table 114):

Table 114: Study Design and Dose groups (DGD-1-6-A)

Groups (Gd-DOTA was administered to groups 2, 3 and 4)	Intravenous dose (mmol/kg/day)	Number of Females
1 (vehicle)	0 (0.9% NaCl)	16
2 (LD)	0.2	16
3 (MD)	0.4	16
4 (HD)	0.8	16

Source: Reviewer's Table adapted from Sponsor's Study Design veh = vehicle; LD, MD, and HD = low, mid and high dose, respectively of Gd-DOTA (Gadoterate meglumine)

Table 115: Gadoterate meglumine human dose multiples (DGD-1-6-A)

Administration	Vehicle	Low Dose	Mid Dose	High Dose
Dose (mmol/kg)	0	0.2	0.4	0.8
Dose (mmol/m ²)	0	2.4	4.8	9.6
Dose multiples (based on BSA)	0	0.65x (0.7x)	1.29x (1.3x)	2.59x (2.6x)

Observations and Results

The procedures were performed on the pregnant females:

Table 116: Methods (DGD-1-6-A)

Protocol	Method, frequency and/or objectives
Artificial insemination	IV injection of 25 IU of chorionic gonadotrophic hormone (HCG, batch 6285 A, ^{(b) (4)} administered into the external marginal vein of the ear
Clinical observations & Mortality of pregnant rabbits	<u>Clinical signs</u> : Animals were observed daily for presence of clinical signs <u>Mortality</u> : Animals were observed 2x/day for dead or moribund animals
Body weight	Animals were weighed on gestation days (GD) 0, 5, 12, 18, 21, 24 and 28
Food consumption	Monitored regularly (frequency was not indicated)
Necropsy	Animals were sacrificed and caesarean section performed on GD28
Organ weights	At necropsy of dams, the ovaries and gravid uterus were weighed
Litter parameters	Litter parameters were recorded and fetuses examined for external,

	visceral and skeletal abnormalities
Dose formulation analysis	Yes (x), No (); Acceptable (page 10 of 76)

Source: Reviewer's Table constructed from sponsor's data. All abbreviations are standard clinical pathology terms.

Results

Table 117: Effects of Gadoterate meglumine on Embryofetal Development in Rabbits (DGD-1-6-A)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	0.2 (LD)	0.4 (MD)	0.8 (HD)
Dams (pregnant rabbits; GD28)				
No. Inseminated	16	16	16	16
No. Pregnant	13 (81.3%)	16 (100%)	15 (93.4%)	12 (75.0%)
No. dead/sacrificed moribund	0	0	0	1 (6.3%)
No. aborted or with Total resorption of litter	2 (15.4%)	1 (6.3%)	1 (6.7%)	0 (8.3%)
No. with complete gestation	11 (84.6%)	15 (93.4%)	14 (93.3%)	11 (91.7%)
Clinical observations	-	-	-	-
Necropsy observation	-	-	-	-
Body weight (%)	4.0 g	-2.5% ↓	-4.7% ↓	-4.7% ↓
Avg. No. of corpora Lutea	10.5	10.4	10.3	9.8
Avg. No. of implantations	6.5	8.7	9.0	7.3
Mean pre-implantation loss (%)	38.1	16.3	12.6	25.5
Litters				
No. of litters evaluated	11	15	14	11
Mean No. Live fetuses	74	130	118	70
Mean No. of Live fetuses/females with complete gestation	6.7	8.7	8.4	6.4
Mean No. of resorptions (early + late)	0.76	0.56	1.13	0.67
No. of dead fetuses	1	0	0	0
Mean post-implantation loss (%)	12.4	6.9	12.3	12.4
Mean fetal body weight (g)	40.7	38.6	37.8	39.7
Fetal sex ratio (% males)	58	52	54	46
Fetal anomalies				
Gross external, visceral and skeletal anomalies	-	-	-	-

Reviewer's Table based on sponsor data (Table 2.6.7.13B, Page 5 of 7, Tabulated summaries, Table 3_10_00103); - = no noteworthy findings; ↑, ↓ = increase or decrease in value assessed

Mortality / Clinical Signs

One animal in the high dose group (0.8mmol/kg/day) died. Macroscopic examination revealed blood in vagina, purulent lungs and autolysis in the liver. No abortions were reported in any study group.

Body Weight

The weight gain in study animals was comparable to that in the controls. The slight % decreases in weight between treated and controls were not statistically significant.

Feed Consumption

No remarkable test-article related effects

Toxicokinetics

Not performed

Dosing Solution Analysis

Acceptable

Necropsy**Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc)**

No remarkable test article-related effect. The mean number of corpora lutea and implantations per female were of the same magnitude for control and treated groups

Offspring (Malformations, Variations, etc)

Live fetuses: The average number of live fetuses per female and the rate of live fetuses were comparable between treated and respective control groups for females achieving implantation and females achieving complete gestation

Mean Litter weight: There was no statistically significant difference (Student's T-test) between the mean weights of treated groups and control group fetuses

Sex ratio: There were no statistically significant differences in the sex ratio between treatment and control groups

Variations: There were no remarkable findings

Evaluation of Teratogenic risk: There were no statistically significant differences between the treated and control group rabbits in the rate of females that died giving birth to fetuses having major or minor anomalies

Discussions and Conclusions

This study evaluated the effect of repeated intravenous administrations of Gadoterate meglumine to pregnant rabbits on gestation day 6 through and including GD18 at three dose levels – 0.2, 0.4 and 0.8mmol/kg/day or (0.7, 1.3 and 2.6-fold MHD). There was no significant difference between the control group and the treated groups in terms of the number of corpora lutea and implantations, the number and mean weight of living fetuses, or the number of post-implantation losses and fetal abnormalities. NOAEL for F0 females (dams) was 0.8 mmol/kg/day (or 2.6 x-MHD) and for F1 litters NOAEL was also 0.8 mmol/kg/day (or 2.6 x-MHD). There was no evidence of maternotoxicity, embryotoxicity or teratogenicity.

Reviewer's comments

I agree with the findings and conclusions of this study

8.3 Prenatal and Postnatal Development**8.2.3 Report No. DGD-1-8-A (continued)**

Introduction: No additional and specific study was conducted to evaluate pre- and postnatal development. However, in the Segment II teratology study in rats (DGD-1-8-A), a subset of females treated with Gadoterate meglumine from GD7 to GD 17 were allowed to spontaneously (naturally) delivered and rear their pups to weaning at day 25 post partum. The offspring were then examined for growth, behavior and neuromuscular function. Some male and female offspring were paired to investigate the reproductive performance of the F₁ generation. Following is a review of the pre- and postnatal development section of study DGD-1-8-A. Details of methods and study design were reviewed under study DGD-1-8-A.

Study title: Teratology study in the rat (Segment II) continued...

Note: Emphasis is on the "Pre- and postnatal development" aspects of Report No. DGD-1-8-A)

Study no.:	88/LAG052/0037
Study report location:	eCTD Module 4 §4.2.3.5.2.1
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	Date of first dose (April 15, 1987)
GLP compliance:	Yes (x), No (); page 2 of 249
QA statement:	Yes (x), No (); pages 4-5 of 249
Drug, lot #, and % purity:	G 449.06 (Gadoterate meglumine drug substance), batch No. 202, % purity – N/A

Key findings

The test article Gadoterate meglumine was not maternotoxic at any of the doses tested on the F₀ dams.

Results indicated no test article-related effect on sensory function in F₁ generation and their mating and reproductive performances were unaffected by the treatment of F₀ females (F₁ generation were not treated with the test article). However, at the high dose of 0.8 mmol/kg (or 1.3-fold the human dose) induced a slight toxic effect on body weight on F₁ generation. Also at the high dose, there was a reduction in offspring survival and mean litter size and mean body weight. NOAEL was therefore considered as 0.4 mmol/kg (or 0.3-fold the human dose).

Considering the entire study comprising segment II (embryotoxicity and Teratology) and segment III (pre- and post development effects), there appeared to be no evidence that Gadoterate meglumine had any adverse effects on F₀ females, breeding performance, or teratogenicity. The fertility and the reproductive performance of the F₁ generation rats were also not affected.

F₀ Dams

Survival:	No treatment-related deaths in F ₀ dams
Clinical signs:	No adverse clinical signs were reported
Body weight:	There was a slight but significant increase (2.1 to 3%) compared to controls
Feed consumption:	No increase in food consumption
Uterine content:	See review of DGD-1-8-A report above
Necropsy observation:	See review of DGD-1-8-A report above
Toxicokinetics:	Not performed
Dosing Solution Analysis	Yes (x), No (); subsection 3.8.2 page 231 of 249
Other:	None

Note: Results for the F₀ generation (21 females) were presented in under DGD-1-8-A above. The remaining 11 of 32 females (study DGD-1-8-A) delivered spontaneously and reared their pups to weaning at day 25.

Table 118: Summary of Methods – Pre-/postnatal development (DGD-18-A)

Protocol	Method, frequency and/or objectives
F₀ females (allowed to deliver spontaneously)	Number = 11 females
Parturition/Duration of Gestation	From GD20, females were inspected 3x each weekday and 2x at weekends for onset, progress and completion of parturition Gestation length = day of mating to day of parturition
Body weight	Recorded on days 1, 4, 7, 11, 14, 18, 21 and 25 post-partum
Maternal behavior	2x/day for evidence of abnormal behavior
Terminal examination of F ₀ females	After weaning or total litter death, F ₀ females were killed by inhaled CO ₂ . All females were subjected to macroscopic external and internal examination. No. of uterine implantation sites were recorded and specimens of abnormal tissues collected and fixed
Litters	
Postnatal observations	Observations at day 1 post-partum: No. born alive or dead / individual body weight of live offspring / individual sexes / observation of individual offspring Litters were then observed daily for evidence of abnormal behavior or appearance
Mortality and litter size	Mortality was recorded daily; litter size adjusted on day 4 post-partum to 8 (4M+4F, where possible)
Body weight	Individual body weight of offspring was recorded on days 1, 4 (before culling), 7, 11, 14,

	18, 21 and 25 post-partum
Sex ratio	On days 1, 4 (before and after culling), 14, and 25 post-partum; sexes were reported for days 1, 4 and 25
Physical development	According to SOP (including but not limited to auditory and visual function, general activity, learning ability, neuromuscular function)
Termination of unselected offspring	After selecting offspring to continue study, unselected offspring were terminated at approximately 8 weeks of age
Post-weaning observation of offspring (F₁ generation)	
Clinical signs	Daily
Body weight	Weekly
Mating	9-10 weeks; Selected F ₁ male and female offspring were mated and the reproductive performance of the F ₁ generation was evaluated
Termination of F ₁ animals	F₁ females (Day 20 post-coitum; inhaled CO ₂ ; uterine contents examined; fetuses discarded after macroscopic external examination) F₁ males (inhaled CO ₂ ; examined externally and internally for macroscopic anomalies and specimens fixed)

Source: Reviewer's Table constructed from sponsor's data.

