CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203684Orig1s000

PHARMACOLOGY REVIEW(S)

MEMO TO FILE

Application number:	NDA 203684
Supporting document/s:	Electronic submission
CDER stamp date:	April 11, 2014
Product:	Lumason (Sulfur hexafluoride)
Applicant:	Bracco Diagnostics Inc.
Review Division:	Medical Imaging Products
Reviewer:	Sunny Awe, Ph.D.
Acting/Team Leader:	Yanli Ouyang, MD, Ph.D.
Division Director:	Libero Marzella, MD., Ph.D.
Project Manager:	Frank Lutterodt, MS.

This memo to file is in respect of NDA 203684 application for Sulfur hexafluoride (Lumason) by Bracco Diagnostics Inc. The NDA application is for this indication: "*For use in echocadiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation"*. This is a class 2 resubmission.

Bracco Diagnostics Inc had submitted NDA 021315 dated on February 29, 2001 for a similar indication. (b) (4) The nonclinical data of the application was reviewed by Dr. Tushar Kokate and the application was recommended for approval from a nonclinical perspective. There are no outstanding nonclinical issues with the application. The second and third review cycles of NDA 203684 submission were in 2011 and 2013, respectively. The present resubmission did not contain new nonclinical information. Thus, previous recommendation of approval of the NDA from a nonclinical perspective stands. No new amendment is recommended on the nonclinical sections of the label.

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/s/

SUNNY O AWE
08/28/2014

YANLI OUYANG
08/28/2014

MEMO TO FILE

Application number:	NDA 203684
Supporting document/s:	Electronic submission-Sequence Number 015.
CDER stamp date:	May 31, 2013
Product:	Sonovue (sulfur hexafluoride) Microbubbles
Applicant:	Bracco Diagnostics, Inc.
Review Division:	Medical Imaging Products
Indication:	"For use in echocardiography (ECHO) in patients with suboptimal ECHO, to obtain left ventricular opacification and improve endocardial border delineation (EBD)"
Reviewer:	Sunny Awe, Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Acting Division Director:	Libero Marzella, MD.
Project Manager:	Frank Lutterodt, M.S.

This memo to file is in respect of Dr. Hausman's comment on page 4 of her pediatric consult review. The Division of Medical Imaging Products (DMIP) requested that Pediatric and Maternal Health Staff (PMHS) review and comment on the sponsor's pediatric development plan (PDP) which includes a proposed study in children 9 to 17 years of age, and a request for partial waiver for children younger than 9 years old. As part of her review, Dr. Hausman recommended that "*nonclinical should review and comment on any pediatric implications of excipients at the concentrations used whether the 'microbubbles' in the reconstituted product present unique pediatric safety concerns".*

There are published papers reporting off-label pediatric use of Sonovue. No serious adverse effects were reported in the children administered doses up to the recommended adult dose of this product as summarized below:

1) Ascenti et al (2004) Harmonic US imaging of vesicoureteric reflux in children: usefulness of a second generation US contrast agent Pediatr Radiol (2004) 34: 481–487. In this clinical study, 80 children (44 girls, 36 boys; age 3 months to 5 years, mean-20 months) were prospectively studied with contrast-enhanced second-harmonic voiding ultrasonography (VUS). The children received a dose of 0.5 ml of SonoVue (Bracco, Milan, Italy). No adverse effect was reported due to the administration of the Microbubbles.

2) Riccabona M (2012) Application of a second-generation US contrast agent in infants and children--a European questionnaire-based survey. Pediatr Radiol. 2012 Dec;42(12):1471-80. Riccabona (2012) surveyed the off-label use in children of a second-generation ultrasound contrast agents by emailing questionnaires to European paediatric radiologists, who were asked about their experience with the second-generation US-CA Sonovue® (Bracco, Milan, Italy). Forty-five centers reported 5,079 examinations in children (age mean: 2.9 years; range: birth-18 years, M/F: 1/ 2.8). No adverse effects had been recorded from intravesical use. Six minor adverse effects (skin reaction, unusual taste, hyperventilation) had been recorded after five intravenous studies (0.52%).

3) Duran et al (2012) Voiding urosonography (VUS) including urethrosonography:high-quality examinations with an optimized procedure using a second-generation US contrast agent. Pediatr Radiol 42:660–667. In this study, 307 VUS examinations performed using SonoVue® in 591 pelvi-ureter units in 295 children of mean age, 27.1 (S.D., 42.5) months, with 154 (50.2%) of the examinations performed in boys; 58 children also underwent VUS using Levovist®. The dosage of I mL (45 mcg) as recommended by the manufacturer was administered to the children during this study. No adverse effects related to the ultrasound contrast agents were observed during or reported 48 h after the examination.

4) Wang et al (2012) Polyethylene glycol 4000 treatment for children with constipation: A randomized comparative multicenter study. Exp Ther Med. 3(5):853-856. This study evaluated the efficacy and safety of polyethylene glycol 4000 (PEG 4000) for the treatment of constipation in 216 children from 7 hospitals over 8 years of age. During the study, a total of 105 patients received oral PEG 4000 (20 g/day) over one to 2 weeks and no significant clinical adverse effects or abnormalities in the laboratory tests were observed in the treatment groups.

Based on previous off-label use of Sonovue in pediatric population, it is unlikely the excipients at the concentrations used or the reconstituted microbubbles would pose any unique pediatric safety concern other than those stated in the labeling.

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/s/

SUNNY O AWE
10/17/2013

ADEBAYO A LANIYONU
10/17/2013

MEMO TO FILE

Application number:	NDA 203684
Supporting document/s:	Electronic submission
CDER stamp date:	October 7, 2011
Product:	SonoVue (Sulfur hexafluoride)
Applicant:	Bracco Diagnostics Inc.
Review Division:	Medical Imaging Products
Reviewer:	Sunny Awe, Ph.D.
Supervisor/Team Leader:	Adebayo Laniyonu, Ph.D.
Division Director:	Dwaine Rieves, MD.
Project Manager:	Frank Lutterodt

This memo to file is in respect of NDA 203684 application for SonoVue (Sulfur hexafluoride) product by Bracco Diagnostics Inc. The NDA application (stamp date-December 21, 2011) was for this indication: "*For use in echocadiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation*"

Bracco Diagnostics Inc had earlier submitted NDA 021315 dated February 29, 2001 for a similar indication (b) (4) The nonclinical data of the application was reviewed Dr. Tushar Kokate and the application was recommended for approval from nonclinical perspective. Nonclinical has no outstanding issues with the NDA application. The review is available in DARRTS.

In the current NDA 203684 submission, the sponsor did not conduct new nonclinical studies and no additional nonclinical studies are required. Therefore, our previous recommendation of approval from nonclinical perspective stands.

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/s/

SUNNY O AWE
08/21/2012

ADEBAYO A LANIYONU
08/21/2012
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203-684 **Applicant:** Bracco Diagnostics Inc **Stamp Date:** December 21, 2011

Drug Name: SonoVue **NDA/BLA Type:** Original

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
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)?	X		
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).	X		
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 submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Sunny Awe — 01/30/2012

Reviewing Pharmacologist — Date

Team Leader/Supervisor — Date 1/30/2012

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

SUNNY O AWE
01/31/2012

ADEBAYO A LANIYONU
01/31/2012

Review and Evaluation of Pharmacology and Toxicology Data
Division of Medical Imaging and Radiopharmaceutical Drug Products

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: Approvable

1.2 Recommendation for nonclinical studies: None

2. Summary of nonclinical findings

SonoVue (BR1) is a lipid-encapsulated sulfur hexafluoride (SF_6) microbubble suspension proposed for visualization of cardiac chambers and delineation of endocardial borders during echocardiography. (b) (4)

The proposed doses are 2 ml (b) (4) for imaging of the endocardial border (b) (4) During a single examination, a second injection (same dose as the first one) may be administered when necessary. Therefore, the maximum clinical dose is (b) (4)

2.1 Brief overview of nonclinical findings:

The original NDA was received on February 29, 2001. (b) (4)

a) CVS safety pharmacology study:

(b) (4) the agency recommended that a comprehensive safety pharmacology study be conducted in a large animal species.

In addition, it was recommended that effects on ECG be evaluated under ultrasound exposure at different mechanical index (MI) values to correlate with the clinical settings. During clinical trials, MI values up to 1.2 were used. Ultrasound exposure at high emission power levels/MI values can cause microbubble oscillations and bursting, which may adversely affect ECG.

In response, the sponsor conducted a comprehensive safety pharmacology study (# 886/019) in conscious cynomolgus monkeys evaluating effects of SonoVue (1, 2 and 5 ml/kg, 3-17 times clinical dose based on surface area) on the arterial blood pressure, heart rate, ECG, body temperature and locomotor activity. No significant effects on various CNS and CVS parameters, including ECG were noted as compared to the control saline group.

QT effect was not significant when normalized for heart rate. The NOAEL for this study was established at 5 ml/kg (17-times clinical dose based on body surface area).

In addition, a CVS safety study (# 886/021-RE) evaluating SonoVue (0.1, 0.3 & 1 ml/kg) effects on cardiovascular parameters after ultrasound exposure at various MI values (0.4, 0.8 & 1.2) in real time imaging or triggered mode was conducted in unanesthetized dogs. SonoVue did not affect blood pressure, heart rate or ECG when compared with pre-dosing baseline levels or control group. Heart histopathological examination did not show any remarkable findings. The NOAEL for this study was 1 ml/kg (5-times clinical dose based on body surface area).

b) Safety study in an animal model with compromised pulmonary function:

In general, microbubbles as a class are characterized by inherent potential to cause pulmonary microembolism with the resultant clinically significant hemodynamic changes. Such hemodynamic changes that may be handled well in healthy humans may aggravate already compromised functions in special populations such as those with chronically compromised pulmonary functions. This concern led the agency to request for a special safety study to evaluate effects of SonoVue on cardiovascular and pulmonary function in an animal model with pulmonary microvascular compromise.

In response, a pre-clinical study (# 886/025) was conducted to evaluate the effect of SonoVue (0.1, 0.3 and 1 ml/kg) on pulmonary and cardiovascular functions in a model of acute pulmonary arterial hypertension in the anesthetized dogs. Pulmonary hypertension was induced by the injection of glass beads. In this study, there were no effects on CVS (including ECG) or pulmonary parameters. The NOAEL for this study was at 1 ml/kg (5-times clinical dose).

c) Effect on microvasculature (Hamster cheek pouch model):

(b) (4)

it was recommended that the study be conducted using intra-arterial administration of SonoVue and observing blood vessels with less than 10 µm diameter to examine entrapment/retention of microbubbles and behavior of retained bubbles in the microcirculation.

