CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203684Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review

NDA	203-684
Submission Date	May 31, 2013 SDN 15
Type/Category	Resubmission (Class-2 - New Molecular Entity)
Brand Name	SonoVue (sulfur hexafluoride lipid microsphere) Kit for Preparation of Injectable Suspension (note: the trade name of the product is being negotiated, it was formerly called SonoVue)
Generic Name	Sulfur hexafluoride lipid microspheres
Proposed Indication	For use in patients with suboptimal echocardiograms to obtain left ventricle and to improve the delineation of the left ventricular endocardial border
Formulation	25 mg sterile, nonpyrogenic lyophilized powder in a (b) (4) sealed vial; upon reconstitution as directed, 1 mL of the resulting suspension contains 8 µL SF_6 in the microbubbles, equivalent to 45 µg
Dose Regimen	2 mL administered as an intravenous bolus injection during echocardiography. During a single examination, a second injection of 2 mL may be administered to prolong contrast enhancement. Each SonoVue injection should be followed by a flush with 5 mL of sodium chloride injection (0.9% w/v), USP
Route of Administration	Intravenous Injection
Applicant	Bracco Diagnostics, Inc.
Reviewing Division	Division of Clinical Pharmacology 5 (DCP 5)
Medical Division	Division of Medical Imaging Products (DMIP)
Primary Reviewer	Christy S. John, Ph.D.
Secondary Reviewer	Gene Williams, Ph.D.

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1. Executive Summary

The applicant, Bracco Diagnostics Inc. (Bracco) has resubmitted a 505(b)(1) new drug application NDA 203-684 for SonoVue™ (note: the trade name of the product is being negotiated, it was formerly called SonoVue) for use in echocardiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation. This is the fourth submission of this NDA. The most recent prior submission occurred on December 21, 2011. Bracco was issued a Complete Response letter due to the failed inspection of the Bracco Suisse manufacturing facility. Bracco was asked to amend the chemistry, manufacturing and controls (CMC) sections in Module 2 and Module 3 of the application to contain applicable information if resolution of the inspectional deficiencies required any new manufacturing and control procedures.

Each of the prior applications was reviewed by clinical pharmacology. The most recent prior review was that of the December 21, 2011 submission which was submitted to DARRTS August 24, 2012. There were no clinical pharmacology related issues. The application was found acceptable from a clinical pharmacology perspective, provided an agreement on package insert language was reached.

1.1. Recommendations

The Office of Clinical Pharmacology, Division of Clinical Pharmacology 5 has reviewed NDA 203-684. The application is acceptable from a clinical pharmacology standpoint, provided an agreement on package insert language can be reached.

1.2. Post-Marketing Requirements and Commitments

We have no recommendations for post-marketing requirements or commitments.

1.3. Summary of Important Clinical Pharmacology Findings

See the attached review of the most recent prior submission of this NDA (DARRTS date August 24, 2012).

2. Question Based Review

See the attached review of the most recent prior submission of this NDA (DARRTS date August 24, 2012).

3. Detailed Labeling Recommendations

See the attached review of the most recent prior submission of this NDA (DARRTS date August 24, 2012).

4. Appendices

4.1. Applicant's Proposed Package Insert (original, annotated)

4.2. Clinical pharmacology review of earlier submission of NDA 203-684 (DARRTS date August 24, 2012)

4.1. Applicant's Proposed Package Insert (original, annotated)

18 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

4.2. Clinical pharmacology review of earlier submission of NDA 203-684 (DARRTS date August 24, 2012)

Clinical Pharmacology Review

NDA:	203-684
Type/Category:	New NDA, Original-1 (Type 1- New Molecular Entity), 1S
Brand name:	SonoVue™
Generic name:	Sulfur hexafluoride microbubbles injection
Proposed indication:	SonoVue™ is indicated for use in echocardiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation
Formulation:	25 mg of lyophilized powder sealed under sulfur hexafluoride gas
Route of Administration:	Intravenous
Applicant:	Bracco Diagnostics Inc.
Reviewing Division:	Division of Clinical Pharmacology V (DCP 5)
Medical Division:	Division of Medical Imaging Products (DMIP)
Submission Dates:	December 21, 2011, SDN 1 July 23, 2012, SDN 10
Primary Reviewer:	Christy S. John, Ph.D.
Secondary Reviewer:	Gene Williams, Ph.D.

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1. Executive Summary

The applicant, Bracco Diagnostics Inc. (Bracco).has submitted a 505(b)(1) new drug application NDA 203-684 for SonoVue™ for use in echocardiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation.

There is no new clinical pharmacology information in this NDA. Most of the clinical pharmacology information was submitted in the initial NDA 21-315 dated January 29, 2001. The data was reviewed by the Office of Clinical Pharmacology and the NDA was found acceptable from the clinical pharmacology perspective. Clinical pharmacology comments were conveyed to the applicant and the applicant adequately addressed all the issues in a resubmission of the NDA dated June 30, 2003.

The current NDA 203-684 submission includes a summary of the data on the effect of sulfur hexafluoride microbubbles on QT_C. The data was consulted to CDER's Interdisciplinary Review Team for QT (the IRT) which elected to not conduct a formal review and made no recommendations for revision to the package insert.

1.1. Recommendations

The Office of Clinical Pharmacology, Division of Clinical Pharmacology V has reviewed the submission NDA 203-684. The application is acceptable from a clinical pharmacology standpoint, provided an agreement is reached on package insert language.

1.2. Phase 4 Requirements and Commitments

We have no recommendations for post-marketing requirements or commitments.

1.3. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Sonovue was administered to healthy subjects by intravenous bolus injections of 0.03 mL/kg and 0.3 mL/kg, corresponding to approximately the recommended dose (a fixed dose of 2.0 mL) and ten times the recommended dose. Concentrations of SF6 (the active moiety) in blood peaked within 1 to 2 minutes. The terminal half-life of SF6 was approximately 10 minutes for the 0.3 mL/kg dose (assay sensitivity was insufficient to estimate the half-life for the 0.03 mL/kg dose).