Results

F₀ Females (i.e. 11 of 32 females that delivered spontaneously)

At necropsy of the pregnant F₀ dams, ovaries and gravid uterus were weighed, litter parameters recorded and fetuses examined for visceral and skeletal anomalies. F₁ generation rats were not dosed with Gadoterate meglumine.

Results revealed no deaths, abortions/litter resorptions in F₀ females or remarkable clinical signs in the subset (11 of 32) F₀ females allowed to deliver spontaneously. There were no effects on lactation body weight, mean number of implantations, mean % implantation loss, mean litter size, live birth index or viability index (see Table 119 below).

Table 119: Effect of Gadoterate meglumine on Pre- and Postnatal development (Maternal data, DGD-18-A)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	0.2 (LD)	0.4 (MD)	0.8 (HD)
F₀ females (allowed to deliver spontaneously)				
No. Pregnant	11	11	11	11
No. dead/sacrificed moribund	0	0	0	0
No. aborted with total resorptions of litter	0	0	0	0
Clinical signs	-	-	-	-
Lactation body weight (%)	301g [@]	-3.7	0	0

Mean implantation sites	14.8	13.8	15.7	15.8
Mean Litter size on lactation day 25	6.3	7.6	7.5	6.5
Mean post-implantation loss (%)	9.0	12.0	5.0	5.0
Avg. No. of implantations	14.6	15.0	15.0	15.9
Live birth index (%)	96	100	96	90
Viability index (%)	82	97	90	62

Reviewer's Table based on sponsor data (Table 2.6.7.14, page 2 of 4, Tabulated summaries, Table 3_10_00104); - = No noteworthy findings; @ = control group mean value; Statistical differences were based on actual data not on percentages; ↑, ↓ = increased or decreased

(F₁ Offspring)

1. Neuromuscular function: Offspring were examined for growth, behavior and neuromuscular function and some males and females paired to investigate reproductive performance of the F₁ generation. Results indicated no test article-related effect on sensory function. There was however a slight effect at the high dose (0.8mmol/kg) on decreased motor activity.

2. Offspring survival and mean litter size: There was a reduction in offspring survival and mean litter size in the high dose group (0.8 mmol/kg or 1.3-fold MHD) on day four (117.0 vs. 92.0). Differences were not statistically significant compared to controls even though values were lower than historical controls.

3. Mean body weight: In the high dose (0.8 mmol/kg/day) group offspring, there was a lower mean body weight (a 7.4 % decrease) when compared with controls until mid lactation. The difference was not statistically significant.

4. Mean body weight in F₁ generation: In the high dose group, there was a lower mean body weight in male and female offspring designated F₁ generation compared to controls up to 5 weeks (see Table 120 below). Thereafter until the end of study body weight was not affected. The mating and reproductive performance of the F₁ generation was not affected by the treatment of F₀ females.

5. F₁ mating and reproductive performance: The mating and reproductive performances of the F₁ generation were unaffected by the treatment of F₀ females (F₁ generation were not treated with the test article).

Table 120: The effect of Gadoterate meglumine on Postnatal Development (DGD-1-8-A)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	0.2 (LD)	0.4 (MD)	0.8 (HD)
F₁ Litters (Pre-weaning data)				
Mean Body weight (%) on lact day 25	65.3 @	+1.7 ↑	-4.6 ↓	-7.4 ↓
Mean No. of live fetuses on lact day 4 (before litter size adjustment)	117	129	142	92 ↓
Mean No. of live fetuses on lact day 4 (after litter size adjustment)	77	85	80	56 ↓
Mean No. of live fetuses on lact day 25	69	84	75	52 ↓
Fetal sex ratio (% males) on lact day 25	53.6	47.6	48 ↓	51.9 ↓
Sensory function	-	-	-	-
Decreased motor activity	-	-	-	+
Necropsy observations	-	-	-	-
Body weight of selected animals (%)				

Males	313g [@]	0	0	-4.8 ↓
Females	201g [@]	-1.5	-2.0	-5.5 ↓

Reviewer's Table based on sponsor data (Table 2.6.7.14, Page 3 of 4, Tabulated summaries, Table 3_10_00104); lact day = lactation day; - = No noteworthy findings; [@] = control group mean value; Statistical differences were based on actual data not on percentages; ↑, ↓ = increased or decreased

Table 121: The effect of Gadoterate meglumine Postnatal (F₁ males) Development (DGD-1-8-A)

Parameters	Gadoterate meglumine (Daily dose in mmol/kg)			
	0 (control)	0.2 (LD)	0.4 (MD)	0.8 (HD)
F₁ Males (Post-weaning data)				
No. evaluated post-weaning	20	20	20	20
Premating body weight change (g)	368	356	371	373
Animals mating within 1-4 days (%)	10 (90)	20 (100)	17 (85)	18 (90)

Reviewer's Table based on sponsor data (Table 2.6.7.14, Page 3 of 4, Tabulated summaries, Table 3_10_00104)

Post-weaning F₁ females: In post-weaning F₁ females, compared to controls, there were no remarkable test article related changes in: pre-mating body weight (g), gestation body weight (g), number of pregnant females, necropsy observations, mean number of corpora lutea, mean number of implantations, mean % pre-implantation loss, mean number of live fetuses, mean number of resorptions, placental weight (g), F₂ fetal body weights (g) and F₂ fetal sex ratio (% males).

Conclusions

The sponsor concluded that Gadoterate meglumine administered at the high dose of 0.8 mmol/kg (or 1.3-fold the human dose) induced a slight toxic effect on body weight on F₁ generation and NOAEL was considered as 0.4 mmol/kg (or 0.3-fold the human dose).

When the entire study comprising segment II (embryotoxicity and Teratology) and segment III (pre- and post development effects) are taken together, there appeared to be no evidence that Gadoterate meglumine had any adverse effects on F₀ females, breeding performance, fertility and the reproductive performance of the F₁ generation rats. Gadoterate meglumine also did not show any evidence of teratogenic effects.

Reviewer's comments

I agree with the findings and conclusions of this study

9 Local Tolerance

9.1 Overview

An overview of local tolerance studies conducted is shown in Table 122:

Table 122: Overview of Local Tolerance Studies

Type of Study / Report No.	Species	Route of Admin.	Dose(s) (mmol/kg)	GLP (Yes/No)	Reviewed (Yes/No)
Acute subcutaneous Toxicity: DGD-1-14-A	Rat	SC	0, 2.5	Yes	No
Acute intramuscular Toxicity: DGD-1-15-A	Rat	IM	0, 2.5	Yes	No
Local Tolerance in the rabbit by the intravenous, perivenous and intra-arterial routes: DGD-33-003	Rabbit	IV, PV, IA	0.9, 0.9, 0.25	Yes	Yes

Reviewer's Table based on sponsor's data; SC, IM, IV, PV, IA = subcutaneous, intramuscular, intravenous, perivenous or intra-arterial administration

9.1.1 Summary of local tolerance studies

Studies were performed in rats to evaluate the local tolerance of Gadoterate meglumine (2.5mmol/kg) following subcutaneous or intramuscular injection, respectively. Macroscopic and microscopic changes at the injection site were similar between control and test article-treated animals on days 1 and 3.

Transient local inflammatory reactions (hematoma, edema and mononuclear/polynuclear cell aggregation) were seen at 6 hours post-dose. Gadoterate meglumine was well tolerated in rats after a single subcutaneous or intramuscular injection.

In another study conducted in rabbits, Gadoterate meglumine was administered at 0.9, 0.9 and 0.25 mmol/animal via intravenous, intra-arterial or perivenous routes respectively. Microscopic findings at the different sites of administration in control and Gadoterate meglumine-treated rabbits included in varying degrees erythema, hemorrhagic infiltration, epidermal vacuolation, presence of inflammatory cells, ulceration of the outer skin and perivascular hemorrhage. The findings were consistent with histopathological findings following the intravenous administration of Gadoterate meglumine in single-dose and repeat-dose toxicity reports.

9.1.2 DGD-33-002**Study Title: Local tolerance in the rabbit by intravenous, perivenous and intra-arterial routes**

Report location: eCTD Module 4 §4.2.3.6.1
 Conducting laboratory and location: (b) (4)
 Study #: 848/098-D
 Date of study initiation: June 24, 2002 (day 0)
 GLP compliance: Yes (x), No ()
 QA report: Yes (x), No ()
 Drug, lot #, and % purity: Gadoterate meglumine, Batch No. 99M077, Purity: considered 100%
 Animal species/strain/sex per dose: Rabbit / New Zealand White, NZW, (b) (4) / 20 males
 Age: Age at initiation of treatment: 10-11 weeks
 Weight: Weight at initiation of treatment: 2.130-2.385 g
 Doses: 0.9, 0.9 and 0.25 mmol/animal for intravenous (IV), intra-arterial (IA) or perivenous (PV), respectively
 Duration/route: Single. Routes - IV, IA, PV

Objective

The purpose of the study was to objective of the study was to evaluate the local tolerance of Gadoterate meglumine in the rabbit following a single intravenous, perivenous and intra-arterial administration.

Key findings

The microscopic findings at the different sites of administration in control and Gadoterate meglumine-treated rabbits included varying degrees of erythema, hemorrhagic infiltration, epidermal vacuolation, presence of inflammatory cells, ulceration of the outer skin and perivascular hemorrhage. These findings were mostly reversible and appeared consistent with histopathological findings following the intravenous administration of Gadoterate meglumine in single-dose and repeat-dose toxicity reports. The intravenous route of administration was predominantly employed for Gadoterate meglumine in the animal studies and has been proposed for the clinical use of Gadoterate meglumine.