In response, sponsor conducted a study (# ECHO 209) examining microvascular behavior of SonoVue (1, 2.5 & 5 ml/kg, 2-8 times clinical dose) microbubbles and the extent of bubble retention in the rat spinotrapezius muscle microcirculation using intravital microscopy. The retained microbubbles (1.2-1.7% of total circulating microbubbles visualized) were primarily observed in capillaries. Retention of bubbles of size smaller than 5 µm was mainly due to sticking to the endothelium. Some of the large sized bubbles (>5 µm) did get entrapped in the capillaries with subsequent deformation (elongation). The bubbles eventually reduced in size and disappeared due to dissolution of the SF_6 gas. The

mean retention time of the bubbles was ~6 minutes and no retained bubbles were observed after 20 minutes post-dosing. No coalescence or aggregation of bubbles was observed.

The entrapment of bubbles did disturb the microvascular circulation flow transiently but there was no RBC modification or leukocyte and/or platelet adhesion observed at the site of retainment. According to the sponsor, this transient disruption in microvascular circulation should not adversely affect the hemodynamic functions due to large reserve capacity for lung (closed capillaries that can be recruited/reopened within 4 sec to cope with a sudden need for increased blood flow).

The effects seen were similar to the ones reported for approved microbubble agent Definity. This reviewer feels that transient disruption in circulation in a small percentage of capillary bed as a whole should not adversely affect the function of lungs. In this respect, there were no adverse microscopic findings in the lungs in toxicology studies or no adverse effects were seen in the respiratory function parameters in a safety study using animal model of acute pulmonary hypertension.

d) Ultrasound effects on SonoVue microbubbles:

This histopathological evaluation study was requested to examine any potential for organ lesions (in particular, heart, kidney, carotids and lungs) after exposure to ultrasound at various MI settings, including settings at which extensive microbubble destruction may occur.

In response, the sponsor carried out a study (# TOX-1-02) evaluating potential for SonoVue to induce internal organ lesions in rats after a single dose (1 & 5 ml/kg, 2-8 times clinical dose) during individual organ exposure to ultrasound at various MI values (0.4, 0.8 & 1.9). Higher MI settings (0.8 & 1.9) caused extensive microbubble destruction as evidenced by the reduction in imaging intensity. Histopathological examination showed minimal areas of blood suffusion/micro-hemorrhages within the pulmonary alveoli in SonoVue-treated as well as saline control groups. No other histopathological lesions in any other organs were reported.

According to the sponsor, the pulmonary lesions observed in this study, were similar to those described in the lungs of rats and mice due to exposure to clinical diagnostic levels of ultrasound. Indeed, there are several published studies reporting RBC suffusions/lung intra-parenchymal hemorrhage in densely micro-circulated organs such as lungs in small animals after ultrasound exposure. This adverse effect may not be related to the SonoVue treatment and may be due to intense pressure and oscillations caused by ultrasound probe in small animals. Considering no adverse histopathological findings for heart and lungs in toxicity studies and no adverse effects reported for respiratory function in various safety studies, it is not necessary to repeat this study using larger animals.

e) Effect on arterial blood gases:

The sponsor conducted this study in the original NDA application with analysis of blood gases starting at 5 minutes post-dosing. Since microbubbles can produce fast and transient effects on arterial blood gases and elimination kinetics for SonoVue are very rapid ($t_{1/2}$<1 min), the agency recommended that this study be repeated with blood sampling immediately after SonoVue administration.

In response, sponsor stated that follow up of blood oxygen saturation was performed in various clinical studies (n=323 patients) using a pulse oximeter with time-points for analysis carried out at 30 sec post-dosing, and thereafter, every 1 or 2 minutes. Some of these studies involved subjects with congestive heart failure and COPD patients. In all these clinical studies, no marked changes in oxygen saturation were noted.

Considering no remarkable effects on oxygen saturation in various clinical studies and no post-dose abnormal respiratory clinical signs noted in pre-clinical safety studies, I recommend that the requirement for this preclinical study be waived.

f) Pharmacodynamic studies:

Although not requested, sponsor conducted an efficacy study using formulation of SonoVue with and without palmitic acid to show that addition of palmitic acid to the SonoVue formulation did not change overall signal intensity and duration of imaging. Based on the data submitted, there were no significant differences in terms of intensity and persistence of contrast effect in batches with or without palmitic acid in the formulation.

Overall, the sponsor has adequately addressed all pre-clinical deficiencies. From pre-clinical perspective, NDA application for SonoVue is considered approvable with no outstanding issues.

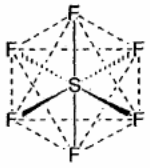
PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number:	21-315
Review number:	002
Sequence number/date/type of submission:	N-000-B2/07-05-03/NDA resubmission
Information to sponsor:	Yes ( ) No (X)
Sponsor and/or agent:	**Bracco Diagnostics, Inc.** Princeton, NJ 08543
Manufacturer for drug substance:	Bracco Diagnostics, Inc.
Reviewer name:	Tushar Kokate
Division name:	Medical Imaging and Radiopharmaceuticals
HFD #:	160
Review completion date:	10/24/03

Drug:

Trade name:	SonoVue
Code name:	BR1
Chemical name:	Sulfur hexafluoride microbubbles
Molecular weight and structure:	146



Relevant INDs/NDAs/DMFs:	21-315 (Original NDA review)
Drug class:	Microbubble ultrasound contrast agent
Route of administration:	Intravenous

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

Studies reviewed within this submission:

Study # Vol # (page #)	Study Type	Species	Lot #	Dose, ml/kg (DoseMultiples)	Review Page
ECHO 207 42 (01)	Imaging study using formulation with and without palmitic acid	Minipigs	1A008 BG 1189	0.004-0.05 (0.1-1)	09
BRG 005 42 (65)	Sensitivity of microbubbles to ultrasound exposure	In vitro study	1A008 BG 1205	----	12
886/019 42 (79)	Safety Pharmacology: Effect on CNS & CVS parameters	Cynomolgus Monkey	2A005	1, 2 & 5 (3, 7 & 17)	14
886/021-RE 43 (01)	Effect on ECG after ultrasound exposure at various MI settings	Beagle Dog	2A005	0.1, 0.3 & 1 (1, 3 & 5)	17
G0005 43 (233)	Safety Pharmacology: Effect on renal system	SD Rat	CA 58/25	0.3 & 1 (0.5 & 2)	20
886/025 44 (41)	Effect on pulmonary pressure in an acute model of pulmonary hypertension	Beagle Dog	2A005	0.1, 0.3 & 1 (1, 3 & 5)	21
ECHO 209 44 (01)	Effect on microvasculature: Rat spinotrapezius muscle microcirculation	Wistra Rat	0A011B 1A008	1, 2.5 & 5 (2, 4 & 8)	25
TOX-1-02 44(189)	Histopathological evaluation: Effect under ultrasound at various MI values	SD Rat	2A005	1 & 5 (2 & 8)	31
BRG 011 45 (83)	Stability of SonoVue microbubbles in gas-saturated media (plasma & saline)	In vitro study	1A008 1A010	----	36
ECHO II 93 44 (154)	Pharmacokinetics of 14C-labeled PEG-ECHO II	SD Rat	JP/Ps 203	2 & 20 (3 & 33)	40

Studies not reviewed within this submission: None

Strength: 8 microliters/ml of SF_6 microbubbles

Indication: SonoVue is indicated for use with ultrasound imaging to enhance the echogenicity of the blood, which results in an improved signal-to-noise ratio.

(a) Endocardial border delineation: For use in echocardiography in patients with suspected or established cardiovascular diseases to improve visualization of cardiac chambers and endocardial border delineation, which assists in the assessment of left ventricular wall motion.

(b) (4)

Clinical Dose:

(a) Imaging of endocardial border: 2 ml (b) (4)
(b) (4)

During a single type of examination, a second injection of 2 (b) (4) ml may be administered if deemed necessary.
Maximum clinical dose: (b) (4)

Clinical Formulation:

SonoVue is formulated in the form of a lyophilizate made up of PEG 4000, highly dispersed phospholipids and palmitic acid, packaged in a (b) (4)-sealed vial containing sulphur hexafluoride (SF_6) in the gas phase. According to the sponsor, following the addition of saline solution (5 ml, just prior to administration to the patient), the lipid molecules readily migrate to the gas/aqueous phase interface leading to the formation of SF_6 microbubbles stabilized by a lipid layer. A stable, milky suspension of highly echogenic gas microbubbles is obtained by vigorous hand agitation/shaking for at least 20 seconds. The pH of the reconstituted product is 4.5 to 7.5.

SonoVue is supplied as a kit with sodium chloride injection 5-ml syringe and a Braun Mini-Spike (b) (4) connector. It is isotonic in human plasma (287 mOsm/kg), and less viscous than blood. Since the product does not contain an antimicrobial preservative, the reconstituted suspension should be used within (b) (4) after reconstitution.

The original SonoVue formulation consisted of two synthetic phospholipids: distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidicglycerol sodium salt (DPPG.Na), and polyethylene glycol 4000 (PEG 4000), which provides the backbone to the lipids for microbubbles formation when 0.9% saline is added. The current clinical formulation has palmitic acid (0.008 mg/ml) added to the formulation to improves long-term stability (shelf life) of the lyophilizate. Phospholipids DSPC, DPPG.Na and palmitic acid are part of the active moiety that form a thin monolayer around the microbubbles.

(b) (4)
Each milliliter of SonoVue contains approximately number of microbubbles. The composition of the formulation with and without palmitic acid is shown in the table below. Clinical formulation of SonoVue contains palmitic acid.

Ingredient	SonoVue without palmitic acid: Amount/5 ml, single dose vial (quantity/ml)	SonoVue with palmitic acid: Amount/5 ml, single dose vial (quantity/ml)

SF_6 microbubbles	40 µl (8 µL/ml)	40 µl (8 µL/ml)
Distearoylphosphatidylcholine (DSPC, (b) (4))	0.19 mg (0.04 mg/ml)	0.19 mg (0.04 mg/ml)
Dipalmitoylphosphatidicglycerol sodium salt (DPPG.Na, (b) (4))	0.19 mg (0.04 mg/ml)	0.19 mg (0.04 mg/ml)
Palmitic acid (physical stabilizer)	----	0.04 mg (0.008 mg/ml)
PEG 4000 (b) (4)	24.56 mg (4.9 mg/ml)	24.56 mg (4.9 mg/ml)
0.9% Saline	qs to 5 ml	qs to 5 ml

All pre-clinical studies in the original NDA (with the exception of the reproductive toxicology studies) were performed using the original formulation of SonoVue, which did not contain palmitic acid. No bridging studies were conducted using new formulation of SonoVue that contains palmitic acid.

Studies in this submission were conducted using the clinical formulation containing palmitic acid. It should be noted that although palmitic acid is an endogenous blood component (29 mg/L) and the 2ml clinical dose (0.0038 mg/L palmitic acid) of SonoVue does not significantly alter the concentration of palmitic acid in the blood, it is a part of the active moiety forming a monolayer around the microbubble along with other phospholipids.