Steady-state volumes of distribution for SF6 were estimated as 341 L and 710 L for the SonoVue doses of 0.03 mL/kg and 0.3 mL/kg, respectively. Preferential distribution to the lung is likely responsible for these high values, which exceed total body volume.

SF6 is eliminated via the lungs. Twenty minutes following injection, the mean cumulative recovery of SF6 in expired air was 82 ± 20% at the 0.03 mL/kg dose and 88 ± 26% at the 0.3 mL/kg dose. SF6 undergoes a substantial degree of first pass elimination within the pulmonary circulation; approximately 40-50% of the SF6 content was eliminated in the expired air during the first minute post-administration.

In patients with pulmonary impairment, blood concentrations of SF6 peaked at 1 to 4 minutes following administration. The cumulative recovery of SF6 in expired air was 102 ± 18%, and the clearance and terminal half-life estimates for of SF6 were similar to those in healthy subjects. Based on pharmacokinetics, dosage adjustment is not necessary for patients with pulmonary impairment.

2. Question Based Review

2.1. What *In Vitro* and *In Vivo* Clinical Pharmacology and Biopharmaceutics studies and Clinical Studies contributed PK and/or PD information to the application?

Seven studies contributed clinical pharmacology information (two pharmacokinetic studies, one safety study, and four exploratory efficacy studies) (**Table 1.**). The formulations used for each of the studies are shown in **Table 2.**

Table 1. Studies contributing clinical pharmacology information, Bracco's Table A from NDA section 2.7.2 Summary of Clinical Pharmacology Studies

Table A: Clinical Pharmacology Studies

Protocol number	No. treated	Study design	SonoVue doses	Control Agent	Dose
Pharmacokinetic Studies					
Healthy Volunteers					
BR1-010[1]	12	Randomized, crossover	0.03, 0.3 mL/kg	--	--
Patients With Impaired Pulmonary Function					
BR1-036	13	Single dose	0.3 mL/kg	--	--
Safety Study					
Healthy Volunteers – B-mode Echocardiography					
BR1-025	33 (8)[a]	Dose escalating, sequential group	15 mg/mL, (0.02 mL/kg/min, for 6, 15, 30, 60 min) 1.8, 4.5, 9.0, 18 mg/kg	0.9% Saline	0.12, 0.3, 0.6, 1.2 mL/kg
Exploratory Studies					
Healthy Volunteers – B-mode Echocardiography					
BR1-001[2]	24 (12)[a]	Single ascending dose	0.003, 0.01, 0.03, 0.06, 0.09, 0.012 mL/kg	0.9% Saline	0.003, 0.01, 0.03, 0.06, 0.09, 0.012 mL/kg
BR1-002[2]	20 (10)[a]	Repeat ascending cumulative dose	0.15, 0.225, 0.325, 0.45, 0.60 mL/kg (cumulative)	0.9% Saline	0.15, 0.225, 0.325, 0.45, 0.60 mL/kg (cumulative)
BR1-007	10 (10)[a]	Repeat fixed dose	2 mL x 4 injections	--	--
Patients With Coronary Heart Disease - B-mode Echocardiography (Transthoracic)					
BR1-005	36	Open-label, crossover	0.5, 1.0, 2.0, 4.0 mL	--	--

[a] Number of patients who received SonoVue (number of patients who received control).
Table data *derived from individual clinical trial reports.*

Table 2. Formulations utilized for the studies in **Table 1.**, Bracco's Table B from 2.7.2 Summary of Clinical Pharmacology Studies

Table B: Formulations of SonoVue used in Clinical Pharmacology Studies

	Formulation 1	Formulation 2	Formulation 3	Formulation 4 - Final (Proposed for market)
Ingredients[a]	[redacted]	[redacted]	(b) (4)	25 mg (reconstituted in 5 mL)[b]
Active ingredient: sulfur hexafluoride (SF_6)				(b) (4)
Other ingredients:				
Polyethylene glycol (PEG, M.W. 4000)				24.56 mg
Distearoylphosphatidylcholine (DSPC)				0.19 mg
Dipalmitoylphosphatidylglycerol sodium (DPPG.Na)				0.19 mg
Palmitic acid				0.04 mg
Clinical pharmacology studies:	BR1-002	BR1-001, BR1-005	BR1-007	BR1-010, BR1-025, BR1-036

a All formulations reconstituted in 0.9% sodium chloride
b Except for study BR1-025 for which the reconstitution volume was reduced from 5.0 mL to 1.7 mL using the same 25-mg size vials of SonoVue.
Table data *derived from individual clinical trial reports.*

2.2. General Attributes of the Drug
2.3. General Clinical Pharmacology

For a detailed review of the data contained in the NDA, see the prior reviews:
Appendix 4.1. Clinical Pharmacology Review of initial NDA 21-315, and
Appendix 4.2. Clinical Pharmacology Review of re-submission of NDA 21-315.

Sonovue was administered to healthy subjects by intravenous bolus injections of 0.03 mL/kg and 0.3 mL/kg, corresponding to approximately the recommended dose (a fixed dose of 2.0 mL) and ten times the recommended dose. Concentrations of SF6 (the active moiety) in blood peaked within 1 to 2 minutes. The terminal half-life of SF6 was approximately 10 minutes for the 0.3 mL/kg dose (assay sensitivity was insufficient to estimate the half-life for the 0.03 mL/kg dose).

Steady-state volumes of distribution for SF6 were estimated as 341 L and 710 L for the SonoVue doses of 0.03 mL/kg and 0.3 mL/kg, respectively. Preferential distribution to the lung is likely responsible for these high values, which exceed total body volume.

SF6 is eliminated via the lungs. Twenty minutes following injection, the mean cumulative recovery of SF6 in expired air was 82 ± 20% at the 0.03 mL/kg dose and 88 ± 26% at the 0.3 mL/kg dose. SF6 undergoes a substantial degree of first pass elimination within the pulmonary circulation; approximately 40-50% of the SF6 content was eliminated in the expired air during the first minute post-administration.