Methods

The study was conducted according to the following design. Other protocols are described in Table 123.

Table 123: Study Design and Dose Groups (DGD-33-002)

Group	Treatment	Route of Admin		Dose (mmol/kg)		No. of rabbits sacrificed	
		Left Ear	Right Ear	Left Ear	Right Ear	24h p.i	96h p.i
1	Saline	IV	PV & IA	0	0 (IV)	5	5
2	Gadoterate meglumine	IV	PV & IA	0.9	0.25 (PV) 0.9 (IA)	5	5

ulceration of the overlying skin and perivascular hemorrhage. There was a slight irritation that was not accompanied by any signs necrosis. The injection site findings, except for edema in one animal, were no longer present at 96 h (4 days)

Conclusions

Based on the results, the sponsor concluded that the microscopic findings at the sites of administration in both control and Gadoterate meglumine-treated rabbits were associated with the administration procedure and were unrelated to treatment. The microscopic findings at the different sites of administration in control and Gadoterate meglumine -treated rabbits included in varying degrees erythema, hemorrhagic infiltration, epidermal vacuolation, presence of inflammatory cells, ulceration of the outer skin and perivascular hemorrhage.

Reviewer's comments

I do not agree that the microscopic findings at the injection sites were associated with the administration procedure and appear unrelated to treatment. The findings appeared consistent with histopathological findings following intravenous administration of Gadoterate meglumine in single-dose and repeat-dose toxicity reports. In view of the fact that local injection site reactions were observed in control and treatment groups, a proportionately greater number of Gadoterate meglumine-treated animals (9/10) than controls (2/10) showed local injection site reactions. This disproportion in incidence may indicate a treatment-related effect not easily explained as effects due to mechanical injury alone. The intravenous route of administration was predominantly employed for Gadoterate meglumine in the animal studies and has been proposed for the clinical use of Gadoterate meglumine.

10 Special Toxicology Studies

10.1 Immunotoxicity

10.1.3 Report No. DGD-33-003

Study Title: Gadoterate meglumine, evaluation of the potential to induce immediate hypersensitivity-induced anaphylactic shock and passive cutaneous anaphylaxis in the guinea pig	
Study no.:	848/096
Study report location:	eCTD Module 4; § 4.2.3.7.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 12, 2002
GLP compliance:	Yes (x), No (), page 4 of 102
QA statement:	Yes (x), No (), pages 5-6 of 102
Drug, lot #, and % purity:	Gadoterate meglumine, batch No. 99M077, purity considered 100%

Objective

The purpose of this study conducted using the guinea pig was to evaluate the potential of Gadoterate meglumine to induce immediate hypersensitivity and passive cutaneous anaphylaxis.

Key findings

Anaphylactic shock and increased antibody titers were observed in the positive control animals but not in guinea pigs that were administered Gadoterate meglumine. Therefore under the conditions of this study, the test article Gadoterate meglumine did not induce any immediate hypersensitivity reactions.

Methods	
Doses:	Induction: 0.25 and 0.5 mmol/animal (SC, IV) Challenge: 0.5 mmol/animal (IV)
Frequency of dosing:	Induction: Single dose on days 0 and 7 Challenge: Single dose days 26 and 33
Route of administration:	SC, IV
Dose site:	Described in methods
Dose volume:	Described in methods
Formulation/Vehicle:	Gadoterate meglumine / Sterile saline (0.9% NaCl; Batch #: CVG20A), (b) (4)
Negative control:	0.9% saline
Positive control:	Ovalbumin ((batch #: 106H7070) (b) (4)
Adjuvant:	Aluminium hydroxide wet Gel(type VAC 30), (b) (4) batch No. 0105069
Species/Strain:	Guinea pigs / (b) (4)
Number/Sex/Group:	Anaphylactic shock phase: 46 males Passive cutaneous anaphylaxis phase: 48 males
Age:	Young adult (at first dose)
Weight:	Anaphylactic shock phase: 336 – 412 g Passive cutaneous anaphylaxis phase: 392 -497 g
Deviation from study protocol:	No significant deviations

Study Design

The assay consisted of an induction phase (sensitization), a rest phase and a challenge phase

Anaphylactic Shock Phase

Animals were allocated to study groups as shown in the following sponsor Table (Table 126):

Table 126: Study Design (Anaphylactic Phase) (DGD-33-003)

Group number	Induction (days 0 and 7)*		Challenge (days 26 and 33)		Number of males
	Treatment	Route	Treatment	Route	
1	Negative control item	SC	Test item - High dose	IV	10
2	Test item - Low dose	SC	Test item - High dose	IV	10
3	Test item - High dose	SC	Test item - High dose	IV	10
4	Test item - High dose	IV	Test item - High dose	IV	10
5	Positive control item	SC	Positive control item	IV	6

Sponsor's Table: On day 0 only adjuvant (Aluminium hydroxide wet gel – Al(OH)₃) was administered by the subcutaneous route just prior to the administration of the negative or positive control or test article.

The assay consisted of a semi-quantitative measurement of the anaphylactic shock antibodies (IgG1a and IgE) produced in the serum of the animals used in the anaphylactic shock phase after the induction phase.

Induction Phase:

The study design for the induction phase is shown in the following sponsor's Table (Table 127):

Table 127: Study Design (Induction Phase: DGD-33-003)

Group number	Treatment (days 0 and 7)*	Dose concentration	Dose volume (ml per animal)	Dose level (per animal)
1	Negative control item	0.9%	1	0
2	Test item - Low dose	0.5 mmol/ml	0.5	0.25 mmol
3	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
4	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
5	Positive control item	4 µg/ml	0.5	2 µg

Sponsor's Table; Negative control = Saline; Positive control = Ovalbumin; on day 0 only, adjuvant (aluminium hydroxide wet gel, $\text{Al}(\text{OH})_3$) was also administered by the subcutaneous route just before administration of the negative or positive control or the test item; Subcutaneous route (shaved retroscapular area) was used for groups 1-3 and 5 and intravenous (into the vein of the penis) for group 4; Frequency of administration: on day 0 (with adjuvant, see above) and day 7 (alone, without adjuvant).

Blood sampling

3ml whole blood was withdrawn from the retro orbital sinus following ketamine HCl + Xylazine HCl anesthesia via the IM route. Serum samples were obtained, frozen and retained for the passive cutaneous anaphylaxis test

Challenge Phase

Table 128: Study Design (Challenge Phase: DGD-33-003)

Group number	Treatment (days 26 and 33)	Dose concentration	Dose volume (ml per animal)	Dose level (per animal)
1	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
2	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
3*	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
4*	Test item - High dose	0.5 mmol/ml	1	0.5 mmol
5 *	Positive control item	200 µg/ml	1	200 µg

*On day 27, group 3 males were sacrificed for ethical reasons due to incidental injuries. Only one group 5 male was treated on day 33, the others having died on day 26 after the first challenge. The IV route was used.

Observations and Results

Observations

Table 129: Summary of Methods (DGD-33-003)

Protocol	Method, frequency and/or objectives
Morbidity/mortality	Twice/day at the beginning and at the end of the day; Moribund or dead animals were killed and necropsied
Clinical observations	All animals were examined within 1 h following IV injection of in the challenge phase and 3h later; All clinical signs were recorded including signs of anaphylactic shock
Body weights	Prior to initiation of treatment on SD0, on the day of blood sampling (day 21) and on the days of challenge (days 26 and 33)
Blood collection	As previously described
Necropsy	According to SOP

Reviewer's Table based on sponsor data

Results

Anaphylactic shock phase:

Mortality: 5 of 6 positive control group animals died a few min (3-6 min) after IV injection after the first challenge on day 26 due to anaphylactic shock. 3 animals were sacrificed in treatment groups 3 and 4 for ethical reasons due to incidental injuries. A description of injuries was not provided

Clinical signs: After the first challenge on day 26, typical signs of anaphylactic shock were observed (convulsions, labored breathing, and retching). The surviving positive control animal showed convulsions, labored breathing, retching and chewing, piloerection and prostration. After the second challenge on day 33, the surviving positive control animal showed minor signs of sneezing, nose licking and major signs of labored breathing, retching, and lacrimation between 12 min and 3h or longer. No clinical signs were observed in the negative controls or test article groups after the 1st or 2nd challenge.

Body weight

Body weight gain occurring between days 0 and 21 was lower in the positive control group and in the group treated with test item at the high dose (group 4), when compared with the negative control group.

Necropsy

No necropsy findings were reported

Passive cutaneous anaphylactic (PCA) phase

Mortality: No mortality was reported in this phase

Results of the PCA assay showed that there were positive cutaneous reactions in animals administered the positive control. No reactions were seen in animals that received Gadoterate meglumine.

Conclusions

Anaphylactic shock and increased antibody titers were observed in the positive control animals but not in guinea pigs that were administered Gadoterate meglumine. Therefore, under the conditions of this study, the test article Gadoterate meglumine did not induce any immediate hypersensitivity reactions.

Reviewer's Comments

The design of the study is acceptable. However, I do not fully agree with the sponsor's conclusion that only the mortality (5/6 animals) observed in positive control animals due to anaphylactic shock, was notable. Three animals were sacrificed in treatment groups 3 and 4 for ethical reasons due to incidental injuries. Since a description of the injuries was not provided, it is difficult to understand the ethical basis to support sacrificing these animals.

10.2 Nephrogenic Systemic Fibrosis (NSF)

10.2.1 Introduction to the clinical syndrome of NSF

General: Nephrogenic systemic fibrosis (NSF) is a recently described, serious, fibrotic and highly debilitating disorder most frequently described in patients with end-stage renal disease or acute renal failure. While NSF primarily affects the skin, other organ compartments may also be involved. Although the etiology of NSF is not fully understood, exposure to gadolinium-

containing contrast agents has been associated with the onset of symptoms of NSF. Considerable progress towards a better understanding of this disease has occurred since the first association between the clinical use of GBCAs and NSF was made (Grobner et al., 2006). Consequently, there are several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate < 30 mL /min/1.73m²). The use of GBCAs is currently contraindicated in such individuals. The commonly reported hypothesis for the involvement of gadolinium in the pathomechanism of NSF is the in vivo dechelation of GBCAs. Although all GBCAs have the potential to release gadolinium ion (Gd³⁺), which in the context of renal failure, is an important trigger for NSF, such a release is thought to more readily occur among the less stable gadolinium chelates. However, the genesis of NSF may be multifactorial (Sieber et al., 2009) and other triggers or co-triggers may play a role in its pathogenesis. A definitive diagnosis of NSF requires deep skin biopsy and histopathology. No effective treatment is currently available for NSF and prevention of disease is of key epidemiological importance. Current understanding seems to reasonably indicate that Gadolinium complexes play a causative role in the pathophysiology of nephrogenic systemic fibrosis or nephrogenic fibrosing dermopathy. Still, the exact pathogenesis and the risk for patients are unclear beside the obvious connection to moderate to severe renal insufficiency (Bongatz, 2007). The author also expressed the opinion that pharmacoepidemiological and preclinical studies will allow a better understanding of the pathophysiology and role of the various MR contrast agents in the near future (Bongatz, 2007).