3.2 PHARMACOLOGY

3.2.1 Introduction:

SonoVue is a second generation liquid contrast medium proposed for visualization of cardiac chambers and delineation of endocardial borders during echocardiography, (b) (4) According to the sponsor, the acoustic impedance of SonoVue microbubble gas is much lower than that of the surrounding aqueous medium, therefore, the interface between the SF_6 bubble and the aqueous medium acts as a reflector of the ultrasound beam. The ultrasound waves that are scattered and reflected at the microbubble-blood interface are visualized in the ultrasound image and result in an increased contrast between the blood and the surrounding tissues. After intravenous administration, SonoVue does not diffuse into the extravasal compartment, but remains within the blood vessels until the gas dissolves and is eliminated in expired air.

According to the sponsor, in clinical studies, a bolus injection of SonoVue produced marked increase in signal intensity of more than 2 minutes for B-mode imaging in echocardiography (b) (4)

3.2.2 Pharmacodynamic Studies:

Two pharmacodynamic studies were conducted using formulation of SonoVue with and without palmitic acid to show that addition of palmitic acid to the SonoVue formulation did not change overall efficacy and duration of imaging. According to the sponsor, palmitic acid is added to increase the shelf life of the product and does not affect efficacy or safety of the product. The two imaging studies conducted are described below.

3.2.2.1: Comparison of the efficacy of SonoVue batches (with palmitic acid) and BR1 batches (without palmitic acid) on left heart opacification in minipigs

Study no.: **ECHO207**
Volume #, and page #: 42, 1
Conducting laboratory and location: Bracco Research SA, Switzerland
GLP compliance: No

Methods:

SonoVue batches with palmitic acid (1A008, 1A009 & 1A010: commercial batches produced on the manufacturing line) and without palmitic acid (BG1189, BG1220 & BG1221) in the formulation were compared in minipigs (n=4) at different doses (0.0004, 0.003, 0.01 and 0.05 ml/kg, 0.1-1 times clinical dose based on body surface area) by assessing left ventricle opacification. The imaging modes used were: B-mode imaging, real time harmonic B-mode and real time B-mode pulse inversion. The ultrasound equipment (ATL HDI 5000), the transducer, the ultrasound frequency and the imaging modes were

similar as used in clinical practice. For each imaging mode, each animal received one vial of SonoVue (with palmitic acid) and one vial of BR1 (without palmitic acid). In addition, bubble size distributions in SonoVue batches were determined using Coutler Multisizer II.

Results:

No substantial differences were observed in bubble numbers and size distributions between SonoVue batches with and without palmitic acid in the formulation. (b) (4)

(b) (4)

Intravenous administration of SonoVue (with and without palmitic acid) produced dose-related contrast enhancement in the left ventricular heart cavity in all three imaging modes tested. No statistical difference ($P < 0.05$) was observed between the batches with and without palmitic acid in terms of intensity and persistence of the contrast effect for all three imaging modes tested. The data for imaging intensity (mean of baseline-subtracted imaging intensity, Imax) and persistence of contrast effect (mean of AUC, area under the time-pixel intensity curve) is summarized.

Dose (ml/kg)	Without palmitic acid: Imax (mean)	AUC (mean)	With palmitic acid: Imax (mean)	AUC (mean)
(A) Fundamental B-mode:	(b) (4)			
0.0004			32	154
0.003			65	684
0.01			77	1379
0.03			75	1971
0.05			79	2608
(B) Harmonic B-mode:				
0.004			81	572
0.003			132	2067
0.01			143	3584
0.03			142	4954
0.05			140	6167
(C) B-mode Pulse Inversion:				
0.004			158	1413
0.003			214	4526
0.01			215	8322
0.03			223	12123
0.05			221	15085

It was concluded that the addition of palmitic acid in the formulation during the development of SonoVue did not significantly ($P < 0.05$) modify the ultrasound performance of SonoVue.

Reviewer's comments:

According to the sponsor, palmitic acid was added to improve the shelf stability of the product and it does not affect efficacy of the product. It seems addition of palmitic acid did not affect the intensity (Imax) of imaging but the persistence of contrast effect (AUC) was lower. However, these differences were not statistically significant ($P < 0.05$) and some variation in duration/persistence of contrast effect is expected due to lingering effect of few of the remaining intact microbubbles in the circulation. We concur with the sponsor's conclusion that addition of palmitic acid does not change significantly the efficacy of the product under different imaging modalities (fundamental B-mode, harmonic B-mode and B-mode pulse inversion) that are clinically relevant.

(b) (4)

The data related to number and size distribution of bubbles in various SonoVue batches has been reviewed in detail by Chemistry Reviewer. Please refer to the chemistry review for more details and comments on the study.

3.2.2.2: In vitro study of the sensitivity of SonoVue microbubbles to ultrasound exposure- Comparison between the formulations with and without palmitic acid

Study no.: **BRG 005**
Volume #, and page #: 42, 65
Conducting laboratory and location: Bracco Research SA, Switzerland
GLP compliance: No

Methods:

The objective of this study was to investigate *in vitro* the interaction between ultrasound and SonoVue microbubbles. The bubble concentrations and size distribution were determined using Coutler counter analysis before and after ultrasound exposure (at 2.5, 3.5, 5 and 7 MHz) at two power levels –9 dB and 0 dB. SonoVue batches with palmitic acid (1A008, 1A009 & 1A010) and without palmitic acid (BG1205, BG1220 & BG1221) in the formulation were used.

Results: Both formulations were sensitive to ultrasound exposure. (b) (4)

(b) (4)

Reviewer's comments:

The study indicates that microbubbles are sensitive to ultrasound exposure, in particular, at high acoustic power levels. At low acoustic power (-9 dB), less microbubble destruction occurred and frequency effect was minimal. However, extensive microbubble destruction was evident at higher acoustic power (0 dB) and lower frequencies. In terms of optimum imaging, low acoustic power level may be preferable and frequency employed should not affect imaging.

Microbubbles containing palmitic acid in the formulation were somewhat more resistant to destruction due to ultrasound exposure at low acoustic power. However, it should be noted that in earlier study (#ECHO 207), no significant differences in imaging were noted in the formulation with or without palmitic acid (as imaging intensity is directly correlated with intact microbubbles). This was an in vitro study and results obtained may not necessarily translate in an in vivo situation.

3.2.3 Safety Pharmacology:

(A) FDA request:

Safety pharmacology studies (CVS, respiratory and renal system):

These studies were carried out using formulation that did not contain palmitic acid, which is a part of the active moiety that stabilizes the lyophilizate. In addition, effects on ECG (continuous: before, during and up to 1-2 hrs post-administration) and renal function were not evaluated. In general, dose-multiples used for these studies were low (0.5-3 times clinical dose, mg/m^2) for establishing adequate safety profile of SonoVue.

To address these deficiencies, provide the following:

A comprehensive safety pharmacology study in a larger species (monkeys or dogs). This study should be carried out using clinical formulation of SonoVue containing palmitic acid, and at various dose levels (with high dose-multiples). The study should include complete battery of CVS (including continuous ECG monitoring, with emphasis on QT interval), CNS, renal and respiratory (pulmonary arterial pressure/resistance etc.) parameters. It should be preferably carried out in unanesthetized animals and statistical analysis should be performed on the whole data generated.

In addition, effects on ECG should be evaluated at different MI (Mechanical index) settings that includes a highest MI value at which some effect is observed (microbubble bursting). Limited histopathology (heart) should be preformed for this study.

Sponsor's response:

In response, sponsor conducted following safety pharmacology studies:

a) Safety pharmacology study in conscious monkeys evaluating effects of SonoVue on CNS and CVS functions, including EKG.

b) A cardiovascular safety study in conscious dogs with 6 ECG leads under ultrasound exposure (at different MI values and systolic triggering).

c) Safety study examining effects of SonoVue on the renal function in rats.

These studies are reviewed below.

3.2.3.1: SonoVue-Effects on arterial blood pressure, heart rate, activity and ECG, following intravenous infusion (bolus) in the conscious cynomolgus monkey monitored by telemetry

Key study findings: No significant effects of SonoVue (1, 2 and 5 ml/kg, 3-17 times clinical dose) on arterial blood pressure, heart rate, various ECG parameters including QT/QTc, body temperature and locomotor activity.

Study no.: **886/019**
Volume #, and page #: 42, 79
Conducting laboratory and location: (b) (4)
Date of study initiation: May 14, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #: 2A005

Methods:

The effects of SonoVue on clinical signs, arterial BP, heart rate, locomotor activity, ECG (RR, PR, QT, QTc and QRS complex duration) and body temperature were examined in the conscious cynomolgus male (n=3) and female (n=3) monkey monitored by telemetry. The blood pressure and body temperature was recorded at pre-dosing, every 2 minutes for first 30 minutes post-dosing and at 1, 3, 6 and 8 hour post-dosing. The ECG recordings were analyzed at following time-points: pre-dosing, 10, 20, 30 minutes, 1, 3, 6 and 8-hour post-administration. The dose levels used were 0 (saline control), 1, 2 and 5 ml/kg and injection rate was 5 ml/min. Each animal received one dose (control or SonoVue dose)/day in a random manner. Each animal received all the doses (0, 1, 2 and 5 ml/kg) with 24-hour recovery period between the doses.

Results:

Clinical signs and locomotor activity: No remarkable observations at any dose levels.

Body Temperature: No significant effects on body temperature.

Arterial blood pressure: Mild but statistically significant increase in mean, systolic and diastolic blood pressure was observed following the administration of saline control as well as SonoVue dosing. This effect was usually <10% and lasting up to 10-15 minutes post-dosing. However, there was no dose-response relationship and this variability was observed at all doses including control. According to the sponsor, this effect is likely due to the presence of technicians in the room for treatments.

The figure below shows the effect of saline control and SonoVue at various dose levels on arterial blood pressure at various time-points for the first 30 minutes post-administration.

Heart rate: No consistent significant effect on heart rate. The minor variations in the heart rate (<10% up to 15 minutes post-dosing) were not dose-response related. These minor effects may be due to recording in the conscious animals and presence of technicians in the room.

ECG parameters: There were no significant effects on any of the ECG parameters as compared to the control group, including QT/corrected QT using Fridercia or Bazett formulae.

Within groups, the decrease in RR (maximum 15%, from 327 msec to 279 msec) and QT interval (maximum 10% or 19 msec) within first 30 minutes was significant as compared to pre-dosing duration. However, this effect was observed in SonoVue-treated as well as in the control group. There was no dose-response relationship for this effect. This effect is not related to the test administration but to an increase in heart rate as difference was not significant when QT interval was normalized using Bazett's correction.

Reviewer's comments:

We concur with sponsor's conclusion that SonoVue (1, 2 and 5 ml/kg) did not significantly affect arterial blood pressure, heart rate, body temperature and locomotor activity as compared to the control group. Although, within group there were minor but significant (<15%) changes in the blood pressure and heart rate (as compared to pre-dosing baseline levels), these effects were transient, and observed in SonoVue-treated as well as control group. There was no dose-response relationship for this effect. The increase in blood pressure was significant at 1 ml/kg but not at 5 ml/kg. These effects are likely due to the recording in conscious animals as earlier studies in anesthetized rats and rabbits did not show any significant effect on the blood pressure or heart rate.