At FDA's request, Bracco submitted a Highlights of Clinical Pharmacology Table in the standard format of CDER's Interdisciplinary Review Team for QT (the IRT). It is reproduced in review section **2.4.3 (Table 3.).**

2.4. Exposure-Response
2.4.1. What are the characteristics of the exposure-response relationship for effectiveness?

For a detailed review of the non-QT exposure-response data contained in the NDA, see the prior reviews:
Appendix 4.1. Clinical Pharmacology Review of initial NDA 21-315, and
Appendix 4.2. Clinical Pharmacology Review of re-submission of NDA 21-315.
A summary of the dose-response studies is presented, below.

Study BR1-001 tested six doses ranging from 0.003 mL/kg to 0.12 mL/kg. The balance between contrast enhancement and attenuation appeared to be optimal at the 0.01 mL/kg dose, while duration of contrast enhancement appeared to be optimal at doses of 0.06 and 0.09 mL/kg. Maximum enhancement of contrast was obtained at doses of 0.01 mL/kg and above. In the lowest dose group (0.003 mL/kg), all post- SonoVue images gave a moderate contrast enhancement. All post-injection images in the placebo group resulted in no visible contrast. The relationship between dose and enhancement score was statistically significant. For endocardial border definition, no dose effects were observed. However, improvement relative to baseline was seen with all doses of SonoVue, while no change in endocardial border definition score was observed with placebo

administration. The duration of contrast enhancement in the left ventricle increased as the dose of SonoVue increased, showing a clear dose-dependent trend from 0.003 to 0.09 mL/kg.

In Phase I Study BR1-002 (single center, single blind, placebo controlled, crossover), five dose groups were studied. A total of 30 healthy male were studied in the following five dose groups (30 minutes between each of the three doses administered):

Group 1: 0.025 + 0.05 + 0.075 mL/kg Total dose: 0.15 mL/kg
Group 2: 0.05 + 0.075 + 0.1 mL/kg Total dose: 0.225 mL/kg
Group 3: 0.075 + 0.1 + 0.15 mL/kg Total dose: 0.325 mL/kg
Group 4: 0.1 + 0.15 + 0.2 mL/kg Total dose: 0.45 mL/kg
Group 5: 0.15 + 0.2 + 0.25 mL/kg Total dose: 0.60 mL/kg.

Two readers concluded that all doses (0.025 to 0.25 mL/kg) resulted in enhancement of myocardial images obtained during echocardiography. Improvement with increasing doses was not mentioned.

In a Phase II Study (BR1-005), a multicenter (five centers), open-label, randomized, crossover study, patients received four doses of SonoVue, 0.5, 1, 2, and 4 mL (fixed doses – no adjustment for body weight), administered in randomized order. A 2D echocardiographic recording was obtained beginning 30 seconds prior to each injection of SonoVue and continuing for up to 5 minutes after each injection or until the end of the contrast effect. All four doses were effective in terms of improving the detection of endocardial border in echocardiographic images. There appeared to be improvement of detection with increasing dose and maximum detection was shown by the highest dose (4.0 mL). Both categorical assessment of endocardial border detection and the total score of endocardial border detection showed similar results.

In a Phase II, open-label, single-center, repeated-dose study (BR1-007), a fixed dose (2 mL) of SonoVue was used to study the efficacy of SonoVue as an echocardiographic contrast agent with multiple transducer frequencies. Ten healthy volunteers, male or female, at least 18 years of age, who underwent an apical four-chamber view echocardiogram (performed with a 5 MHz transducer) yielding images of acceptable quality were enrolled into the study. Two-dimensional echocardiographic images were obtained starting at least 30 seconds prior to each injection and continuing for at least 5 minutes or until the end of the contrast effect. The interval between injections was at least 10 minutes or until the contrast effect had disappeared. For the first three injections, three different transducer frequencies were employed (2.5, 3.5, and 5.0 MHz). The effect of trandsducer frequency on outcome is not mentioned, but it is stated that “good” opacification occurred with 3.5 MHz.

In conclusion, a range of doses from (0.025 – 0.09 mL/kg) appeared to be effective. A dose-response was observed: a 4 mL dose (approximately 0.06 mL/kg) resulted in higher contrast enhancement as compared to 2 mL. Nonetheless, the sponsor elected to study the lower dose of 2 mL for Phase 3 studies and marketing of SonoVue. As the lower dose proved clinically effective, the reviewer finds a dose of 2 mL acceptable.

2.4.2. Does this drug prolong QT/QTc Interval?

Bracco writes,
"Overall, the post dose changes in ECG parameters include small increases and decreases from baseline in the majority of patients across all time points. Most of the changes in QT and QTc values were <30 msec. Changes >60 msec were few and occurred at sporadic time points."
Bracco also writes,
"No clinically meaningful mean preinjection to postinjection changes in any ECG parameter values were observed."

No pharmacokinetics data were collected in the studies that contribute to the QT database. Review of the QT data was consulted to CDER's Interdisciplinary Review Team for QT (the IRT). At FDA's request, Bracco submitted a Highlights of Clinical Pharmacology Table using the IRT's standard format. It is reproduced, below (**Table 3.**).