Progress has been made towards a better understanding of this disease since the first association between the clinical use of GBCAs and NSF was made in 2006. Consequently, there have been several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate [GFR] <30 mL/min/1.73m²).

Table 130: Gadolinium-Based Contrast Agents (GBCAs)

Product		NDA	Sponsor	Approval Yes (Year)
Trade Name	Established Name			
Magnevist	Gadopentetate dimeglumine	N 019-596 N 021-037	Bayer	Yes (2000)
ProHance	Gadoteridol	N 020-131 N 021489	Bracco	Yes (1992)
Omniscan	Gadodiamide	N 020-123 N 022-066	GE Healthcare	Jan (1993)
OptiMARK	Gadoversetamide	N 020-937 N 020-975 N 020-976	Covidien	Dec (1999)
MultiHance	Gadobenate dimeglumine	N 021-357 N 021-358	Bracco	Nov (2004)
Eovist/Primovist	Gadoxetate disodium	N 022-090	Bayer	Jul (2008)
Ablavar/Vasovist	Gadofosveset trisodium	N 021-711	Lantheus	Dec (2008)
Gadavist	Gadobutrol	N 201-277	Bayer	March (2011)

Gadoterate meglumine	Gadoterate	N204-781	Guerbet	NDA submission
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Table adapted from “Post-marketing Safety Evaluation of Gadolinium-Based Contrast Agents” for MRI by Dr. Lucie Yang

Table 131: Classification of GBCAs Based on Structure and Charge

Trade Name	Established Name	Structure	Charge
Omniscan	Gadodiamide	Linear	Nonionic
OptiMARK	Gadoversetamide	Linear	Nonionic
Magnevist	Gadopentetate dimeglumine	Linear	Ionic
MultiHance	Gadobenate dimeglumine	Linear	Ionic
Eovist	Gadoxetate disodium	Linear	Ionic
Ablavar/Vasovist	Gadofosveset trisodium	Linear	Ionic
ProHance	Gadoteridol	Macrocylic	Nonionic
Gadovist	Gadobutrol	Macrocylic	Nonionic
Gadoterate meglumine	Gadoterate	Macrocylic	Ionic

Table adapted from “Postmarketing Safety Evaluation of Gadolinium-Based Contrast Agents” for MRI by Dr. Lucie Yang

10.2.2 Published Nonclinical Studies on NSF

According to the sponsor, nonclinical studies were conducted by the Research Department of Guerbet in collaboration with academic institutions with the aim of obtaining a better understanding of the NSF disease mechanism. Two published articles (Fretellier et al, 2011a, b) were reviewed.

10.2.3 Summary of NSF Publications

Two published articles (Fretellier et al, 2011a, b) were reviewed to provide a better understanding of the disease mechanism in Nephrogenic Systemic Fibrosis (NSF).

In the first study, following intravenous injection in rats with impaired renal function, neither Gadoterate meglumine nor gadodiamide induced macroscopic skin lesions, in contrast with nonformulated gadodiamide which was associated with systemic toxicity. It was concluded that the stable gadoterate was associated with less pathologic and biochemical effects than linear ionic compound, gadodiamide.

In the second study, the levels of dissociated versus chelated Gadolinium (Gd) in plasma, skin, and bone was assessed in Wistar rats with impaired renal function following intravenous administration of Gadoterate meglumine, Gadodiamide (Omniscan) or nonformulated gadodiamide (without excess caldiameter ligand). Omniscan and gadodiamide, unlike Gadoterate meglumine induced microscopic skin lesions renally-impaired rats. A higher Gd concentration was observed in the skin and bone (femur) in Omniscan- and nonformulated gadodiamide-treated rats when compared to rats treated with Gadoterate meglumine.

10.2.3.1 Fretellier et al (2011a) - Clinical, Biological, and Skin Histopathological Effects of Ionic Macrocytic and Nonionic Linear Gadolinium Chelates in a Rat Model of Nephrogenic Systemic Fibrosis, *Investigative Radiology*, 46(2):85-93.

Introduction: This study was designed to compare an ionic, macrocyclic GBCA (Gadoterate; Gadoterate meglumine) - a compound with high thermodynamic and kinetic stabilities, with the nonionic and linear compound, Gadodiamide (with low thermodynamic and kinetic stabilities). Gadodiamide was tested in its formulated and non-formulated (with free ligand, Caldiamide) forms. Using a rat model of impaired renal function, the compounds were compared with respect to their clinical, histopathologic, biochemical, and hematological effects. The aim of the nephrectomy was to induce chronic kidney disease in the rat.

Objective: The purpose of this study was to compare the clinical, pathologic, and biochemical effects of repeated administrations of ionic macrocyclic or nonionic linear gadolinium chelates (GC) in rats with impaired renal function.

Key findings:

Following intravenous injection in rats with impaired renal function, neither Gadoterate meglumine nor gadodiamide induced macroscopic skin lesions, in contrast with nonformulated gadodiamide which was associated with systemic toxicity. It was concluded that the stable gadoterate was associated with less pathologic and biochemical effects than linear ionic compound, gadodiamide.

Methods

Animal model:

Animal species/strain/sex:

Rat/Wistar/males

Source:

(b) (4)

Age:

Adult (6 weeks)

Weight:

235 ± 23 g

Subtotal (5/6) nephrectomy (SNx): All rats underwent surgery for subtotal (5/6) nephrectomy (SNx). Briefly, the right kidney of rats anesthetized with ketamine and Xylazine, was exposed through a flank incision. The adrenal gland was separated from the upper pole, and the kidney decapsulated. The renal pedicle (or renal helium) was ligated and the right kidney removed. The left kidney was also decapsulated and the adrenal gland separated from the upper pole. The upper and lower poles were ligated and the poles excised. Saline (1.0 mL/kg) was administered IV on completion of surgery to compensate for blood loss.

Test articles:

1. Gadoterate meglumine (Dotarem)

0.5M, Batch No. 8GD051B (Guerbet, Villepinte, France)

2. Gadodiamide (Omniscan)

0.5M. Batch No. 10758384

(b) (4)

- | | |
|------------------------------|--|
| 3. Nonformulated Gadodiamide | 0.495 M (Without the 25nM excess ligand, Caldiamide; Synthesized at Guerbet Research Department) |
| 4. Normal Saline | 5.0 mL/kg/rat |

Study design and dose groups:

Two main studies were performed: (1) A Short-Term comparative study of Gadoterate vs. Gadodiamide and (2) A Long-Term Histopathologic study.

The comparative study of Gadoterate vs. Gadodiamide was conducted as follows:

Table 132: Short-Term Study Design and Dose groups (Fretellier et al, 2011a)

Group	Dose (mmol/kg/day) x 5 days	No. of Rats
1. Saline (5.0ml/kg)	0	7
2. Gadoterate meglumine	2.5	8
3. Gadodiamide (Omniscan)		8
4. Nonformulated Gadodiamide		10

Reviewer's Table based on sponsor's data in Fretellier et al (2011); Saline or GBCAs were administered IV via the tail vein at the rate of 1.0 ml/min/day for 5 consecutive days starting 10 days after subtotal nephrectomy surgery. All injections and blood samples were performed under anesthesia (Isoflurane/O₂).

The rats were administered saline or 2.5 mmol/kg of each Gd-based contrast agent intravenously for 5 consecutive days starting 10 days after the SNx surgery. The 10-day post surgical period was defined after a preliminary study in which 10 SNx rats were compared with 4 sham-operated rats. The creatinine clearance of the SNx rats was significantly ($p < 0.05$) reduced by 66% when compared with the sham-operated rats. The creatinine clearance in SNx rats (0.38 - 0.48 mL/min/100g) was stable throughout the study. The creatinine clearance in sham-operated rats was 1.0 – 1.2 mL/min/100 g.

Sacrifice: Rats were sacrificed by exsanguination under isoflurane anesthesia 11 days after the first administration.

Biochemistry and Hematology:

Blood samples were obtained from the sublingual vein on days 0, 3, 9 and 11 and the following parameters were measured: Erythrocyte, Leukocyte and Platelet counts; Hematocrit and Hemoglobin concentration; Total calcium, phosphorus, transferrin-bound iron, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine, Sodium, Chloride, and Potassium. All assays were performed in duplicate.

Immunochemistry:

- C-reactive protein levels were measured by Enzyme-linked Immunosorbent Assay (ELISA) before the first injection and on day 3.
- Plasma levels of cytokines (interleukin-1- β ; IL1- β ; monocyte chemotactic protein-1; MCP-1; tumor necrosis factor- α ; TNF α and transforming growth factor β 1; TGF β ₁) were measured by ELISA on days 3 and 9.

- Plasma levels of fibroblast growth factor-23 (FGF-23) was measured on day 11. The FGF-23 levels of SNx rats fed with a high phosphate diet (1.0%) and sham-operated rats on normal diet were also measured at day 35 post-surgery. The plasma phosphorus levels in these rats were 3.9 (n=2) and 2.3 ± 0.4 mmol/L, respectively.

Macroscopic and microscopic examination of the skin:

Rats were checked for macroscopic skin changes before the first injection and thereafter daily until the end of the study. On day 4, the back of the rats was shaved for better visualization of potential lesions. The back and abdominal areas were shaved again at the end of the study. 2 cm² skin samples were taken from the backs of the rats in areas considered normal and areas with lesions.