There were no significant differences in any of the ECG parameters between SonoVue-treated animal groups and control group. Although, decrease in RR and QT interval was observed (pre-dosing versus 30-minute post-dosing), this effect was seen in both control and SonoVue-treated animals, and QT effect was not significant when corrected for heart rate. No dose-response relationship was observed for this effect.

The ECG recording was conducted up to 8 hours post-dosing and analyzed at 10, 20 and 30 minutes post-dosing for the first 30 minutes. More frequent time points should have been selected for the first 30 minutes (every 1 or 2 minutes) to properly analyze the effect on ECG since PK profile of SonoVue shows that it is eliminated rapidly with half-life for the elimination of SF_6 gas ~2 minute. In another study (#886/021-RE) involving ultrasound exposure at various MI levels (comparable to clinical situation), sponsor did evaluate effects on ECG with analysis at more frequent time-points (every 2 minutes) for the first 30 minutes. In this study, no effect on ECG, including QT/QTc interval was observed. In addition, another study (#886-025) with more frequent time-points was conducted evaluating effects on ECG and pulmonary pressure in an animal model with acute pulmonary hypertension, and no effects on ECG parameters were reported. Thus, although present study lacks frequent time-points, taking into consideration all studies conducted for ECG evaluation, it is likely that SonoVue does not affect ECG.

The sponsor has conducted a clinical study (n=40) specifically looking at effects on ECG parameters at 0.1 and 0.5 (5-times clinical dose) ml/kg dose-levels. According to the sponsor, some minor QT prolongation as well as reduction was observed in this study, however, there was no significant difference between placebo and SonoVue-treated patients. Please see the Medical Officer's review for more details.

The NOAEL for this safety pharmacology study evaluating effects on cardiovascular and central nervous system was established at 5 ml/kg, which is 17 times the human dose based on body surface area.

3.2.3.2: Effects on ECG of SonoVue and ultrasound exposure at various mechanical index values in the conscious dog

Key study findings: No effects of SonoVue (0.1, 0.3 and 1 ml/kg, 0.5-5 times clinical dose) on cardiovascular function including ECG during ultrasound exposure at various MI values.

Study no.: **886/021-RE**
Volume #, and page #: 43, 01
Conducting laboratory and location: (b) (4)
Date of study initiation: May 21, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #: 2A005

Methods:

The objective of this study was to evaluate combined effect of SonoVue (0, 0.1, 0.3 and 1 ml/kg) and exposure to ultrasound at various mechanical index values (0.4, 0.8 and 1.2). 1.2 was the maximum mechanical index possible with the machine (ATL HDI 5000) used in real time imaging or triggered mode. Four male and female Beagle dogs each were used for this study. Each dog was tested at all the dose levels and MI values utilized. The experimental design of the study was as follows:

Sex and animal number	Male No. 361 and Female No. 365		Male No. 362 and Female No. 366		Male No. 363 and Female No. 367		Male No. 364 and Female No. 368	
Day	Dose	Mode	Dose	Mode	Dose	Mode	Dose	Mode
0	Control	MI 0.4	Control	MI 0.8	Control	Max MI FC	Control	Max MI FT
1	LD	MI 0.4	LD	MI 0.8	LD	Max MI FC	LD	Max MI FT
2	MD	MI 0.4	MD	MI 0.8	MD	Max MI FC	MD	Max MI FT
3	HD	MI 0.4	HD	MI 0.8	HD	Max MI FC	HD	Max MI FT
4	Control	MI 0.8	Control	MI 0.4	Control	Max MI FT	Control	Max MI FC
7	LD	MI 0.8	LD	MI 0.4	LD	Max MI FT	LD	Max MI FC
8	MD	MI 0.8	MD	MI 0.4	MD	Max MI FT	MD	Max MI FC
9	HD	MI 0.8	HD	MI 0.4	HD	Max MI FT	HD	Max MI FC
10	Control	Max MI FT	Control	Max MI FC	Control	MI 0.8	Control	MI 0.4
11	LD	Max MI FT	LD	Max MI FC	LD	MI 0.8	LD	MI 0.4
14	MD	Max MI FT	MD	Max MI FC	MD	MI 0.8	MD	MI 0.4
15	HD	Max MI FT	HD	Max MI FC	HD	MI 0.8	HD	MI 0.4
16*	HD	None	HD	None	HD	None	HD	None

Control: saline 1 mL/kg, intravenous route at rate of 4-6 mL/min
LD: low dose: SonoVue 0.1 mL/kg, intravenous route at rate of 4-6 mL/min
MD: mid dose: SonoVue 0.3 mL/kg, intravenous route at rate of 4-6 mL/min
HD: high dose: SonoVue 1.0 mL/kg, intravenous route at rate of 4-6 mL/min
Max MI FC and/or Max MI FT: maximum mechanical index of 1.2 available with the machine used in real time imaging (FC) or triggered mode (FT) (see below)

The doses were administered in a sequential manner. The frequency of administration was one dose and one MI value/day with a minimum of 24 hours recovery period between testing at next dose level and different MI value. Heart was exposed to ultrasound 1-minute pre-dosing and ultrasound was carried out for duration of approximately 5 minutes post-administration.

Clinical observations and body weight: Performed daily and body weight was recorded pre-dosing and on Day 10 and 16 (last day of testing).

CVS examinations: Heart rate, BP and continuous ECG recording were carried out up to 1-hour post-dosing. The ECG parameters evaluated were RR, PR, QT/QTc (Fridericia's correction formula) and QRS complex. The recording was continuous with analysis of time-points at 2-minute interval.

Clinical laboratory parameters: Total WBC count and aspartate aminotransferase were performed on each treatment day before and after the last cardiovascular recording.

Histopathology: All animals were sacrificed at the end of study and histopathological examination was performed for heart, aorta and lungs.

Results:

Clinical observations and body weight: No remarkable effects except agitation, salivation and mild tremors due to restraining of dogs for recordings.

CVS parameters: One female dog (1 ml/kg) out of total eight tested had consistent bradycardia (maximum 40% reduction in heart rate compared to pre-dosing) during first 10 minutes of post-administration, irrespective of the intensity of the ultrasound exposure. In the same dog, transient profound hypotension (60% decrease compared to pre-test value) was also observed. The sponsor states that this CVS adverse reaction is similar to the reaction sometimes observed in dogs with gaseous contrast agents. These effects were not seen in other animals, and no effect on blood pressure or heart rate was observed.

ECG parameters: There were no statistically significant effects at any dose level for SonoVue on any of the ECG parameters (including QT/QTc) during ultrasound exposure at various MI levels. The maximum QT prolongation seen was 18 msec in average but there was no dose-response relationship for this effect and this effect was normalized when corrected for heart rate using Bazzet or Fridercia's formula.

The figure below shows the effect of SonoVue at various dose levels on QT interval at maximum emission power/MI (1.2) employed. Similar results were obtained for other MI settings employed.

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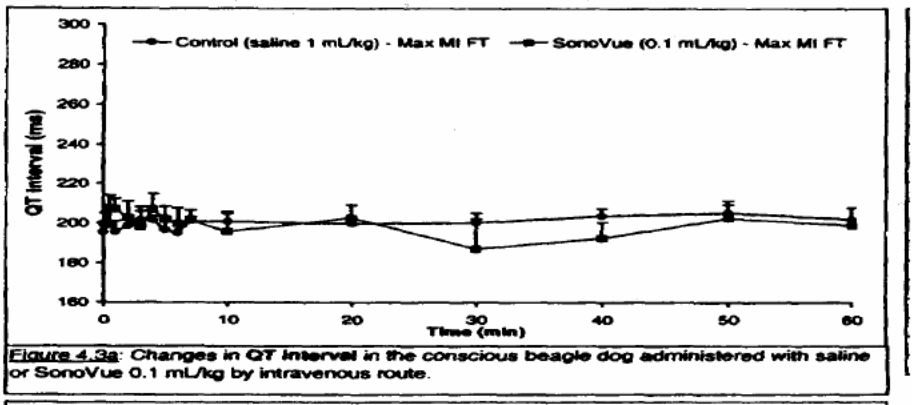

Figure 4.3a: Changes in QT interval in the conscious beagle dog administered with saline or SonoVue 0.1 mL/kg by intravenous route.

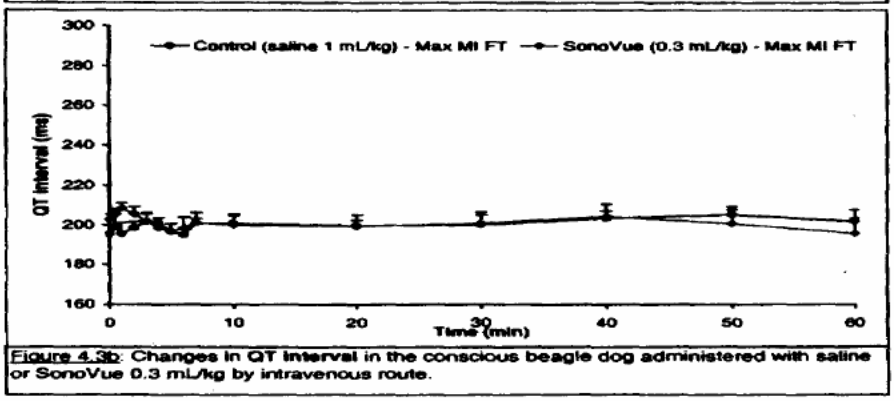

Figure 4.3b: Changes in QT interval in the conscious beagle dog administered with saline or SonoVue 0.3 mL/kg by intravenous route.

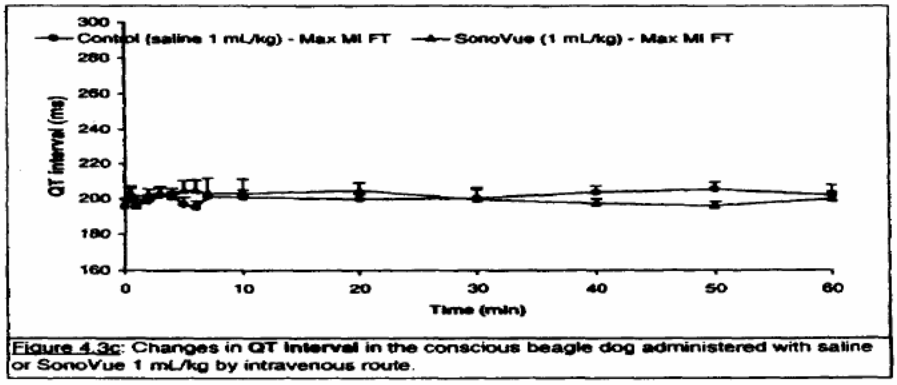

Figure 4.3c: Changes in QT interval in the conscious beagle dog administered with saline or SonoVue 1 mL/kg by intravenous route.