Table 3. Highlights of Clinical Pharmacology Table, as submitted by Bracco

Therapeutic dose	SonoVue (sulfur hexafluoride microbubbles) is formulated as a 25 mg sterile, non-pyrogenic lyophilized powder (combination of pharmaceutical grade polyethylene glycol [PEG] 4000, phospholipids [PLs] and palmitic acid). The gas phase in the vial is SF6. After dispersion in 5 mL of sodium chloride injection, USP (0.9% w/v), 1 mL of the dispersion contains 8 µL SF6 in the microbubbles, equivalent to 45 µg. The recommended dose of SonoVue for left ventricular opacification and endocardial border delineation is 2 mL administered as an intravenous bolus injection during echocardiography. During a single examination, a second injection of 2 mL may be administered when deemed necessary. Each SonoVue injection should be followed by a flush with 5 ml of sodium chloride injection (0.9% w/v), USP.	
Maximum tolerated dose	No mortality, clinical signs or pathological findings seen in rats at 20 mL/kg. (BIO 1/93)	
Principal adverse events	In the 10 clinical pharmacology studies, the most frequently reported adverse reactions were: • headache (6 patients) • injection site pain (5 patients)	
Maximum dose tested	Single dose	The highest single dose tested was 0.3 mL/kg, approximately 10 times the recommended dose (BR1-010).

<table>
<tr><td></td><td>Multiple dose</td><td>In a repeated-dose study (BR1-002) in healthy subjects, 3 doses of increasing SonoVue volumes were administered by intravenous bolus injections to each subject.
Thirty minutes elapsed between injection of each dose.
Subjects in the highest dose group received the following multiple doses: 0.15, 0.2, 0.25 mL/kg.</td></tr>
<tr><td rowspan="2">Exposure achieved at maximum tested dose</td><td>Single dose</td><td>Cmax : Mean 3.531 ng/mL (50.2% CV)
AUC_{0-60} : Mean 10.26 ng.min/mL (32.7 % CV)
(Study BR1-010)</td></tr>
<tr><td>Multiple dose</td><td>Pharmacokinetic parameters were not assessed at multiple doses.</td></tr>
<tr><td>Range of linear PK</td><td colspan="2">0.03 to 0.3 mL/kg (BR1-010)</td></tr>
<tr><td>Accumulation at steady state</td><td colspan="2">Not applicable</td></tr>
<tr><td>Metabolites</td><td colspan="2">SF6 does not undergo biotransformation. Palmitic acid and the phospholipids, distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylglycerol (DPPG) sodium, present in trace quantities in SonoVue to form a monolayer around the gas bubble, are metabolized via normal metabolic routes in the body.</td></tr>
<tr><td rowspan="3">Absorption[a]</td><td>Absolute/Relative Bioavailability</td><td>• Mean 0.543 (28% CV) (note : high first pass lung extraction)</td></tr>
<tr><td>Tmax for parent</td><td>• Median 1.5 min (Range: 1.0-2.0 min)</td></tr>
<tr><td>Tmax for metabolites</td><td>Not applicable</td></tr>
<tr><td rowspan="2">Distribution[a]</td><td>Vd/F or Vd</td><td>Mean 710 L (52.7 % CV)</td></tr>
<tr><td>% bound</td><td>The binding of SF6 to plasma proteins and the partitioning into blood cells have not been studied.</td></tr>
<tr><td rowspan="3">Elimination[a]</td><td>Route</td><td>• Expired air : 93.9%</td></tr>
<tr><td>Terminal t½</td><td>• Gas (SF6) : Mean 9.88 min (88.4 % CV)</td></tr>
<tr><td>CL/F or CL</td><td>• Gas (SF6) Mean 8298 L/h (41.8 % CV)</td></tr>
<tr><td rowspan="3">Intrinsic Factors</td><td>Age</td><td>Not assessed</td></tr>
<tr><td>Sex</td><td>No gender differences in pharmacokinetics parameters
Although values of $AUC_{0-60min}$, C_{max}, apparent clearance and apparent volume of distribution were highly variable at each dose level, no consistent or clinically meaningful differences in any of these parameters were observed between males and females. (BR1-010)
<table>
<tr><th>Parameter</th><th>Subjects</th><th>Mean at 0.03 mL/kg</th><th>Mean at 0.3 mL/kg</th></tr>
<tr><td>$AUC_{0-60min}$ (ng.min/mL)</td><td>Males
Females</td><td>0.874
1.094</td><td>9.934
9.498</td></tr>
<tr><td>C_{max} (ng/mL)</td><td>Males
Females</td><td>0.390
0.541</td><td>3.823
2.436</td></tr>
</table></td></tr>
<tr><td>Race</td><td>Not assessed</td></tr>
</table>

<table>
<tr><td></td><td>Impaired pulmonary function</td><td>Compared with healthy subjects:<table><tr><th>Subjects</th><th>Mean Cmax (ng/mL)</th><th>AUC (ng.min/mL)</th></tr><tr><td>Healthy volunteers[a] (Study BR1-010)</td><td>3.17 (geometric)</td><td>AUC_{0-60} = 9.75</td></tr><tr><td>Impaired pulmonary function (Study BR1-036)</td><td>1.45 (arithmetic)</td><td>$AUC_{0-\infty}$ = 5.87</td></tr></table></td></tr>
<tr><td></td><td>Hepatic & Renal Impairment</td><td>Because SF6 is eliminated via the lungs, no studies of SF6 pharmacokinetics were performed in patients with renal or hepatic impairment. Negligible amounts of SF6 were recovered in the urine of rabbits injected with 0.3 or 1.0 mL/kg of SonoVue.</td></tr>
<tr><td>Extrinsic Factors</td><td>Drug interactions</td><td>No specific interaction studies have been performed in humans.
In preclinical studies, SonoVue did not interact with the action of aspirin in vitro, or with the action of antihypertensive drugs (captopril, propranolol, or nifedipine), heparin, isosorbide dinitrate, or digoxin in rats in vivo. (Studies SAF 3/97, BRF100, BRF101, BRF102, BRF104)
There was no apparent relationship with respect to occurrence of adverse events in the clinical studies for patients receiving various categories of the most common concomitant medications.</td></tr>
<tr><td></td><td>Food effects</td><td>Not assessed</td></tr>
<tr><td>Expected High Clinical Exposure Scenario</td><td colspan="2">Preclinical data in rabbit show that pharmacokinetics of SF6 is not saturated at least up to a dose of 1 mL/kg approximately 30 times the recommended dose (BIO 1/93). Therefore, Cmax and AUC are expected to increase linearly with the dose up to a dose of 1 mL/kg.</td></tr>
<tr><td colspan="3">a Values that are reported are for the 0.3 mL/kg dose in Study BR1-010.</td></tr>
</table>

After summarizing the submitted QT data, the IRT reviewer generated "Reviewer Comments" and "QT-IRT Comments for DMIP." They are reproduced (indented).