Skin samples were fixed in 4% neutral buffered formalin, dehydrated, embedded in paraffin and sectioned (5 μ m thick). Staining was with hematoxylin-eosin-saffron (for topographic analysis), Picrosirius red (to detect collagen), and Alcian blue (for acidic mucopolysaccharides).

Immunohistochemical staining was performed to detect CD34 and TGF β ₁-positive cells using anti-CD34 goat antibody (diluted 1:1000) and anti-TGF β ₁ rabbit antibody (diluted 1:250).

Blinded histopathologic examinations were performed. Pathologic lesions including distribution of thin or thick collagen fibers in the extracellular matrix and CD34 and TGF β ₁-positive cells were examined semi-quantitatively using a 5-point severity grading scale ranging from 0 (absent) to +++ (very severe) in each rat.

Long-Term Histopathologic study:

To determine long-term histopathologic effects, 10 Wistar rats aged 6 weeks and weighing 222 ± 17 g were administered intravenously once per day for 5 consecutive days with saline (2 rats), or 2.5 mmol Gd/kg body weight Gadoterate (4 rats) and Gadodiamide (4 rats), beginning 10 days after SNx nephrectomy.

Table 133: Long-Term Study Design and Dose groups (Fretellier et al, 2011)

Group	Dose (mmol/kg/day) x 5 days	No. of Rats
1. Saline (5.0ml/kg)	0	2
2. Gadoterate meglumine (Dotarem)	2.5	4
3. Gadodiamide (Omniscan)	2.5	4

Reviewer's Table based on sponsor's data in Fretellier et al (2011); Saline, Gadoterate or Gadodiamide were administered IV for 5 consecutive days starting 10 days after subtotal nephrectomy surgery. All injections were performed under anesthesia (Isoflurane/O₂).

Skin biopsies, using a 6 mm diameter biopsy punch, were taken on days 4, 11, 18 and 25 following the first injection, under isoflurane/O₂ anesthesia. Wounds were sutured and the samples fixed in 4% neutral buffered formalin for histological analysis. The rats were sacrificed as previously described (exsanguination under isoflurane anesthesia) 32 days after the first administration of test articles.

Statistical Analysis: Data were expressed as mean \pm SD.

Results

Short-Term Study (Comparative Gadoterate vs. Gadodiamide Study; Table 134 below):

Nonformulated gadodiamide group: Skin lesions (scabs and ulcerations in the neck, back and abdominal skin were observed in 4/10 rats treated with nonformulated gadodiamide on day 5 (i.e. 5 days after the first dose) for 2 rats and on day 9 for another 2 rats. In this group, a total of 8/10 rats died or were sacrificed for ethical reasons notably severity of skin lesions or loss of body weight. No skin scratching was observed.

Table 134: Macroscopic lesions and other symptoms after treatment (Short-Term Study)

Group	No. of Rats	No. of Rats alive at End of study (day 11)	No. of Rats with skin lesions	Other symptoms
1. Saline (5.0 ml/kg)	7	7	0	-
2. Gadoterate meglumine	8	8	0	-
3. Gadodiamide (Omniscan)	8	8	0	-
4. Nonformulated Gadodiamide	10	2 (5 rats euthanized for ethical reasons; 3 rats died on Day 3, 7 & 9)	4 Scab formation & ulceration starting on Day 5	Prostration, piloerection, loss of body weight

Reviewer's Table adapted from Fretellier et al (2011) page 87

Saline, Gadoterate and Gadodiamide groups: There were no macroscopic lesions in the rats administered saline, Gadoterate meglumine (Dotarem) or Gadodiamide (Omniscan). No significant differences in body weight changes were observed between these groups. Mean body weight was 287.8 g on day 1 and 270.6 g on day 11.

Histopathology: Few microscopic lesions were observed in Gadoterate meglumine -treated rats or in saline controls.

Biochemistry and Hematology: No significant differences were observed between the groups for plasma Na, K, Cl, ALT and AST levels. Plasma phosphorus decreased in gadodiamide-treated rats compared with controls on day 9. Plasma phosphorus was also significantly lower in the gadodiamide group compared to Gadoterate meglumine -treated animals. There was a decrease in plasma iron levels in the nonformulated gadodiamide group. No changes were observed in other groups. No changes in hematology parameters were observed except an increase in WBC count in the nonformulated group when data for day 1 and day 11 were compared. There were no significant changes in plasma C-reactive protein on day 3 between the groups. Plasma IL1- β was significantly higher in the nonformulated gadodiamide group when compared with the other test groups on day 3 and significantly higher on day 9 compared to gadodiamide group. There was a significant increase in plasma MCP-1 in the nonformulated gadodiamide group on days 3 and 9. The test compounds did not alter TGF β 1 and TNF α plasma levels were not affected, regardless of treatment.

Higher FGF-23 levels were seen in SNx rats administered a high phosphate diet compared with sham-operated animals. Plasma FGF-23 levels were also higher in the gadodiamide-treated group compared to saline controls.

Long-Term Study (Comparative Gadoterate vs. Gadodiamide Study):

No differences in body weight or macroscopic skin lesions were observed in saline-, gadoterate- or gadodiamide-treated rats. The histological lesions reported on days 4 and 32 are summarized in the sponsor's Table (Table 135):

Table 135: Histopathologic lesions of the dorsal skin observed in the Long-Term Study

Treatment	No. Rats	Histopathological Lesions on Rat Dorsal Skin at	
		Day 4	Day 32
Saline	2	N = 2	SN = 2
Gadoterate	4	N = 3, SN = 1	SN = 4
Gadodiamide	4	N = 1, SN = 2, + = 1	± = 1, + = 3

N indicates normal; SN, subnormal (rare, small areas of altered sub-epidermal dermis, minimal inflammatory reaction); ±, locally thickened epithelium associated with several isolated necrotic cells, heterogeneous dermal matrix (presence of both dense and loose collagen bundles); +, locally thickened epithelium associated with some macrophage foci and small inflammatory foci, small clusters of damaged collagen bundles.

Sponsor's Table (Table 3) of Fretellier et al (2011).

More lesions were observed on day 32 than on day 4. More lesions were also observed in gadodiamide –treated rats than in the Gadoterate meglumine -treated group. Thickened epithelium in association with inflammatory foci and damaged collagen were observed in 3 of 4 gadodiamide-treated rats. Gadoterate or gadodiamide did not have any effect on TGFb1 or CD34 immunostaining when compared to the control group.

Brief Discussion and Conclusions

According to the authors, macroscopic skin lesions were detected in 4/10 rats administered the nonformulated gadodiamide. A high mortality was also associated inflammatory signs and skin lesions were observed with this group. The findings of this study were consistent with the results of other published studies in rats with impaired renal function in which visible skin lesions following the administration of formulated or nonformulated gadodiamide were reported.

The sponsor concluded that neither Gadoterate meglumine nor Gadodiamide (Omniscan) induced macroscopic skin lesions after an intravenous administration in rats with impaired renal function in contrast with nonformulated gadodiamide, which was associated with a high systemic toxicity. Histopathologic lesions were greater with the nonformulated gadodiamide compared to

gadodiamide. Only limited histopathological skin changes were observed in rats treated with Gadoterate meglumine.

In the long-term study, damaged collagen bundles were found in 3 of 4 gadodiamide-treated rats. Significantly higher plasma levels of FGF-23 were found in association with a decreased phosphate level in the gadodiamide group than were obtained in the saline and gadoterate groups.

Overall, it was concluded that the high stability macrocyclic gadoterate was associated with less pathologic and biochemical effects than linear ionic compound, gadodiamide.

Reviewer's Comments

I agree with the findings and conclusions of this published paper.

10.2.3.2 Fretellier et al (2011b) - Fretellier N, Ide'e J-M, Dencausse A, Karroum O, Guerret S, Poveda N, Jestin, G, Factor C, Raynal I, Zamia P, Port M, and Corot C (2011b). Comparative In Vivo Dissociation of Gadolinium Chelates in Renally Impaired Rats: A Relaxometry Study Investigative Radiology, 46: 292–300.

In this study the levels of dissociated versus chelated Gd in plasma, skin, and bone was assessed in Wistar rats with impaired renal function following intravenous administration of Gadoterate meglumine, Gadodiamide (Omniscan) or nonformulated gadodiamide (without excess caldiameter ligand). Sub-totally nephrectomized (5/6 SNx) rats were surgically prepared as described in Fretellier and coworkers (2011a) after which they were administered 2.5mmol/kg/day Gadoterate meglumine, Gadodiamide (Omniscan) or nonformulated gadodiamide for 5 days. Each study group comprised of seven 5/6 SNx rats. Gd concentration in plasma, skin, and femur (epiphysis) was measured by liquid chromatography inductively-coupled plasma mass spectrometry (LC-ICP-MS) at the completion of study on day 11. The r_1 relaxivity constant was measured in skin on days 4 and 11 and in bone on day 11 to evaluate the dissociated or chelated form of Gd in tissue samples. In addition, clinical and skin histopathology assessments were also conducted.

Results:

- Creatinine clearance was decreased by 60% in SNx rats
- There were no macroscopic skin lesions in Gadoterate meglumine-treated rats in contrast to Omniscan- and nonformulated gadodiamide-treated rats. In rats treated with non-formulated gadodiamide, 2/7 survived and 4/7 had skin ulcerations and scabs
- Skin histopathology lesions were in the order: nonformulated > Omniscan > Gadoterate meglumine
- At the end of study on day 11, skin Gd concentration was lower in the Gadoterate meglumine group (161.0 ± 85.5 nmol/g) compared to the Omniscan group (490.5 ± 223.2 nmol/g) and gadodiamide groups (mean value: 776.1 nmol/g; n = 2).
- The total Gd in the femur was greater in the Omniscan group compared to Gadoterate meglumine group.
- On day 11, the plasma concentration of dissociated Gd^{3+} was lower than the limit of detection in rats treated with Gadoterate meglumine (1.5 ± 0.7 μ mol/L) in contrast to the Omniscan group (62% \pm 15% of total Gd). Gd concentration was 1.1 ± 0.7 μ mol/L in gadodiamide rats

- In the skin, the in vivo relaxivity (r_1 ; measured in $\text{mM}^{-1}\text{s}^{-1}$) increased ($p < 0.05$) from 4.8 ± 0.7 on day 4 to 10.5 ± 3.9 on day 11 in the gadodiamide group. No significant change was observed in rats treated with Gadoterate meglumine (2.8 ± 0.2 and 4.9 ± 2.8 , on days 4 and 11, respectively). In the femur, in vivo relaxivity was higher with Omniscan (8.9 ± 2.1) and gadodiamide ($8.8 \text{ mM}^{-1}\text{s}^{-1}$, $n = 2$) compared to gadoterate meglumine ($3.8 \text{ mM}^{-1}\text{s}^{-1}$, $n = 1$)

Conclusions

Omniscan and gadodiamide, unlike Gadoterate meglumine induced microscopic skin lesions 5/6 SNx rats. A higher Gd concentration was observed in the skin and bone (femur) in Omniscan- and nonformulated gadodiamide-treated rats when compared to rats treated with Gadoterate meglumine. Relaxometry revealed a gradual in vivo dechelation and release of dissociated Gd^{3+} in soluble form in 5/6 SNx (renally-impaired) rats administered Omniscan or nonformulated gadodiamide. Gadoterate meglumine was stable over the study duration.