Clinical laboratory parameters: No significant differences from the control values for total WBC count and aspartate aminotransferase, which is nonspecific biomarker for damage/disease to heart.

Histopathology: There were no remarkable findings in the microscopic examination of heart.

The sponsor concluded that SonoVue at doses up to 1 ml/kg does not produce any deleterious effect on the cardiovascular function during ultrasound exposure at various MI values.

Reviewer's comments:

In clinical settings for optimal imaging purpose, microbubbles may be administered at different emission power levels/MI settings depending on the machine used (availability of maximum MI depends on machine employed). During clinical trials, MI values up to 1.2 were used. Ultrasound exposure at high intensity/MI settings can cause microbubble oscillations and sometimes bursting, which may adversely affect ECG. Therefore, study evaluating potential effects on ECG after ultrasound exposure at various MI settings is important.

Based on the data submitted, this reviewer concurs with the sponsor's conclusion that SonoVue in combination with ultrasound exposure at various MI levels did not adversely affect cardiovascular functions including ECG. Microscopic examination of heart did not reveal any remarkable findings. No increase in aspartate aminotransferase level was observed, which is nonspecific biomarker for damage to heart. The NOAEL for this study was established at 1 ml/kg, which is 5 times the human dose based on body surface area.

3.2.3.3: Effects of E7210 on water and electrolyte metabolism in rats

Key study findings: SonoVue (0.1, 0.3 and 1 ml/kg, 0.2-2 times clinical dose) did not affect renal function.

Study no.: **G00005**
Volume #, and page #: 43, 233
Conducting laboratory and location: (b) (4)
Date of study initiation: November 14, 2000
GLP compliance: Yes
QA report: Yes
Drug, lot #: CA58/25

Methods:

Species: Male SD rats (191-211 g).
Dose levels: 0 (saline), 0.3 and 1 ml/kg, intravenous. 8 rats/group.
Urinalysis: Urine volume and concentrations of urinary electrolytes (Na, K and Cl). The urine was collected for 5 hours post-dosing.

Results:

No statistically significant effects of SonoVue (0.3 & 1 ml/kg) on urine volume or concentrations of any of the urinary electrolytes as compared to control group. The highest dose tested (1 ml/kg) is 2-times the clinical dose based on body surface area.

The table below shows the effects of SonoVue on urine volume and the amount of urinary electrolytes in comparison with the saline control group.

Treatment	Mean Urine volume	Mean urinary electrolyte concentration

(intravenous)	(ml/kg/5 hr)	(mEq/kg/5 hr)		
		Sodium	Potassium	Chloride
Saline control (1 ml/kg)	10	1.82	0.79	2.07
SonoVue (0.3 ml/kg)	13.2	2.22	0.88	2.47
SonoVue (1 ml/kg)	13.7	2.17	0.89	2.47

Reviewer's comments:

We concur with sponsor's conclusion that SonoVue did not produce any significant effect on renal function. Based on the data submitted in the original NDA, SonoVue is unlikely to have adverse effect on renal function. Majority of SonoVue gas (>95%) is eliminated by exhalation and <1% is eliminated through urine.

(B) FDA request:

Animal model with compromised pulmonary function:

No studies were conducted in an animal model with a compromised pulmonary function. Therefore, it is not clear if certain population with pulmonary disease (such as patient with COPD) may be at greater risk for certain toxicities, such as increased pulmonary artery pressure resulting from microembolic events. A study in an appropriate animal model with pulmonary microvascular compromise is therefore recommended. Parameters such as pulmonary artery pressure/resistance, respiratory and cardiac functions (such as ECG) should be assessed.

Sponsor's response:

In response to above deficiency, a safety study evaluating effects of SonoVue in an acute model of pulmonary hypertension in anesthetized dogs was conducted. The study is reviewed below. Sponsor also stated that a clinical study in COPD patients was conducted and no adverse effects were seen.

3.2.3.4: A rising dose cardiovascular assessment of intravenously administered SonoVue in an acute model of pulmonary hypertension in anesthetized dogs

Key study findings: No adverse effects of SonoVue (0.1, 0.3 and 1 ml/kg, 0.5-5 times clinical dose) on pulmonary pressure or ECG.

Study no.: **886/025**
Volume #, and page #: 44, 41
Conducting laboratory and location: (b) (4)
Date of study initiation: October 4, 2002
GLP compliance: Yes

QA report: Yes
Drug, lot #: 2A005

Methods:

The objective of this study was to evaluate the effect of SonoVue on cardiovascular and pulmonary functions in a model of acute pulmonary arterial hypertension (PAH) in the anesthetized dogs.

Species: Male Beagle dogs (9-13 kg)
Dose levels: Three increasing doses of 0.1, 0.3 and 1 ml/kg administered at 15 minute interval resulting in a cumulative dose of 1.4 ml/kg (n=4). Additional four dogs were tested as control (saline) group. The drug was administered via cephalic vein.

PAH model: Pulmonary hypertension was induced by the injection, at 10-minute interval, of two sets of glass beads (150-212 micrometer). Each of these sets was administered at the dose of 4 g/m^2 body area through a catheter advanced up to the right ventricle of heart. PAH was considered as achieved when mean PAH increased by at least 35% from the pre-dosing baseline. The total amount of glass beads administered induced a sustained increase in pulmonary artery pressure on average from 12.1 to 22.8 mm Hg in the linear/sub-maximal region of the dose response.

SonoVue administration: SonoVue was administered after stabilization of the hemodynamic parameters. Also at the end of experiment, two sets of glass beads at the dose of 8 g/m^2 were injected again (at 10 minute interval) to 'check' further rise in PAH, indicating that SonoVue was administered in a sub-maximal region of the PAH dose-response curve.

CVS and respiratory parameters: Arterial blood pressure, heart rate, pulmonary arterial pressure, LVP, ECG, respiratory flow and rate were recorded continuously and analyzed every 1.5 minutes.

Results:

PAH model: During the testing period, the mean baseline PAP was 12.1 mm Hg (n=4) and administration of glass beads increased it to 22.8 mm Hg (92% increase). This increase in PAP was in the sub-maximal region as administration of glass beads again at the end of testing caused PAP to rise further to 40 mm Hg with 2/4 animals dead due to right ventricular failure (see below).

Effects of glass beads on CVS parameters in PAH model: Administration of beads itself produced significant fall in arterial pressure (~28%) and increase in the heart rate (~16%). As a consequence, the QT interval had a minor but consistent trend to shorten (from 211 at baseline to 202 msec after PAH). The glass beads had no remarkable effects on LVP but decreased the tidal volume (from 139 ml to 83 ml after PAH).

Effect of SonoVue on hemodynamic and pulmonary parameters in dogs with pre-existing pulmonary hypertension (PAH model): SonoVue in doses of 0.1, 0.3 and 1 ml/kg administered at 15 minute intervals had no significant effect on arterial blood pressure, heart rate or ECG including QT/QTc interval. Increasing doses, did not modify the myocardial (LVP and myocardial contractility) or the pulmonary (tidal volume and respiratory rate) functions.

Effects of SonoVue on Pulmonary artery pressure: At the highest dose tested (1 ml/kg), transient but significant mean increase of 2.5 mm Hg in pulmonary artery pressure was observed between 4.5 to 7.5 minutes post-administration in one of the four animals tested. This effect was more marked (increase by 7 mm Hg) in 1 out of 4 animals tested. There were no other remarkable effects at any dose level in terms of increase in PAP.

The figures below show the effects of SonoVue (0.1-1 ml/kg) on pulmonary arterial pressure in an animal model of acute pulmonary hypertension in dogs.

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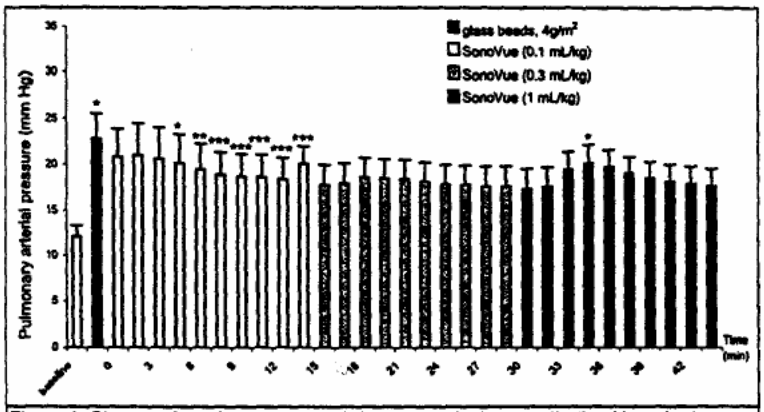


Figure 1: Changes in pulmonary arterial pressure in the anesthetized beagle dogs administered with SonoVue by intravenous route (n = 4).
Statistical results: * = p≤0.05; ** = p≤0.01; *** = p≤0.001

After administration of SonoVue (0.1 ml/kg, first dose administration), pre-existing pulmonary hypertension tended to somewhat decrease for the first 15 minutes (see figure below, decrease from mean 22.8 to 17.8 mm Hg) as compared with the level reached after the glass bead administration. According to the sponsor, this effect is unlikely due to the SonoVue administration but rather to an intrinsic tendency for the PAP sometimes to slightly drift over time as seen with the control animals.

At the end of testing, glass beads were administered again to check if the increase in PAP was not maximal and SonoVue effects were evaluated in the sub-maximal region. This second administration of glass beads caused further increase in PAP from mean of 22.8 mm Hg to 40 mm Hg, suggesting the PAP pressure increase was not maximal during the testing. Two of the four animals died subsequently of right ventricular failure due to second administration of glass beads. The table below shows the increase in PAP induced by glass beads pre- and post-SonoVue testing.

Glass beads administration	Maximum PAP increase/animal (mm Hg) #1	#2	#3	#4
Baseline	11.1	13.5	13.6	17.5
1st administration (4 g/m2, pre-testing)	22.0	14.8	17.0	24.5
2nd administration (4 g/m2, pre-testing): 10-minutes after 1st administration	34.4	18.5	21.2	29.1
1st administration (8 g/m2, at the end of testing)	40.8	31.9	27.8	54.5
2nd administration (8 g/m2, at the end of testing): 10-minutes after 1st administration	RVF	40.8	44.7	RVF

RVF: Right ventricular failure.

Reviewer's comments:

Patients with pulmonary disease such as COPD may be at greater risk for certain adverse events, such as increased pulmonary artery pressure resulting from microembolic events due to microbubble administration. Therefore, evaluating effects of SonoVue in an animal model with compromised pulmonary function was important.

Based on the data submitted, SonoVue at clinically equivalent doses (0.1 & 0.3 ml/kg, 1-2 times human dose) did not produce any increase in the PAP. At the highest dose tested (1 ml/kg, 5-times clinical dose), transient mean increase of 2.2 mm Hg was observed mainly due to one dog having PAP increase by 7 mm Hg. This effect lasted about 3 minutes post-dosing. Overall, there seems to be no adverse effect of SonoVue on PAP in an animal model with acute pulmonary hypertension.