> Reviewer Comments: In studies BR1-112 and 113, two prospective controlled studies, continuous 12-lead ECG recordings were obtained from 3 hours prior to each dose of study drug through 24 hours postdose. ECGs were centrally read and interval measurement was performed by manual digitization with verification of interval measurements by blinded, board-certified cardiologists.
>
> In both studies mean QTc duration was similar in the placebo and treated groups, post dose QTcI did not exceed 500 ms. Maximal increases from baseline in study 112 were < 30 ms (upper bound of CI was 20 ms). A similar trend was seen in study 113. One subject had a

post-baseline increase > 60 ms in study 112; no subject had an increase > 60m s in study 113.

In study 112 one subject, experienced a transient ventricular extrasystoles (PVCs) 1 minute after administration of SonoVue 0.5 mL/kg; the Investigator considered the relationship to study agent to be unknown. This subject also had PVCs at screening ECG. In study 113, thirteen subjects reported PVCs, this was noted at pre and post-dose with similar incidence in placebo and study drug arms. No AEs of concern as per ICHE14 guidance were reported in these studies".

QT-IRT Comments for DMIP
We do not consider that a TQT study for SonoVue is needed. SonoVue will be given once and its systemic exposure is limited. The initial elimination is rapid (approximately 75% of the dose is eliminated by 11 minutes post-dose) and the terminal half life is 10 minutes. Since we have not performed a formal review of the ECG data proposed in the label we defer label revision to the review division.

2.5. Pharmacokinetics
2.6. Intrinsic Factors

For a detailed review of the data contained in the NDA, see the prior reviews:
Appendix 4.1. Clinical Pharmacology Review of initial NDA 21-315, and
Appendix 4.2. Clinical Pharmacology Review of re-submission of NDA 21-315.

In patients with pulmonary impairment, blood concentrations of SF6 peaked at 1 to 4 minutes following administration. The cumulative recovery of SF6 in expired air was 102 ± 18%, and the clearance and terminal half-life estimates for of SF6 were similar to those in healthy subjects. Based on pharmacokinetics, dosage adjustment is not necessary for patients with pulmonary impairment.

2.7. Extrinsic Factors
2.8. General Biopharmaceutics
2.9. Analytical Section

For a detailed review of the data contained in the NDA, see the prior reviews:
Appendix 4.1. Clinical Pharmacology Review of initial NDA 21-315, and
Appendix 4.2. Clinical Pharmacology Review of re-submission of NDA 21-315.

3. Detailed Labeling Recommendations

The reviewer's recommendations for changes to sections (b) (4) and **12 CLINICAL PHARMACOLOGY** of Bracco's proposed package insert are performed as "track changes" and begin on the next page.

2 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

4. Appendices

4.1. Clinical Pharmacology Review of initial NDA 21-315

4.2. Clinical Pharmacology Review of re-submission of NDA 21-315

4.3. Applicant's Proposed Package Insert (original, annotated)

4.4. Office of Clinical Pharmacology New Drug Application Filing and Review Form

Appendix 4.1. Clinical Pharmacology Review of initial NDA 21-315

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 21-315 REVIEWER: David J. Lee, Ph.D.

DRUG: SonoVue™
(Sulfur hexafluoride microbubbles for injection)
(2 mL [redacted] (b) (4) volume/injection)

SUBMISSION DATE: 1/29/01
STAMPED DATE: 1/31/01
REVIEW DATE: 8/31/01

APPLICANT: Bracco Diagnostics Inc. Princeton, NJ.

TYPE OF SUBMISSION: Original NDA (1S)

I. EXCUTIVE SUMMARY

Bracco Diagnostics Inc. has submitted a New Drug Application (21-315) on 1/29/01, and is seeking approval of SonoVue™ (Sonovue) as an ultrasound drug.

SonoVue (sulfur hexafluoride microbubbles) is indicated as an i.v. ultrasound contrast agent for use in echocardiography in patients with suspected or established cardiovascular diseases to improve visualization of cardiac chambers and endocaridial border delineation, which assists in the assessment of left ventricular wall motion. [redacted] (b) (4) The recommended dose stated in the Applicant's initial proposed package insert for endocardial border delineation is 2 mL administered as a bolus injection during echocardiography performed at rest or stress. During a single examination a second injection of 2 mL may be administered if deemed necessary by the physician. [redacted] (b) (4)

The to-be-marketed formulation was used in most of the clinical studies. To support the Human Pharmacokinetics and Bioavailability section (No.6) of NDA 21-315, the Applicant has submitted 2 studies (See appendix for individual study reviews).

The Applicant's proposed labeling appears to be adequate. With respect to safety, the Applicant stated that there were no clinically significant changes while receiving 0.03 and 0.3 mL/kg doses.

The following conclusions were made from the crossover study of single doses in healthy volunteers:

- SF_6 T_{max} in blood occured 1 to 2 minutes after intravenous administration of SonoVue and after reaching the maximum peak, it declined rapidly.
- For 0.03 mL/kg dose, SF_6 concentrations were usually below quantifiable levels 12 minutes after injection.
- For 0.3 mL/kg, the elimination half-life of SF_6 in blood averaged 9.9 minutes in healthy volunteers.
- The pharmacokinetics (PK) of SF_6 appeared to be dose-linear over 0.03 mL/kg to 0.3 mL/kg doses as observed by similar values for dose-normalized SF_6 $AUC_{(0\text{-}60min)}$ in blood.
- Most or all of the SF_6 from a SonoVue dose is eliminated by the lungs. The cumulative recovery of SF_6 in expired air averaged 86% to 94% of the administered dose.
- The first-pass extraction of SF_6 from blood as it passes through the lungs is estimated to be 41% to 46%, based on the fractional recovery of SF_6 in expired air during the first minute following SonoVue injection.
- The calculated total body clearance of SF_6 in blood was very high.