10.3 Studies in Juvenile Animals

Juvenile animal toxicity studies were not submitted in support of the pediatric indication in patients from neonates 0-2 years of age. The agency has recommended that sponsors of all gadolinium-based contrast agents proposing a pediatric indication conduct a juvenile animal study to support their application. The sponsor subsequently submitted a juvenile animal study protocol for review and comments during the review cycle.

10.4 Impurities

10.4.1 Introduction and Overview

1. Overview:

Gadoterate meglumine consists of DOTA (b) (4) gadolinium oxide (paramagnetic agent) and meglumine (b) (4). Since gadolinium oxide and meglumine are not known sources of impurity, the discussion on impurities in the drug product is focused on DOTA (b) (4).

Impurities that could arise during manufacture or during storage of Gadoterate meglumine include (b) (4)

(b) (4)

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11 Integrated Summary and Safety Evaluation

Introduction: Gadoterate meglumine (Dotarem) is a paramagnetic, macrocyclic and ionic gadolinium (Gd) compound is proposed for use as a non-specific contrast agent for magnetic resonance imaging (MRI). As a macrocyclic compound, the gadolinium ion (Gd^{3+}) is bound in a stable complex. The macrocyclic structure in Gadoterate meglumine is 1,4,7,10-tetraazacyclododecane-N, N',N'',N'''-tetraacetic acid or DOTA).

Gadoterate meglumine is intended for intravenous use in adults and pediatric patients (neonates to 17 years) at a recommended dose of 0.1mmol/kg (or 3.7 mmol/m² based on body surface area) to detect and visualize areas with disrupted blood brain barrier (BBB) and/or abnormal vascularity. Following administration, Gadoterate meglumine rapidly distributes to the extracellular fluid. Similar to other MRI contrast agents, Gadoterate meglumine is water-soluble, does not cross the intact blood-brain barrier and is excreted unchanged in the urine.

Safety pharmacology

CNS safety: Behavioral tests were conducted in conscious animals to evaluate the neurological safety of Gadoterate meglumine. There was no effect on motility; No central depressant, extrapyramidal or cataleptic effect was observed. Gadoterate meglumine did not alter the body temperature. Gadoterate meglumine solution (1350mOsm/kg) had a pro-convulsive effect following an intravenous injection of 4mmol/kg in picrotoxin-treated mice and after an intracisternal administration of Gadoterate meglumine in pentylenetetrazole-treated rats.

CVS safety: In a pivotal cardiovascular study in conscious telemetered beagle dogs, Gadoterate meglumine was administered intravenously at doses of 0.6, 2.4, 3.6 and 5.5mmol/kg (or 3.2x, 13x, 20x and 30x the clinical dose adjusted for body surface area, respectively). ECG (Lead II) was recorded continuously for up to 24 hours. At 0.6mmol/kg, no remarkable effects were observed on the heart rate (HR), arterial blood pressure (BP) and ECG parameters (PR, QT and QRS complex). There was a slight increase in HR and arterial BP at 2.4 and 3.6mmol/kg compared to the saline control. At 4h following the 3.6 mmol/kg dose, QTc was slightly reduced (-2 msec). At the highest dose (5.5.mmol/kg), there was a reduction in QTc (-8 to -13msec or 2-6% change) at 1-4 hours after dosing. NOAEL was established as 0.6mmol/kg (or 3.2x the clinical dose).

The potential effect of Gadoterate meglumine on cardiac action potential was evaluated *in vitro* on isolated canine purkinje fibers. The results showed that Gadoterate meglumine had no effect on action potential duration in Purkinje fibers.

Respiratory system: Gadoterate meglumine was administered intravenously to mongrel dogs at doses of 0.1 to 1.0 mmol/kg (or 0.5x to 5.4x the human dose). The high dose (1.0 mmol/kg) resulted in an increase of 4-16% increase in respiratory rate. Lower doses (0.1 and 0.5 mmol/kg) did not elicit any pulmonary effects. Based on the results, the NOAEL was determined as 0.5mmol/kg (or 2.7x the human dose).

Renal system – The effect of Gadoterate meglumine on renal function was evaluated in a glycerol-induced renal failure model in the rat. Gadoterate meglumine was administered as single intravenous dose of 2mmol/kg (or 3.2x the human dose). Gadoterate meglumine (or its comparator Magnevist administered at the same dose), did not cause an exacerbation of the impairment of renal function induced by glycerol in rats. As expected, intramuscular injection of glycerol caused a significant increase in urinary excretion of proteins, plasma creatinine, and urea.

Since radiocontrast media are recognized to induce changes in renal hemodynamics comprising of an initial vasodilatation followed by pronounced vasoconstriction, a safety pharmacology study was conducted to evaluate potential effects of Gadoterate meglumine on renal function normal rats and rats pretreated with (L-NAME (N- ω -nitro-L-arginine methyl ester) administered Gadoterate meglumine (2 mmol/kg or 3.2x the clinical dose adjusted for body surface area) intravenously for 14 days. Gadoterate meglumine was well tolerated with or without L-NAME. L-NAME is an inhibitor of nitric oxide. Inhibition of nitric oxide and prostanoids may result in renal vascular effects including nephrotoxicity. Results showed no adverse effect on renal function. Renal kidney hypertrophy observed 24h following the last treatment was no longer evident after a 28-day treatment-free period. Proximal convoluted tubule vacuolation, seen in all animals euthanized 24h after the last treatment with Gadoterate meglumine, was also not evident after the recovery period. By the end of the treatment-free (reversibility) period, all notable and significant changes in plasma and urinary parameters and histological findings were reversible.

Pharmacokinetics

Studies were conducted to determine the PK profile of Gadoterate meglumine when administered in single- or repeat-dose intravenous administration to mice, rats, rabbits and dogs and goats. Gadolinium (Gd) retained in the body was determined in tissues and biological samples using the Atomic Emission Spectrophotometry (AES) method. The Gadoterate meglumine formulation used in animal PK studies was the same as the to-be-marketed formulation tested in clinical trials. In multiple studies, the PK of Gadoterate meglumine (Gd-DOTA) was compared to that of Magnevist (Gd-DTPA), the gadolinium-based contrast agent that was available at the time these PK studies were performed.

Single-dose PK: Results of nonclinical PK single-dose studies of Gadoterate meglumine in different animal species revealed: 1) a rapid distribution of Gd-DOTA in several organs, 2) the highest Gd concentrations were observed in the kidneys and bone, 3) half-life ($t_{1/2}$) across species was rapid (approximately 1 hr), 4) there was no protein binding or metabolism of Gadoterate meglumine, 5) there was a rapid urinary elimination and a low biliary excretion of Gd, 6) results indicated negligible excretion of Gadoterate meglumine in milk, 7) there was evidence of transplacental transfer of Gadoterate meglumine, 8) Gadoterate meglumine was poorly absorbed via the oral route.

Repeat-dose PK: Following repeated dose administration of Gadoterate meglumine at doses of 0.5, 0.7 and 1.5 mmol/kg in rats (or 0.5, 1.14 and 2.4-fold MHD) over a period of 28 days followed by a 28-day treatment-free period. Gadolinium was detected in the kidneys, liver and femur 1 day after the end of the 28-day treatment period.

Gd concentration was dose-dependent and highest amounts were obtained in the kidney. A linear relationship was obtained between Gd concentration in tissues and the dose administered. Gd concentration was considerably decreased following treatment-free period with slight amounts measurable in high dose animals. The low and mid dose groups were not evaluated for Gd content after the treatment-free period.

Gadoterate meglumine was administered to dogs in doses of 0.5, 0.7 and 1.5 mmol/kg (or 2.70, 3.78, and 8.11-fold MHD) over 28 days of treatment followed by a 28-day treatment-free period. Similar to the finding in rats, Gadolinium was detected in the kidneys, liver and femur. Samples were obtained 24 h following the first injection, after the last injection, and at the end of the treatment-free period (day 56). Plasma and urine were evaluated for Gd content.

As for rats, PK was also linear. At the end of the treatment-free period, highest amounts of Gd were obtained in the kidneys. The repeat dose PK studies confirmed the findings of the single-dose PK studies. Of note, in the single dose studies, a small fraction of Gd was detected in the liver and bone.

Under conditions of repeated exposure, higher levels of tissue Gd were obtained with implications for a greater, long-lasting retention of Gadolinium in the body. It has been shown in the literature that bone tissue may serve as a site for Gd storage. Long-term persistence and slow release of Gd^{3+} from bone stores could therefore enhance Gd-associated toxicity.

It is also noteworthy that the skin was not evaluated for Gd content in view of the importance of the role of skin Gd content in the pathophysiology of the onset and propagation of NSF.

Toxicology

Single-dose toxicity: Expanded single-dose toxicity studies were performed in rats and dogs.

In rats, Gadoterate meglumine was administered intravenously at dose levels of 7, 10.1, and 14.5mmol/kg (or 11x, 16x or 24x the human dose, respectively). There were no treatment-related mortalities. Treatment-related clinical signs included piloerection and half-closed eyes were observed in rats of both sexes; swollen muzzle was observed in 6/10 males and 6/10 females and decreased physical activity (3/10 males) and respiratory difficulties (2/10 males). Gadoterate meglumine did not cause remarkable findings in hematology, ophthalmoscopy, food consumption or body weight. Biliary proliferation was observed in animals administered the high dose (14.5 mmol/kg or 24x the human dose). Findings were reversible at post-recovery day 15 sacrifice. Based on these results, the NOAEL for the single-dose toxicity study in rats was established at 7mmol/kg (or 11x the clinical dose).