There were no effects on blood pressure or heart rate in anesthetized dogs. This seems to be consistent with the studies conducted in the original NDA indicating no effect on CVS functions in anesthetized rats and rabbits. There were no adverse effects on ECG, including QT/QTc interval.

(C) FDA Request:

(b) (4)

5 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

(D) FDA request:

Ultrasound effects on SonoVue and the endothelium:

(b) (4)

provide the following:

a) Histopathological data after exposure to the range of available MI values. Areas studies must include the myocardium, kidney, carotids, lungs, abdominal vessels etc. Possible considerations should include endothelial injury.

b) Data to document fragility of the microspheres after exposure to the maximally available range of MI values.

c) Potential effects on cardiac electrophysiology (e.g., hERG potassium channel, action potential duration).

Sponsor's response:

a) A study evaluating histopathological examination of organs was carried out in rats after an intravenous administration up to 5 ml/kg and exposure to ultrasound at various MI values (0.4, 0.8 & 1.9). In this study, organs were individually exposed to the ultrasound.

b) The MI settings used for the above study was maximum commercially available and did produce microbubble destruction.

c) The FDA request is satisfied by the studies on ECG in animals based on the following from the consensus draft ICH guidelines S7B: Studies showed that there is no effect of SonoVue when administered alone or in combination with ultrasound exposure.

Reviewer's response:

a) Histopathological study evaluating potential for organ lesions induced by SonoVue after exposure to ultrasound at various MI settings has been reviewed in this section below.

b) The MI seetings in this study did produce microbubble destruction at MI values of 0.8 and 1.9.

c) Three CVS safety studies were conducted in this submission and no significant effects of SonoVue (3-15 times human dose) on ECG parameters, including QT/QTc, were noted. Microbubble agents act by acoustic energy mechanisms and are not expected to selectively affect/block hERG potassium channels. Potential effects on ECG are more likely due to nonspecific action involving microbubble oscillations or bursting during ultrasound exposure. In this regard, sponsor has conducted an ECG study evaluating effects of SonoVue microbubbles after ultrasound exposure at various emission power levels. No effect on ECG was noted in this study.

In addition, the sponsor has conducted a clinical study (n=40) evaluating effects on ECG in cardiac patients at 0.1 ml/kg and 0.5 ml/kg (5-times clinical dose). In this study, there were no remarkable differences in terms of effects of SonoVue on ECG parameters as compared to placebo. Please refer to Medical Officer's review for more details.

Based on above-mentioned reasons, I recommend that an in vitro electrophysiological study looking into effect on hERG potassium channel be waived.

3.2.3.6: Histopathological evaluation of organs in rats after an intravenous injection up to 5 ml/kg and exposure to ultrasound at various mechanical index values:

Key findings: Micro-hemorrhages/RBC suffusions in alveoli were noted in all animals including control groups. The presence and intensity of RBC suffusions was similar in control and SonoVue-treated animals. There are several published reports describing ultrasound-induced lung hemorrhage in small animals at clinically relevant and higher frequencies.

Study no.: **TOX-1-02**
Volume #, and page #: 44, 189
Conducting laboratory and location: Bracco Research SA, Switzerland
Date of study initiation: June 18, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #: 2A005

Methods:

The objective of this study was to evaluate whether a single injection of SonoVue induces internal organ lesions (in particular, heart and lung) during exposure to ultrasound at various mechanical index values.

Species: Female SD rats (average weight: 200 g)
Doses: 0 (saline), 1 and 5 ml/kg, Intravenous
Experimental design: Anesthetized animals were exposed to ultrasound (Acuson Sequoia, Siemens) starting ten seconds before the administration of SonoVue. The ventral face of the animal was shaved and covered with ultrasound gel. The organs were individually exposed in the following order: liver, spleen, left kidney, intestines and abdominal vessels, right kidney, stomach, pancreas, heart, thymus, lung and carotid arteries. The ultrasound exposure was repeated on these organs for a total duration of 5 minutes post-dosing. Three emission powers or Mechanical Index values (0.4, 0.8 and 1.9) were tested. There were total 9 groups with 6 rats/group. The animals were grouped as follows:

Group	Product	Dose (ml/kg)	Mechanical Index value
1	Saline	5	0.4
2	SonoVue	1	0.4
3	SonoVue	5	0.4
4	Saline	5	0.8
5	SonoVue	1	0.8
6	SonoVue	5	0.8
7	Saline	5	1.9
8	SonoVue	1	1.9
9	SonoVue	5	1.9

Histopathology: Rats were sacrificed one hour after the ultrasound exposure to perform necropsy and histopathological evaluation. Microscopic evaluation was carried out for following organs: Heart, lung, carotid arteries, thymus, kidneys, liver, spleen, stomach, intestines (duodenum, jejunum, ileum, colon and cecum) and pancreas.

Results:

Ultrasound effect on the microbubbles: Higher MI values (0.8 and 1.9) produced rapid disappearance of the echo signal indicating rapid destruction of the microbubbles at high ultrasound intensity. The signal intensity, on the same location in the liver under ultrasound

exposure was decreased by 80% at MI value of 0.8 or higher (SonoVue: 5 ml/kg). However, the lower MI value of 0.4 did not produce any decrease in the signal intensity suggesting that the microbubbles were not destroyed at lower MI value. No additional signal intensity data was provided.

Macroscopic findings: Small red areas/spots were observed on the lung surface (indicative of RBC accumulation/micro-hemorrhages) in all groups except groups 1 (saline control at 0.4 MI) and 8 (SonoVue, 1 ml/kg at 1.9 MI). These lung macroscopic lesions were observed in both saline (at MI 0.8 and 1.9) and SonoVue-treated animals irrespective of SonoVue dose employed. There were no other remarkable findings reported.

Microscopic findings: Examining the lung sections in a blinded fashion showed RBC suffusion in the alveoli in all animals including saline control groups. The intensity of it was graded on a 1 to 5 scale with 1 being minimal RBC presence and 5 being maximal RBC presence. Using this qualitative scale, the RBC accumulation in various groups was graded either 1 (minimal presence of few RBC in some dispersed alveoli) or 2 (foci of 10-20 alveoli filled with RBC). The intensity of RBC suffusion in the alveoli was a general phenomenon not related to SonoVue dose employed or the MI value of the ultrasound exposure. According to the sponsor, pulmonary lesions/lung hemorrhage in rats and mice after pulsed ultrasound exposure has been reported in the literature (references provided), and therefore, it is not related to SonoVue administration. The following table summarizes the histopathological findings for lung:

Group	1	2	3	4	5	6	7	8	9
MI value	0.4	0.4	0.4	0.8	0.8	0.8	1.9	1.9	1.9
SonoVue dose (ml/kg)	0*	1	5	0*	1	5	0*	1	5
RBC in alveoli/ micro-hemorrhage**: # of animals with minimal (Grade 1) RBC presence:	5	6	4	5	4	4	3	6	3
# of animals with slight (Grade 2) RBC presence:	--	--	2	1	2	2	3	--	3

*Saline control, 5 ml/kg, **Qualitative grading for RBC presence in alveoli on a scale of 1 to 5. Grade 1: minimal presence, 2: slight, 3: marked, 4:severe, 5:maximal.

In addition to micro-hemorrhages in lung, minimal blood suffusions were noted for mesenteric blood vessels (control & SonoVue-treated groups). The blood suffusion was mostly noted close to a broken vessel suggesting a possible post-mortem artifact during necropsy.

There were no other remarkable microscopic findings reported that could be attributed to the treatment of SonoVue and/or ultrasound exposure. In particular, there were no lesions noted for heart, intestinal tract (including cecum) or carotid artery.

Reviewer's comments:

The main findings in this study were presence of RBC suffusion or micro-hemorrhages in the pulmonary alveoli. The intensity and presence of suffusion was similar in both control and SonoVue-treated groups.

According to the sponsor, the pulmonary lesions observed in this study, were similar to those described in the lungs of rats and mice due to exposure to clinical diagnostic levels of ultrasound. Indeed, there are several published reports, including those noted by the sponsor (Kramer et al., J. Ultrasound Med., 1197-1206, 20, 2001; O'Brien et al., 267-277, 27, 2001 and Zachary et al., 829-839, 27, 2001), showing ultrasound-induced lung hemorrhage. The published studies reported lesions consisted of alveolar hemorrhage without apparent injury of alveolar septa or visceral pleura. Alveoli were packed with erythrocytes and occasional accretions of plasma proteins. This suggests that ultrasound exposure in small animals induces RBC suffusions/lung intra-parenchymal hemorrhage in densely micro-circulated organs such as lungs and this effect is not related to SonoVue dosing.

It is not clear why higher emission power levels (MI) did not produce hemorrhages stronger in intensity than those caused by lower MI values. However, some published studies do suggest that the lung hemorrhage during ultrasound exposure is more related to pressure and/or frequency employed than the MI used.

Perhaps larger animals would have been better suited for this study than rats as high ultrasound vibration/oscillation intensity can be more damaging in the small animals. Considering no adverse histopathological findings in heart and lungs (acute & repeat dose toxicity studies) and no adverse effects reported for respiratory function in various safety studies (including in an animal model with acute pulmonary hypertension); it is not necessary to repeat this study using larger animals.

(E) FDA request:

Effects on arterial blood gas:

Blood samples should have been taken within the first minutes of SonoVue administration, as some of the effects of microspheres on blood gases are fast and transient. The first time-point for blood sampling in this study was 5 minutes post-dosing. Also the blood elimination kinetics for SonoVue is very rapid ($t_{1/2}<1$ min). It is recommended that this study be repeated using higher doses of SonoVue (>1 ml/kg) and blood sampling carried out at multiple time-points immediately after SonoVue administration.

Sponsor's response:

The follow up of blood oxygen saturation was performed in various clinical studies (n=323 patients and 132 placebo) using a pulse oximeter. This method has the advantage to

produce real time measurements of blood oxygen saturation during and after injection of SonoVue. These measurements also involved three special population studies: subjects with congestive heart failure, COPD subjects and subjects with diffused interstitial pulmonary fibrosis. The time-points for the analysis in these studies were 30 sec post-dosing, and thereafter, every 1 or 2 minutes for the first 15 minutes post-dosing.

In all these clinical studies, no marked changes in oxygen saturation were noted from pre-dose to post-dose and no trends were observed across the dose levels (0.1-0.5 ml/kg) of SonoVue. In particular, in a clinical study in COPD patients no marked changes from baseline in oxygen saturation were observed at any time-points and the mean changes in oxygen saturation after SonoVue and placebo were similar in both COPD severity groups. Data from humans are particularly more valuable because they represent the circumstances of clinical use of the product.

In addition, pre-clinical data are in agreement with data from clinical studies, there were no post-dose abnormal respiratory clinical signs in rats or monkeys at high doses in toxicology studies, and no histopathological effects in lung were observed.

Since there were no changes in oxygen saturation in various clinical studies conducted, there is no point in measuring other blood gases or pH.