- No consistent or clinically relevant differences in pharmacokinetic parameters have been observed between men and women.

The following conclusions were made from the single-dose 0.3 mL/kg pharmacokinetic study in patients with pulmonary impairment:

- SF_6 T_{max} in blood occurred 1 to 4 minutes after administration.
- The mean elimination half-life (11.6 minutes) for SF_6 in blood was not markedly different from that observed in healthy subjects.
- The recovery of SF_6 in expired air averaged 102%. This finding may indicate that the patients eliminate virtually all of the SF_6 via the lungs.
- The total exposure to SF_6 appeared to be lower in patients with impaired lung function as compared with healthy volunteers (AUC of 5.87 and 10.26 ng.min/mL, for patients and healthy volunteers, respectively).
- The calculated total body clearance of SF_6 from blood in patients with pulmonary impairment was higher than observed in healthy volunteers.
- Within the group of patients with impaired lung function, the calculated total body clearance of SF_6 decreases with increasing degree of pulmonary dysfunction. However, nearly 100% of the gas was collected over time, adjustment of SonoVue dosage in patients with pulmonary impairment may be not needed.

A. RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation II (OCPB/DPE-2) has reviewed NDA # 21-315 for SonoVue which was submitted by the Applicant on 1/29/01.

The current NDA submission, The Human Pharmacokinetics and Bioavailability Section, contains sulfur hexafluoride (SF_6) pharmacokinetic information and negligible PK/PD data. Ideally "intact" microbubble pharmacokinetic information should have been provided with respect to the dosage proposed in the package insert.

In order to obtain this information in the future, the Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method so it could definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. Once such information is obtained the data/information should be submitted to the agency for review.

Regarding the approval status of the application, this submission is considered acceptable. The items covered under 'Comments to the Applicant' should be conveyed to the Applicant as appropriate.

_____ _____

David J. Lee, Ph.D. Date
Team Leader
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

_____ _____

FD – John Hunt, Deputy Director Date

CPB Briefing Meeting: ; Attendees:

CC: NDA 21-315 (orig., 1 copy)
HFD-160 (Division file, THarper)

HFD-850 (Lesko, Huang, LEEP)
HFD-870 (Malinowski, LeeD, HUNT)
Central Document Room

B. GENERAL REVIEW COMMENTS

1. SF_6 GAS PROTEIN BINDING INFORMATION

There is no SF_6 gas binding and distribution information in the submitted application. In the past, fluorocarbon gas binding information has been asked from the applicants. However, it should be noted that in-house and literature information suggests that fluorocarbon gases do not to bind to plasma proteins. Therefore, there is no concern on the fluorocarbon protein binding issue.

2. PHARMACOKINETICS OF THE MICROBUBBLE SHELL (DSPC*, DPPG* and palmitic acid)

It should be noted that lipid the PK of the components of the microbubble shell have not been characterized in the current submission. Regarding metabolism, the natural, semi-synthetic and synthetic lipids or surfactants undergo usual phospholipid metabolism processes.

The Applicant should submit any supporting data, e.g., in vitro metabolism on DSPC, DPPG. Additionally, the Applicant should submit information on palmitic acid metabolism. In the past, phospholipid metabolism information has been requested and submitted by the applicants.

Note: Distearoylphosphatidylcholine (DSPC)
Dipalmitoylphosphatidylglycerol sodium (DPPG.Na)

3. PHARMACOKINETICS OF THE *INTACT* MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of SonoVue microbubbles, i.e., the "intact SF_6- filled" microbubbles. However, the Applicant did assess the pharmacokinetics of SF_6 gas, some of which may or may not have been encapsulated.

Currently this reviewer is not aware of any analytical assay method, which could be utilized to detect this product's microbubbles in vivo. Therefore, it may not be possible at this time to characterize "intact" microbubbles in vivo due to the lack of assay methodology. However, the Applicant is encouraged to explore in vitro methods to provide information on the microbubbles in terms of microbubble "fragility and stability." One such in vitro method that can be explored is the microbubble "fragility" test: addition of the microbubbles to blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. In addition, the Applicant may explore relevant animal models (e.g., microscopic examination of nail-bed capillary or cannulated cat mesenteric artery), if any. The Applicant is encouraged to correspond with the Pharm/Tox review team to explore the feasibility of using animal models to obtain microsphere fragility information.

4. Blood and expired air sample assay

For BR1-010 study (healthy subject PK study), the Applicant needs to submit the assay information, e.g., standard curves, QC runs, etc., for the days the biological samples were actually analyzed. Additionally, individual subject SF_6 profiles for blood and expired air should be submitted.

However, the assay validation information (Validation of an analytical procedures for SF6 in blood and air samples) appears acceptable from the submitted data (standard curve generation, peak separation between SF_6.

5. Proposed Labeling

The Applicant's proposed Clinical Pharmacology and Dosage Administration sections of the Labeling appear to be adequate.

C. COMMENTS TO THE APPLICANT

The following comments should be forwarded to the Applicant, as appropriate.

1. PHARMACOKINETICS OF INTACT MICROBUBBLES

The NDA submission does not contain any pharmacokinetic data to describe the "fate" of SonoVue bubbles, i.e., the "intact SF_6- filled" microbubbles. Ideally "intact" microbubble pharmacokinetic information is needed with respect to the dosage proposed in the package insert. The Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method in order to definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. In addition, the Applicant is encouraged to explore in vitro methods to provide information on the microbubbles in terms of microbubble "fragility and stability." One such in vitro method that can be explored is the microbubble "fragility" test: Addition of the microbubbles to blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. Furthermore, the Applicant may explore relevant animal models (e.g., microscopic examination of nail-bed capillary or cannulated cat mesenteric artery), if any. Once such information is obtained the data/information should be submitted to the agency for review.