In dogs, Gadoterate meglumine was administered as a single intravenous dose at the dose levels of 2.5, 5 or 7.5 mmol/kg. There was no mortality. Treatment-related clinical signs of vomiting and urination were observed in males and female dogs following injection at all administered doses.. The vomiting and urination were dose-related. According to the sponsor, the urination was due to the high osmolarity of the test article. There were no remarkable effects on body weight, food consumption, ocular signs, hematology, and serum chemistry parameters. Urinalysis showed an

increased urinary volume on day 2 in males administered the high dose (7.5mmol/kg). High urine volume was associated with reduced sodium and chloride excretion. No abnormalities in urine volume, sodium or chloride at the end of the observation period. There were also no treatment-related changes in organ weight. On day 2, vacuolation of renal tubules occurred at the mid and high dose levels and in hepatocytes at the high dose level. The vacuolation, which was no longer evident at day 15 in males and females, was not associated with degeneration or tubular necrosis. Based on the results, NOAEL for single-dose toxicity in dogs was established at 2.5 mmol/kg (or 14-fold MHD) since no renal tubular vacuolation was observed at this dose level.

Repeat-dose toxicity: Following repeated administration of Gadoterate meglumine in the rat at dose levels of 2, 4 and 8 mmol/kg (or 3.2x, 6.5x and 13x the human dose) over a 4-week period followed by a 13-week treatment-free period, no test article-induced mortality was reported. Findings, where present, were observed at the mid 4mmol/kg) and high dose (8mmol/kg) levels. The findings were mostly reversed after the 13-week post-treatment, treatment-free period. NOEL was not established since vacuolation was also observed at the low dose (2 mmol/kg/day or 3.2x MHD).

Similar to the rat, Gadoterate meglumine was administered in repeated doses (0.3, 0.7 and 1.5mmol/kg (or 1.6x, 3.8x and 8.1x the human dose) over a 4-week period followed by a 13-week treatment-free period in the dog. Cytoplasmic vacuolation of proximal renal tubules was observed in all treated animals. There was also a dose-related increase in severity of this finding in males. Renal tubular vacuolation was no longer present after the post-treatment 4-week reversibility/recovery period. Based on the finding of renal tubular vacuolation in all treated animals, NOEL was not established in this study.

Genetic toxicity: Gadoterate meglumine was not mutagenic (with or without metabolic activation) in the Ames test, Chromosomal aberration test on Chinese Hamster ovary cells, a gene mutation test in Chinese Hamster lung cells and in the *in vivo* micronucleus test in mice.

Carcinogenicity: No studies were conducted.

Reproductive toxicity

i). Fertility and early embryonic development: In a study to evaluate the effect of Gadoterate meglumine on fertility and early embryonic development, Gadoterate meglumine was administered intravenously to rats at the doses of 2, 4 and 10 mmol/kg/day (or 3.2, 6.5 and 16-fold the clinical dose adjusted for body surface area, respectively). Males were treated for 63 days prior to mating and throughout mating while females were treated 2 weeks prior to mating and throughout mating until gestation day (GD) 17.

Renal tubular vacuolation was observed in rats in all the treatment groups. Pale and enlarged kidneys were observed in males and females administered the mid and high dose levels. There was a reduction in the rate of body weight gain during gestation in the 4 and 10 mmol/kg dose groups.

Based on these findings, the no-observed-adverse-effect-level (NOAEL) in this study was established as 2 mmol/kg (or 3.2-fold the human dose based on body surface area) for general

toxicity in F₀ males and females and F₁ litters and ≥ 10 mmol/kg (or 16.2-fold human dose) for fertility and reproductive performance. Overall, there were no adverse effects on fertility or reproductive function/performance. There was no evidence of teratogenic effects following the daily intravenous administration of Gadoterate meglumine to male and female rat fetuses.

In rabbits, the NOAEL for F₀ females (dams) was 0.8mmol/kg/day (or 2.6x-MHD) and for F₁ litters NOAEL was also 0.8 mmol/kg/day (or 2.6x-MHD). There was therefore no evidence of maternotoxicity, embryotoxicity or teratogenicity in rabbits.

ii). Pre- and postnatal development in the rat: Gadoterate meglumine did not appear to have any effect on breeding performance, fertility or reproductive performance in the F₁ litters. It did not demonstrate any adverse effects on the progress and outcome of pregnancy or on the development of the F₁ litters in the period of organogenesis. At the high dose of 0.8 mmol/kg (or 1.3-fold MHD) administered to F₀ dams, Gadoterate meglumine caused a slight toxic effect notably on body weight of the F₁ generation hence the NOAEL for F₀ dams was determined as 0.4 mmol/kg/day (or 0.7-fold MHD).

Studies in Juvenile animals: Juvenile animal toxicity studies were not submitted in support of the pediatric indication in patients from neonates 0-2 years of age. The agency has recommended that sponsors of all gadolinium-based contrast agents proposing a pediatric indication conduct a juvenile animal study to support their application. The sponsor subsequently submitted a juvenile animal study protocol for review and comments during the review cycle.

Special Toxicology

i). Nephrogenic Systemic Fibrosis (NSF): Nephrogenic systemic fibrosis (NSF) is a recently described, serious, fibrotic and highly debilitating disorder most frequently described in patients with end-stage renal disease or acute renal failure. While NSF primarily affects the skin, other organ compartments may also be involved. Although the etiology of NSF is not fully understood, exposure to gadolinium-containing contrast agents has been associated with the onset of symptoms of NSF. Considerable progress towards a better understanding of this disease has occurred since the first association between the clinical use of GBCAs and NSF was made. Consequently, there are several reports associating the incidence of NSF with the clinical use of GBCAs in patients with acute kidney disease or severely impaired kidney function (Glomerular Filtration Rate < 30 mL /min/1.73m²). The commonly reported hypothesis for the involvement of gadolinium in the pathomechanism of NSF is the in vivo dechelation of GBCAs. Although all GBCAs have the potential to release gadolinium ion (Gd³⁺), which in the context of renal failure, is an important trigger for NSF, such a release is thought to more readily occur among the less stable gadolinium chelates. Current understanding seems to indicate reasonably that Gadolinium complexes play a causative role in the pathophysiology of nephrogenic systemic fibrosis or nephrogenic fibrosing dermopathy. Still, the exact pathogenesis and the risk for patients are unclear.

Two published articles (Fretellier et al, 2011a, b) were reviewed to provide a better understanding of the disease mechanism in Nephrogenic Systemic Fibrosis (NSF).

In the first study, following intravenous injection in rats with impaired renal function, neither Gadoterate meglumine nor gadodiamide induced macroscopic skin lesions, in contrast with nonformulated gadodiamide which was associated with systemic toxicity. It was concluded that

the stable gadoterate was associated with less pathologic and biochemical effects than linear ionic compound, gadodiamide.

In the second study, the levels of dissociated versus chelated Gadolinium (Gd) in plasma, skin, and bone was assessed in Wistar rats with impaired renal function following intravenous administration of Gadoterate meglumine, Gadodiamide (Omniscan) or nonformulated gadodiamide (without excess caldiameter ligand). Omniscan and gadodiamide, unlike Gadoterate meglumine induced microscopic skin lesions renally-impaired rats. A higher Gd concentration was observed in the skin and bone (femur) in Omniscan- and nonformulated gadodiamide-treated rats when compared to rats treated with Gadoterate meglumine.

ii). *Antigenicity*: Gadoterate meglumine did not induce any immediate hypersensitivity reactions.

iii). *Toxicological Characterization of Impurities*: Gadoterate meglumine consists of DOTA

(b) (4) gadolinium oxide (paramagnetic agent) and meglumine (b) (4)

Since gadolinium oxide and meglumine are not known sources of impurity, the discussion on impurities in the drug product is focused on (b) (4) (b) (4)

(b) (4) Impurities that could arise during manufacture or during storage of Gadoterate meglumine include (b) (4)

(b) (4)

Local Tolerance

Studies were performed in rats to evaluate the local tolerance of Gadoterate meglumine (2.5mmol/kg) following subcutaneous or intramuscular injection, respectively. Macroscopic and microscopic changes at the injection site were similar between control and test article-treated animals on days 1 and 3.

Transient local inflammatory reactions (hematoma, edema and mononuclear/polynuclear cell aggregation) were seen at 6 hours post-dose. Gadoterate meglumine was well tolerated in rats after a single subcutaneous or intramuscular injection.

In another study conducted in rabbits, Gadoterate meglumine was administered at 0.9, 0.9 and 0.25 mmol/animal via intravenous, intra-arterial or perivenous routes respectively. Microscopic findings at the different sites of administration in control and Gadoterate meglumine-treated rabbits included in varying degrees erythema, hemorrhagic infiltration, epidermal vacuolation, presence of inflammatory cells, ulceration of the outer skin and perivascular hemorrhage. The findings were consistent with histopathological findings following the intravenous administration of Gadoterate meglumine in single-dose and repeat-dose toxicity reports.