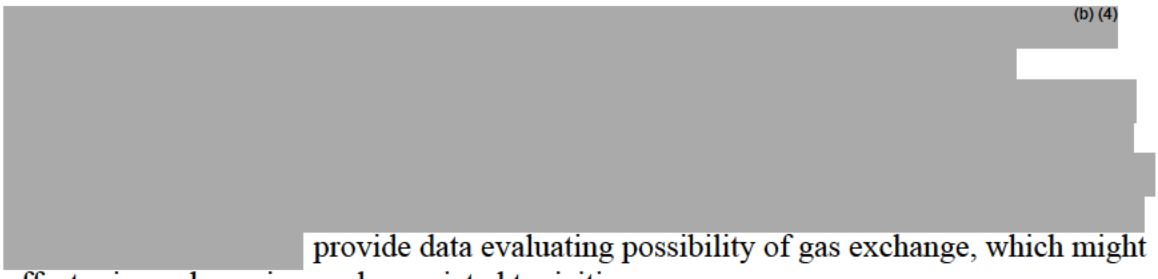
Reviewer's comments:

Considering no marked changes in oxygen saturation were noted in various clinical studies (including those involving congestive heart failure and COPD patients) involving frequent time-points (in particular, every 1 or 2 minutes for first 15 minutes post-dosing) for analysis, it is not necessary to repeat this study evaluating effects on arterial blood gases.

Please note no effects of microspheres on blood gases (PO2, PCO2 and pH) were noted in the original study with blood sampling time-points at 5 to 30 minutes post-dosing. Also safety pharmacology studies did not reveal any specific concern in terms of respiratory function. Therefore, repeating this study is not necessary.

(F) FDA request:

Exchange of gas and duration of microspheres:



provide data evaluating possibility of gas exchange, which might affect microsphere size, and associated toxicities.

Sponsor's response:

A research in vitro study examining stability of SonoVue microbubbles in gas-saturated media was performed. This study is reviewed below.

3.2.3.7: Stability of SonoVue microbubbles in gas-saturated media (plasma and saline)

Study no.: **BRG 011**
Volume #, and page #: 45, 83
Conducting laboratory and location: Bracco Research SA, Switzerland
Date of study initiation: May, 2002
GLP compliance: No
Drug, lot #: 1A008 and 1A010

Methods:

The objective of this in vitro study was to evaluate the behavior of SonoVue microbubbles after introduction into O_2 or CO_2-saturated human plasma and saline, and to establish whether gas exchange between bubbles and the surrounding medium could affect the bubble concentration, size distribution and stability.

After air evacuation using a vacuum pump, saline and plasma samples were instilled with O_2 or CO_2 under pressure and solution was vigorously shaken to ensure saturation. SonoVue samples (0.1 ml) were mixed with the gas saturated medium (0.9 ml) by rotary mixer. The bubble suspension was kept under agitation until analysis. 10-µL samples of bubble suspension were obtained at 0, 10 and 20 minutes post-mixing and evaluated for bubble concentration and size distribution using optical microscope. For each sample, 25 optical fields were scanned and a total of more than 10,000 microbubbles of all sizes combined were counted. Following parameters were evaluated: total microbubble concentration in number and in volume (MVC), bubble concentration between 2-8 µm, bubbles larger than 8 µm and smaller than 2 µm, mean number diameter (Dn) and largest bubble diameter detected in the samples (Dmax).

Results:

The stability of SonoVue microbubbles over time was similar for saline and plasma. Small bubbles (<2 µm) tended to disappear more rapidly in CO_2-saturated plasma than O_2-saturated plasma with a resultant decrease in the total concentration of bubbles. No bubble swelling as evidenced by an increase in MVC, Dn or Dmax as a function of time was noticed in saline or plasma irrespective of gas used. Actually decreases in MVC was observed consistent with corresponding decrease in total bubble concentrations. The sponsor concluded that increases in size of SonoVue microbubbles as a consequence of their exposure to environments saturated with O_2or CO_2 do not occur.

The table below summarizes the stability and other characteristics of SonoVue microbubbles in air, O_2 and CO_2-saturated human plasma as a function of time.

Time (min)	Bubble concentration (10^8/ml) Total	<2μm	2-8 μm	>8 μm	MVC (μl/ml)	Diameter (μm) Dn	Dmax
	Air-saturated plasma:						
0	3.3	1.6	1.7	0.05	9.4	2.7	20.1
10	3.1	1.5	1.5	0.04	8.0	2.6	14.6
20	3.0	1.4	1.5	0.04	8.7	2.8	20.7
	O_2-saturated plasma:						
0	3.0	1.5	1.5	0.04	8.3	2.7	16.5
10	2.8	1.4	1.4	0.04	7.7	2.7	14.9
20	2.7	1.3	1.3	0.04	7.5	2.8	17.6
	CO_2-saturated plasma:						
0	3.8	2.1	1.7	0.04	8.5	2.5	15.3
10	3.1	1.6	1.5	0.04	8.0	2.7	16.7
20	2.4	1.1	1.2	0.04	7.2	2.9	16.9

Reviewer's comments:

In vivo study directly evaluating gas exchange in a CO_2 saturated environment is difficult to perform. Therefore, sponsor performed an in vitro study using gas-saturated medium to mimic potential for in vivo gas exchange. Based on the data submitted, SonoVue microbubbles do not swell or increase in size in an O_2- or CO_2-saturated environment. Whether microbubbles behave in a similar fashion in vivo (i.e., no enlargement of bubble size) can not be confirmed, as such an in vivo study is not technically feasible. Given the technical limitations, the sponsor has adequately addressed the microsphere gas exchange issue.

The occurrence of cecal lesions in animals due to possible swelling/enlargement of bubbles in a high CO_2 environment is strain and species specific. It is observed in certain strains and mostly in smaller (mice or rats) but not larger animals (monkeys and dogs). The lesion occurrence in small animals is also dependent on the diet and microbial flora of the GI system. It has been suggested that the cecal lesions occur in certain species due to tissue supersaturation with CO_2 resulting from the intraluminal microbial gas production in the intestines. Clinical relevance of this adverse finding seen specifically in small animals is unclear.

3.3 PHARMACOKINETICS/TOXICOKINETICS

(G) FDA request:

The application lacks PK studies using a formulation of SonoVue with palmitic acid, which is a part of the active moiety and stabilizes the lyophilizate. To address this deficiency, provide the following the following:

1) A comprehensive PK study using clinical formulation of SonoVue containing palmitic acid.

2) The PK study in rabbits evaluated pharmacokinetic profile of SF6 gas, and not that of phospholipid-encapsulated SF6 microspheres. Please address (data/published articles) the fate of phospholipids and PEG 4000 after SonoVue administration.

3) Data on any microspheres (with or without SF6 gas as intact phospholipid shells) taken up by the liver cells (evaluated using light/electron microscopy).

4) Validation of the SF6 gas detection method (gas chromatography). Provide detailed data such as standard curve, linearity, sensitivity, limit of detection etc. for this method.

5) Clarification why the PK data indicating that majority of the SF6 gas is eliminated within the first minute of administration (>85% of ID) yet the echogenicity of tissues is maintained for a significantly longer time (5-10 minutes).

Sponsor's response:

1) Two PK studies with the SonoVue formulation containing palmitic acid performed in humans (BR1-010 and BR1-036) as well as imaging study in minipigs (Echo 207) did not show differences in pharmacodynamics between batches with and without palmitic acid.

During the Type C meeting held on April 23, 2002, FDA mentioned that PK study in animals using formulation containing palmitic acid may or may not be needed depending on resolution of CMC issues. As discussed in the CMC section, we consider that this issue has been resolved.

2) As discussed with FDA, the metabolic fate of the phospholipids is discussed based on the literature. This literature review is provided in Clinical Pharmacology section under Pharmacokinetics of the microsphere shell.

For the PK profile of PEG, data from published literature in mice showed that half-life of PEG 6000 is about 17 minutes after intravenous administration and that half-life increases with the PEG molecular weight. Published studies show that PEG 6000 does not accumulate in the carcass, and is not taken up by pinocytosis by Kupffer cells and peritoneal microphages as are PEGs of higher molecular weight (relevant references provided by the sponsor). A PK study in dog done with PEG 4000 showed that majority portion of it is excreted via the urine in 24 hours. Fully approved parenteral administration products containing PEG 5000 to pegylated the parent protein are Oncaspar, Pegademase and Ceredase.

In addition, a PK study using ^{14}C-PEG in the microbubble formulation that was close to SonoVue was performed in the early stages (ECHO II/93); the report of it is submitted with this submission.

3) Extensive histopathological studies of the liver have been performed in a number of single and repeat dose toxicity studies in rats and monkeys. No abnormal findings resulting from the uptake of phospholipid shells in liver cells have been detected. In conclusion, no abnormal findings, and particularly not the presence of microbubbles or phospholipids accumulation in the Kupffer cells or hepatocytes, were observed in the livers of rats, rabbits or monkeys after treatment with SonoVue.

4) Two validation reports for the clinical study BR1-010 were provided in the original NDA. For the methodology used for the pre-clinical study BIO 1/93 (SF6 PK in rabbits) the procedures and the assay precision data of the analysis of SF6 in expired air, heparinized whole blood, urine and dosing solution sampled during the study using a specific gas chromatography procedure are submitted.

5) Although, most of the SF6 gas is eliminated rapidly (1-2 minutes), echogenicity is still present at relatively longer time (5-10 minutes) because the ultrasound method is sensitive enough to detect only a few circulating microbubbles. The published literature suggests that a few thousands of microbubbles per ml are sufficient to generate a strong ultrasound backscatter signal. (b) (4) Thus, the low SF6 blood levels present should still provide ultrasound contrats enhancement at this time point (5 minutes). Contrast is still detectable at later time points (10 minutes) but is weaker.

Reviewer's Comments:

1) Based on the two PK studies conducted in humans with the formulation containing palmitic acid and resolution of CMC issues (please see, Chemistry review), PK study in animal using the formulation containing palmitic acid is not necessary.

Palmitic acid was added to increase the shelf-stability of the product. The efficacy study in minipigs indicated that palmitic acid in the formulation does not change the duration or intensity of imaging.

2) The study (ECHO II/93) examining PK profile of 14C-labeled PEG 4000 was reviewed and presented in this section below.

3) Although, sponsor did not perform study specifically looking into microspheres taken up by the liver cells, the acute and repeat dose studies in rat, rabbit and monkey conducted showed no abnormal histopathological findings in the liver and no evidence of microbubble presence or accumulation of phospholipids in the hepatocytes or Kupffer cells.

4) Validation data for the analytical procedure for SF6 gas detection method (gas chromatography) has been provided.

5) This reviewer concurs with the sponsor's explanation with regard to echogenicity of tissues maintained for a longer time (5-10 minutes) even though majority of SF6 gas is eliminated within the first minute of administration (>85% of ID).