2. PHARMACOKINETICS OF THE MICROSPHERE SHELL [lipids (DSPC, DPPG), and palmitic acid]

It should be noted that the pharmacokinetics of the lipid components of the microbubble shell have not been characterized in the current submission. Regarding metabolism, it is requested that supportive information, e.g., in vitro metabolism regarding lipids be submitted.

Note: Distearoylphosphatidylcholine (DSPC)
Dipalmitoylphosphatidylglycerol sodium (DPPG.Na)

3. BR1-010 STUDY SF_6 GAS ASSAY INFORMATION

For BR1-010 study (healthy subject pharmacokinetic study), assay information, e.g., standard curves, QC runs, etc., for the days biological samples were actually analyzed should be provided. Additionally, individual subject SF_6 profiles for blood and expired air should be submitted.

D. Phase IV Commitment

Ideally "intact" microbubble pharmacokinetic information should have been provided with respect to the dosage proposed in the package insert.

In order to obtain this information in the future, the Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method so it could definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. Once such information is obtained the data/information should be submitted to the agency for review.

II. Table of Contents

III. Summary of Clinical Pharmacology and Biopharmaceutical Findings

A. INTRODUCTION

Bracco Diagnostics Inc. has submitted a New Drug Application (21-315) on 1/29/01, and is seeking approval of SonoVue™ (Sonovue) as an ultrasound drug.

SonoVue (sulfur hexafluoride microbubbles) is indicated as an i.v. ultrasound contrast agent for use in echocardiography in patients with suspected or established cardiovascular diseases to improve visualization of cardiac chambers and endocaridial border delineation, which assists in the assessment of left ventricular wall motion. (b) (4) The recommended dose stated in the Applicant's initial proposed package insert for endocardial border delineation is 2 mL administered as a bolus injection during echocardiography performed at rest or stress. During a single examination a second injection of 2 mL may be administered if deemed necessary by the physician.

(b) (4)

Clinical trials with SonoVue were developed and performed under IND 46,958. The to-be-marketed formulation was used in most of the clinical studies. To support the Human Pharmacokinetics and Bioavailability section (No.6) of NDA 21-315, the Applicant has submitted 2 studies (See appendix for individual study reviews).

Drug Formulation

TABLE A
FORMULATIONS OF SONOVUE USED IN CLINICAL TRIALS

	Formulation 1	Formulation 2	Formulation 3	Formulation 4 - Final (Proposed for market)
Ingredients[a] Active ingredient: sulfur hexafluoride (SF_6) Other ingredients: Polyethylene glycol (PEG, M.W. 4000) Distearoylphosphatidylcholine (DSPC) Dipalmitoylphosphatidylglycerol sodium (DPPG.Na) Palmitic acid	[redacted] (b) (4)			25 mg (reconst. in 5 mL)[b] [redacted] (b) (4) 24.56 mg 0.19 mg 0.19 mg 0.04 mg
Clinical studies:	BR1-002	BR1-001	BR1-007, BR1-008, BR1-009	BR1-010, BR1-025, BR1-026, BR1-036

a All formulations reconstituted in 0.9% sodium chloride
b Except for study BR1-025 for which the reconstitution volume was reduced from 5.0 mL to 1.7 mL using the same 25-mg size vials of SONOVUE

It should be noted that there are minor differences if Formulations 3 and 4 are compared.

B. BACKGROUND- MICROBUBBLE DYNAMICS

In general, ultrasound contrast agent development has focused on increasing the ultrasound reflectivity of blood, thereby enhancing the signals in proportion to the amount and velocity of moving blood within a vessel, tissue, or cavity, which allows for a more accurate assessment of anatomic definition. In this regard, the microbubble must be of a size to allow it to pass unimpeded through the pulmonary circulation, be nontoxic, and remain in concentrations that permit vascular and tissue augmentation of signals for a period of time sufficient to obtain the diagnostic information.

Microbubbles in general relies on the compressibility of gases enclosed within microbubbles to enhance the reflectivity of ultrasound waves. In the past some microbubbles used air only as enclosed gas within microbubbles. It is speculated that SF6 gas provides longevity to the microbubble. Additionally, it is speculated in the microbubble theories that the internal gas will exert a pressure which is equivalent of the external pressure applied by the environment (Laplace pressure). The phospholipid layer acts as a skin of the microbubble, maintaining the surface tension.

C. INDICATIONS AND USAGE (According to the Package Insert)

[redacted] (b) (4)

(b) (4)

D. DOSAGE AND ADMINISTRATION (According to the Package Insert)

(b) (4)

(b) (4)

(b) (4)

E. CHEMISTRY

1. SF_6 GAS CHEMISTRY

Description and Characterization

Chemical Name:	Sulfur Hexafluoride
Generic Name:	Sulfur Hexafluoride
CAS registry number:	2551-62-4
USAN name:	Sulfur Hexafluoride

Physical Characteristics

Color and appearance:	colorless, odorless gas
Molecular Weight:	146.05
Solubility in saline:	0.0045 v/v 367 torr @ 37°C
Stability:	Inert.

IUPAC: International Union of Pure and Applied Chemistry
CAS: Chemical Abstracts Service
USAN: United States Adopted Name

2. TO-BE-MARKETED FORMULATION

INGREDIENTS IN SONOVUE FOLLOWING RECONSTITUTION OF THE LYOPHILIZED POWDER WITH STERILE 0.9% SODIUM CHLORIDE FOR INJECTION.