Table 145: NOAEL, Safety Margin and Prominent Finding(s) in Gadoterate meglumine Toxicity Studies

Toxicity	Species	NOAEL* (mmol/kg) in M/F)	Safety margin (x-fold MHD)	Prominent findings (if any)
Single-Dose	Rat	10.1	16x	Liver vacuolation
		NOAEL not established	Not established; kidney vacuolation was seen at all levels tested	
		Overall NOAEL = 7.0	11x	See below*
	Dog	5.0	27x	Liver vacuolation
		2.5	14x	Kidney vacuolation
		Overall NOAEL = 2.5	14x	
*Notable findings at the high dose (14.5mmol/kg or 24-fold the clinical dose) include: reversible, treatment-related renal cortical tubule and hepatic vacuolation. Reversible increased biliary proliferation was also present				
Repeat-Dose	Rat	NOAEL not established	Kidney vacuolation, the prominent finding in this study, was observed at all doses tested	
	Dog	NOAEL not established	Kidney vacuolation, the prominent finding in this study, was observed at all doses tested	
Reproductive Toxicity				
Fertility and Early Embryofetal Devt.	Rat	F ₀ males and females: NOAEL = 2.0 F ₁ litters: NOAEL ≥ 10mmol/kg	3.2x ≥16.2x	M/F: Pale and enlarged kidneys at the intermediate and high doses F ₀ females: Decreased body weight
Rat Embryofetal Devt.	Rat	F ₀ dams: NOAEL = 0.4mmol/kg	0.7x	Slight toxic effect on body weight of F ₁ litters at high dose (0.8 mmol/kg)
Rabbit Embryofetal Devt.	Rabbit	F ₀ dams: NOAEL ≥ 0.8mmol/kg	2.6x	No evidence of maternotoxicity, embryotoxicity or teratogenicity
Prenatal and	Rat	F ₀ dams and F ₁	≥ 1.3x	No evidence of pre-

postnatal Devt. in Rat		generation: NOAEL \geq 0.8mmol/kg		and postnatal toxicity
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Reviewer's Table. * = Unless stated otherwise, the NOAEL is the top dose administered

11.1 Overall Conclusions and Recommendations

11.1.1 Overall Conclusion

The proposed indication for Gadoterate meglumine (Dotarem) – “Magnetic resonance imaging (MRI) in brain (intracranial), spine and associated tissues in adults and pediatric patients (from neonates to 17 years of age) to detect and visualize areas with disruption of the blood brain barrier (BBB) and/or abnormal vascularity” involves both adult and pediatric components.

The sponsor submitted a complete nonclinical package to support the adult indication. The results of the nonclinical studies did not reveal any significant safety concerns for the proposed indication for Gadoterate meglumine in adults and children 0-17 years of age. Consequently, nonclinical review indicates that the safety and toxicity profiles of gadoterate meglumine were similar to other approved gadolinium-based contrast agents.

However, no juvenile animal toxicity studies were provided in the original NDA submission to support the use of Gadoterate meglumine in the pediatric population 0-2years of age. The agency has recommended that sponsors of all gadolinium-based contrast agents proposing a pediatric indication conduct a juvenile animal study to support their application. The sponsor subsequently submitted a juvenile animal study protocol for review and comments during the review cycle. It is highly unlikely that the result of this study will be available before the end of this review cycle.

Based on the sufficiency of nonclinical data to support the use of Gadoterate meglumine in adults and children 2-17 years of age and a lack of juvenile animal toxicity studies to support proposed indications in pediatric population aged 0-2 years, the potential recommendation from a nonclinical perspective is to approve Gadoterate meglumine for use in adults and children 2-17 years of age.

11.1.1 Recommendation

Pharmacology/Toxicology is recommending approval meglumine in adults and children 2-17 years of age based on the sufficiency of nonclinical data to support the use of Gadoterate meglumine in these age groups. Nonclinical juvenile animal toxicity studies to support proposed indications in pediatric population aged 0-2 years were not provided. The potential recommendation from a nonclinical perspective is to approve Gadoterate meglumine for use in adults and children 2-17 years of age.

12 Appendix/Attachments

12.1 References

Agmon Y, Peleg H, Greenfeld Z, Rosen S, and Brezis M (1994) Nitric oxide and prostanoids protect the renal outer medulla from radiocontrast toxicity in the rat. *Amer Soc Clin Investig* 94:1069-1075.

American College of Radiology Manual on Contrast Media (2012) ACR Committee on Drugs and Contrast Media Version 8, Appendix A. pages 1-90.

Bongatz G (2007). Imaging in the time of NSF/NFD: Do we have to change our routines concerning renal insufficiency? *MAGMA*, 20(2):57-62.

Dailiana HZ, Kotsaki D, Varitimidis S, Moka S, Barkarozzi M, Oikonomou K, and Milizos NK (2008). Injection injuries: Seemingly minor injuries with major consequences. *Hippokratia* 12(1):33-36.

Erley, CM, Heyne, N, Burgert, K, Langanke, J, Risler, T, and Osswald, H (1997). Prevention of radiocontrast-induced nephropathy by adenosine antagonists in rats with chronic nitric oxide deficiency. *J Am Soc. Nephrol.* 8:1125–1132.

Fekete A, Kiss B, and Spitzar E (1975) Renal function in rats after unilateral ligation renal artery *Acta Med Sci Hung*, 32(1):55-62.

Fretellier N, Ide'e J-M, Guerret S, Hollenbeck C, Hartmann, D Gonza'lez W, Robic C, Port M, and Corot C (2011a). Clinical, Biological, and Skin Histopathologic Effects of Ionic Macrocylic and Nonionic Linear Gadolinium Chelates in a Rat Model of Nephrogenic Systemic Fibrosis, *Investigative Radiology*, 46(2):85-93.

Fretellier N, Ide'e J-M, Dencausse A, Karroum O, Guerret S, Poveda N, Jestin, G, Factor C, Raynal I, Zamia P, Port M, and Corot C (2011b). Comparative In Vivo Dissociation of Gadolinium Chelates in Renally Impaired Rats: A Relaxometry Study *Investigative Radiology*, 46: 292–300.

Grobner, T (2006). Gadolinium – a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrol Dial Transplant*, 21(14):1104-1108.

Kimura J, Ishiguchi T, Matsuda J, Ohno R, Nakamura A, Kamei S, Ohno K, Kawamura T and Murata K (2005). Human comparative study of Zn and copper excretion via urine after administration of magnetic resonance imaging contrast agents. *Radiat Med.*, 23(5):322-325.

Morpugo C (1971). A new design for the screening of CNS-active drugs in mice. A Multi-dimensional observation procedure and the study of pharmacological interactions

Arzneim Forsch, 1971, *ZI*, (II):1727-1734.

Okutur SK, Borlu F, Ersoy CY, and Paksoy F (2006) Acute isoniazide intoxication: Convulsion, Rhabdomyolysis and metabolic acidosis. *Turk J Med. Sci.* 36: 397-399.

Salomon, R (1998). Contrast medium-induced acute renal failure. *Kidney Int.* 53:230–242.

Sieber MA, Lengsfeld P, Walter J, Schirmer H, Frenzel T, Siegmund F, Weinmann HJ, Pietsch H (2008) Gadolinium-based contrast agents and their potential role in the pathogenesis of nephrogenic systemic fibrosis: the role of excess ligand, *27(5):955*.

Taupitz M, Stolzenburg N, Ebert M, Schnorr J, Hauptmann R, Kratz H, Hamm B, Wagner S (2013) Gadolinium-containing magnetic resonance contrast media: investigation on the possible transchelation of Gd(3+) to the glycosaminoglycan heparin. *Contrast Media Mol Imaging*, 2:108-16.

Telgmann, L, Wehe, CA, Kunnemeyer, J, Bulter, AC, Sperling, M and Karst, U (2012) Speciation of Gd-based MRI Contrast agents and potential products of transmetallation with iron ions or parenteral iron supplements. *Anal Bioanal Chem* 404(8):2133-41.

Thakral, C, Alhariri, J and Abraham, JL (2007) Long-term retention of Gadolinium in tissues from nephrogenic systemic fibrosis patient after multiple gadolinium-enhanced MRI scans: case report and implications. *Contrast Media Imaging* 2(4):199-205.

Yao K, Heyne N, Erley CM, Risler T, Osswald H (2001). The selective adenosine A1 receptor antagonist KW-3902 prevents radiocontrast media-induced nephropathy in rats with chronic nitric oxide deficiency. *Eur J Pharmacol.* 414(1):99-104.

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/s/

OLAYINKA A DINA
02/21/2013

ADEBAYO A LANIYONU
02/21/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 204-781

NDA Number: 204-781. **Applicant: Guerbet LLC.** **Letter Date: 09/20/2012.**

Drug Name: DOTAREM® **NDA Type: 505(b)(i).** **Stamp Date: 09/20/2012.**

On **initial** overview of the NDA 204-781 (Dotarem) application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		Acceptable
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Acceptable
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		Acceptable
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	✓		<p>Juvenile studies: No Juvenile studies were submitted in Module 2.4 (§2.4.4.5.3, pre and post natal development). However, the sponsor made reference to clinical trials and post-marketing experience in children (age not specified).</p> <p>Other studies (§2.4.4.7): Subsection 2.4.4.7.2 focused on Nephrogenic Systemic Fibrosis (NSF). The sponsor cited, among other studies, a recent study describing the clinical, biological and skin histopathological effects of ionic macrocyclic and non-ionic linear gadolinium chelates in a renally-impaired rat model of NSF (Fretellier et al., 2012). However, no juvenile animal studies were submitted.</p>
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			Formulation to be marketed is not different from the formulation used in the toxicology studies
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	✓		The route of administration (intravenous, IV) used in the animal studies is the same as the intended human exposure route.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA 204-781**

	Content Parameter	Yes	No	Comment
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	✓		According to the sponsor (Nonclinical Overview, §2.4, page 6 of 48, paragraph 6), all pivotal Toxicology studies comply with Good Laboratory Practices (GLP). Safety pharmacology studies were not performed in compliance with GLP except the additional studies requested by the FDA in year 2000. Earlier studies, though not GLP compliant were done according to the state of the art at the time of performance. According to the sponsor, repeating these studies for GLP compliance purpose would not provide additional scientific information
8	Has the applicant submitted all special Studies/data requested by the Division during pre-submission discussions?	✓		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	✓		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	✓		Two impurities ((b) (4)) potentially present in DOTA and in Gadoterate meglumine was addressed in § 2.4.4.7.3 (Impurity profile of drug substance, pages 38-41 of 48, Nonclinical overview). The qualification of (b) (4) was described in § 2.4.4.7.3.2 of the nonclinical overview. New toxicity studies may not be needed.
11	Has the applicant addressed any abuse potential issues in the submission?		✓	
12	If this NDA is to support an Rx to OTC switch, have all relevant studies been submitted?		✓	N/A. This NDA has not been submitted to support a prescription to OTC (Form 356h indicates Dotarem as a prescription product)

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

N/A

File name: Pharmacology/Toxicology Filing Checklist for NDA 204-781 (DOTAREM)

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA 204-781**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

No issues have been identified

<u>Olayinka A. Dina</u>	<u>October 23, 2012</u>
Reviewing Pharmacologist	Date
<u>Adebayo A. Lanionu</u>	<u>October 23, 2012</u>
Team Leader/Supervisor	Date

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OLAYINKA A DINA
10/23/2012

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10/23/2012