Sponsor's explanations are satisfactory and a waiver to conduct PK study is acceptable.

3.3.1: Pharmacokinetics of ^{14}C-labeled PEG-ECHOII in the rat

Study no.: **ECHO II 93**
Volume #, and page #: 44, 154
Conducting laboratory and location: Bracco Research SA, Switzerland
Date of study initiation: February 1993
GLP compliance: No
QA report: No
Drug, lot #: JP/Ps 203

Methods:

Echo II was a research formulation of SonoVue of which 1% of the lyophilizate was constituted of the two phospholipids (b) (4) and DSPC instead of the phospholipids DSPC and DPPG in the clincial SonoVue formulation. The concentration of PEG 4000 in Echo II (995) was approximately same as in Sonovue ((b) (4)%).

14C-labeled PEG 4000-ECHO II (10 and 100 mg/kg corresponding to 2 and 20 ml/kg SonoVue doses) and PEG 4000 (10 mg/kg) itself were administered to rats intravenously. The experimental design for determining the PK profile was as follows:

Compound	# of animals	Type of study	Dose (mg dry matter/Kg)	Blood sampling times	Sacrifice times
14C-PEG-ECHO II	3	Blood kinetics/ Biodistribution	10	5, 15 min, 1, 2, 4, 7, 24 h	24 h
14C-PEG-ECHO II	3	Elimination/ Biodistribution	10	0-2, 2-4, 4-7, 7-24, 24-32, 32-48 h	48 h
14C-PEG-ECHO II	3	Blood kinetics/ Biodistribution	100	5, 15 min, 1, 2, 4, 7, 24 h	24 h
14C-PEG-ECHO II	3	Elimination/ biodistribution	100	0-2, 2-4, 4-7, 7-24, 24-32, 32-48 h	48 h
14C-PEG	2	Blood kinetics/ Biodistribution	10	5, 15, 30 min, 1, 2 h	24 h
14C-PEG	3	Elimination/ Biodistribution	10	0-2, 2-4, 4-7, 7-24, 24-32, 32-48 h	48 h
14C-PEG	2	Blood kinetics/ Biodistribution	10	0-2, 2-4, 4-7, 7-24, 24-32, 32-48 h	7 days

*For tissue distribution following tissue samples were taken: blood, liver, lung, kidney and spleen. Urine samples were collected up to 48 hour to examine elimination.

Results:

Blood kinetics: The radioactivity from 14C-labeled PEG ECHO II was rapidly eliminated from the blood circulation with concentrations falling to trace levels by 4 hour (<0.1% ID/ml-blood). Blood level decays were similar to those for 14C-labeled PEG 4000.

Time (min)	14C-PEG-ECHO II (10 mg/kg): %ID/ml-blood	14C-PEG-ECHO II (100 mg/kg): %ID/ml-blood	14C-PEG-4000 (10 mg/kg): %ID/ml-blood
5	1.1 9 (12.7% ID/blood)	1.1 (11.9% ID/blood)	0.9 (9.5% ID/blood)
15	0.33	.39	.37
30	--	--	.22
60	0.07	.08	.1
120	0.03	.02	.06
420	0.02	0	--
1440	0.01	0	0
AUC_{5-2440} [(mg/ml) min]	3.9	26	6.3
Clearance (ml/min/kg)	2.6	4.1	1.6

Tissue distribution: Very low concentrations of remaining radioactivity (<0.1% ID/g-tissue) observed for both 14C-labeled-ECHO II and 14C-PEG 4000 in liver, spleen, kidneys and lung at 24 and 48 hour sacrifice times. There was no remarkable difference between 10 and 100 mg/kg doses for ECHO II in terms of tissue distribution. The highest quantity of residual radioactivity was observed in liver with less than 0.5% of ID.

Elimination: More than 85% ID was recovered in urine by 24 hour (cumulative, first time-point for analysis) at 10 mg/kg dose level. At 100 mg/kg dose, the elimination was slow with 58% and 66% of ID recovered by 24 and 48 hours, respectively. While 70% of 14C-PEG 4000 was eliminated within first 2 hours (first time point of analysis) and 83% by 24 hours.

Reviewer's comments:

The blood kinetics, tissue distribution and elimination for ^{14}C-PEG contained in ECHO II formulation were similar to ^{14}C-PEG (non-pegylated). Although, this study is not comprehensive and formulation is somewhat different (see the methods section), it may be concluded that pegylated PEG 4000 does not accumulate in the tissues at clinically relevant doses (2 ml/kg, 3-times clinical dose), it is primarily eliminated via urine and its pharmacokinetic profile is similar to the free PEG.

3.4 TOXICOLOGY:

(H) FDA request:

The application lacks adequate information from the single dose acute toxicity study in CD (SD) rats. To address this deficiency, provide the following:

1) Any evidence of cecal lesions detected in this study (including histopathology) using the clinical formulation with palmitic acid.

2) Detection of any lung lesions/redness in any of the single or repeat-dose toxicology studies.

Sponsor's response:

1) A special single dose study was performed in rats at a dose of 20 ml/kg (33-times the human dose) to evaluate the presence of cecal lesions. This study (TOX-2-99) was carried out using a batch containing palmitic acid and submitted in the original NDA apllication. No cecal lesions were observed in this study after intravenous single-dose administration in rats.

2) No lesions in the lung that can be attributed to the administration of SonoVue have been observed in the single or repeat dose toxicity studies performed in rats and in monkeys. Also there were no indications of disturbance of pulmonary vascular or gas exchange functions in the studies conducted in monkeys and dogs.

Reviewer's comments:

1) Cecal lesions:

Microbubble agents can cause cecal lesions after acute and/or repeat administration in animals. The clinical significance of cecal lesions is however unclear since this adverse effect seems to be strain (observed in some strain of rats but not other, see below) and species specific (seen in smaller animals like rats and mice but not in bigger animals such as dog and monkey). The lesion occurrence in small animals is also dependent on the diet and microbial flora of the GI system. It has been suggested that cecal lesions in small animals occur due to tissue supersaturation with CO_2 resulting from the intraluminal microbial gas production in the intestines.

In case of SonoVue, the occurrence of cecal lesions seems to be strain and species specific. Cecal lesions characterized by erosions/ulceration of the mucosa were observed (at all dose levels) after repeat-dose administration in CD (SD) rats but not in Sprague-Dawley rats (single or repeat dose). No cecal lesions were detected in acute and repeat-dose study in monkeys.

Since the repeat dose in CD (SD) rats caused cecal lesions, the sponsor was specifically asked whether such adverse effect is also seen after single-dose administration in the CD (SD) strain of rats. The sponsor did conduct single-dose study in CD (SD) rats (submitted in original NDA), however, histopathology was limited and no cecum evaluation was done.

In response to this question, the sponsor referred to the study conducted in Sprague-Dawley rats (TOX-2-99) specifically examining presence of cecal lesions after single dose (20 ml/kg) administration. This study was reviewed in the original NDA. No cecal lesions were noted in this study.

Considering cecal lesion occurrence seems to be strain (seen in CD (SD) but not in Sprague-Dawley rats) and species (seen in rats but not in monkeys) specific, and clinical importance of it is unclear; the single-dose study in CD (SD) rats specifically examining ceccum is not necessary.

2) Lesions in lungs:

The acute and repeat dose toxicology studies were reviewed in the original NDA submission. Histopathological evaluation did not show any lung lesions after acute or repeat dose administration of SonoVue in rats and monkeys. The sponsor was asked this question to reconfirm that no lung lesions were detected since some microbubble agents can produce lesions in lung. The sponsor provided summary of histopatholgy evaluations conducted as part of single and repeat dose toxicity studies. These studies have already been reviewed in the original NDA submission. This reviewer agrees with the sponsor's conclusions about no lesions in the lung that can be attributed to the administration of SonoVue were observed in the single or repeat dose study.

3.4.1 Overall toxicology summary:

The only outstanding issue related to toxicological studies from original NDA application for SonoVue was about occurrence of cecal lesions in CD(SD) strain of rats. Cecal lesions characterized by erosions/ulceration of the mucosa were observed after repeat-dose administration in CD (SD) rats but not in Sprague-Dawley rats (single or repeat dose). No cecal lesions were detected in acute and repeat-dose study in monkeys or reproductive studies in rabbits. Since cecal lesion occurrence seems to be strain (seen in CD (SD) but not in Sprague-Dawley rats) and species (seen in rats but not in monkeys) specific, the single-dose study in CD (SD) rats specifically examining ceccum is not necessary. Such strain and species specific cecal lesions have been reported for other microbubble agents. Clinical relevance of this adverse effect finding observed in small animals remains unclear.

3.4.2 Single-dose toxicity: N/A

3.4.3 Repeat-dose toxicity: N/A

3.4.4. Genetic toxicology: N/A

3.4.5. Carcinogenicity: N/A

3.4.6. Reproductive toxicology: N/A

3.4.7 Local tolerance: N/A

3.4.8 Special toxicology studies:

(I) FDA request:

Immunotoxicity studies:

Please provide any pre-clinical data available evaluating the potential of SonoVue to cause an anaphylactic response or to exhibit antigenic properties.

Sponsor's response:

No signs of effects on the immune system were observed in any of the general toxicology studies. In particular, there were no abnormal findings in the histopathology of the lymphoid organs (lymph nodes, spleen, thymus). No change in body temperature or inflammatory reactions with infiltration of polymorphonuclear cells in the lungs was observed in the animal studies performe on SonoVue. Therefore, no additional testing is needed. This position is consistent with the guidance provided in the CDER draft document entitled, "Guidance for industry: Immunotoxicology evaluation of investigational new drugs".

Reviewer's comment:

We concur with sponsor's response that no immunotoxicology study is necessary since no signs of effects on the immune system were observed in any of the general toxicology studies. The sponsor was not asked to perform any specific immunotoxicity study but to provide any data if available.

3.5: Characteristics of SonoVue microbubbles:

(J) FDA request:

Provide data on the approximate number of microspheres injected, concentration and size range of the bubbles for pre-clinical studies conducted. Information about how the number of microbubbles injected was estimated.

Sponsor's response:

Concentration of microbubbles were determined using a Coutler Multisizer II (for details see the CMC section of the NDA).

The sponsor provided detailed data regarding characteristics of SonoVue microbubbles for all the batches used in pre-clinical studies and compared it with the industrial production batches. This data is briefly summarized in the table below.

(b) (4)

[redacted]

Reviewer's comments:

The total number of microbubles per ml was (b) (4)

[redacted]

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

In conclusion, the sponsor has satisfactorily addressed all the pre-clinical issues in this submission, and therefore approval is recommended for SonoVue from pharmacology and toxicology perspective (see executive summary for details).

Unresolved toxicology issues (if any): **None**

Recommendation: **Approvable**

3.7. APPENDIX/ATTACHMENTS: N/A

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

/s/

Tushar Kokate
2/27/04 08:49:11 PM
PHARMACOLOGIST
SonoVue Review (resubmission)

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3/1/04 09:53:20 AM
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