Ingredient	Quantity (per mL of Reconstituted SONOVUE)
SF_6 within microbubbles	(b) (4)
Distearoyl phosphatidylcholine (DSPC)	
Dipalmitoyl phosphatidylglycerol (DPPG.Na)	
Palmitic acid	
Polyethylene glycol (PEG) 4000	
0.9% Sodium chloride for injection	

Formulations used in PK studies:

Study Number	Injection Batch No.	Dosage Form and Container[a]	Manufacturer	Formulation				Production		
				PEG (mg/mL)	Phospholipids (DSPC/DPPG 1:1 w/w) (mg/mL)	Palmitic Acid (mg/mL)	SF_6 (µL/mL)	Batch Size (Scale)	Batch Size	Terminal Sterilization
BR1-010	CA39	SONOVUE (25 mg of lyophilized powder) in gaseous atmosphere in a (b) (4) vial	Bracco Research S. A. Geneva, Switzerland	(b) (4)						
BR1-036	CA54/25	SONOVUE (25 mg of lyophilized powder) in gaseous atmosphere in an (b) (4) vial	Bracco Research S. A. Geneva, Switzerland	(b) (4)						

Abbreviations: PEG = polyethylene glycol, DSPC = distearoyl phosphatidylcholine, DPPG = dipalmitoyl phosphatidylglycerol, SF_6 = sulfur hexafluoride.
[a] Supplied with a 5 mL ampoule of sterile 0.9% sodium chloride for injection to be used to reconstitute the powder

IV. QUESTIONS

What is SonoVue?

SonoVue is formulated in the form of a lyophilizate made of PEG 4000, highly dispersed phospholipids and palmitic acid, packaged in a (b) (4) sealed vial containing sulfur hexafluoride (SF_6) in the gas phase. Following the addition of saline and vigorously shaking for (b) (4) seconds, the phospholipids readily migrate to the gas/liquid phase interface leading to the formation of SF_6 microbubbles stabilized by a lipid layer. It is thought that microbubbles are stable for 6 hours at room temperature. Microbubbles used for contrast enhancement during ultrasound imaging procedures. Microbubbles are filled with gas that is presumed to provide a highly compressible surface which reflects ultrasound waves.

According to the Applicant, clinical studies have shown that SonoVue is effective when administered at doses of 0.5, 1, 2 and 4 mL (approx. 0.007 to 0.06 mL/kg for a 70 kg subject).

How does SonoVue work?

SonoVue is presumed to increase the ultrasound reflectivity of blood which enhance the ultrasound signals. As with other microbubbles, SonoVue relies on the compressibility of gases enclosed within microbubbles to enhance the reflectivity of ultrasound waves. In the past some microbubbles used air only as enclosed gas within microbubbles.

The diluted vapor of SF_6 is used to provide longevity to the microbubbles, as compared to that of air-filled microbubbles.

Is there a PK/PD relationship for SonoVue?

There is no studies conducted to collect pharmacokinetic parameters with dynamic data for SonoVue. A typical dynamic end point is decrease or increase in signal intensity.

What is the optimal dose for SonoVue?

The recommended dose is 2 (b) (4) mL as an intravenous bolus. A second dose may be permitted. No injection rate information has been submitted in the NDA. In the submitted PK studies, the Applicant utilized 0.03 and 0.3 mL/kg doses. Presuming an average adult weight of 70 kg, the total volume injected should be approximately 2 mL. Additionally, the PK parameters from the two doses showed dose linearity.

Is metabolism information available for phospholipids or palmitic acid used in the microbubbles?

No. The Applicant did not submit any specific information regarding the phospholipids (distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylglycerol sodium (DPPG.Na)) or palmitic acid.

It appears that both lipids exist naturally in the biological systems. The literature data* showed that phospholipids are nontoxic. Phospholipids, which are the main constituents of natural cellular membranes, have been used in a wide variety of pharmaceutical, nutritional, and cosmetic products.

The metabolism of phospholipids involve the phospholipases A1, A2, C, and D. Phospholipases A1 and A2 are responsible for cleaving the fatty acid ester bonds, while phospholipases C and D cleave at the head group phosphoester bonds. The hydrolysis products formed are available for further degradation or for reincorporation into the same or other lipids. Thus, phospholipases A1 and A2 release fatty acids that can be degraded by beta-oxidation or reincorporated into phospholipids, triglycerides, sphingomyelin, etc. Phospholipase C forms diacylglycerol, which can act as a mediator of cell signal transduction and/or may be further metabolized by diacylglycerol lipase to generate free fatty acids. Phosphatidic acid, which is initially generated by the action of phospholipase D, is also active in cell signal transduction and can be further metabolized to diacylglycerol.

***Literature references:**

1. Dodd DE, Brashear WT, Vinegar A. Metabolism and pharmacokinetics of selected halon replacement candidates. *Toxicol Lett.* 1993; 68: 37-47.
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3. Lopez-Berestein G, Kasi L, Rosenblum MG, et al. Clinical Pharmacology of 99mTc-labelled liposomes in patients wi h cancer. *Cancer Res.* 1984; 44: 375-378.

4. Allen, TM. Toxicity and systemic effects of phospholipids. In: Cevc G, ed. *Phospholipids Handbook.* New York, NY: Marcel Dekker; 1993; 801-816.
5. Scherphof, GL. Phospholipid metabolism in animal cells. In: Cevc G, ed. *Phospholipids Handbook.* New York, NY: Marcel Dekker; 1993; 777-800.
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7. Alliance Pharmaceutical Corp., Internal Report IMUS-041-ADME. Elimination of perfluorohexane from AFO150 expired air in rats with pharmacokinetic evalua ion of perfluorohexane in blood. August 1999. *(Included in Section* 5, *Nonclinical Pharmacology and Toxicology*)
8. Wei K, Jayaweera AR, Firoozan S, et al. Basis for detection of stenosis using venous administration of microbubbles during myocardial contrast echocardiography: bolus or continuous infusion? *J Am Coll Cardiol.* 1998; 32:252-60.

Is metabolism information available for SF6 gas?

SF_6 is an inert gas and is expected not to undergo metabolism. It should be eliminated rapidly as an unchanged drug in expired air.

What is the conclusion from the PK Studies?

With respect to safety, the Applicant stated that there were no clinically significant changes while receiving 0.03 and 0.3 mL/kg doses.

The following conclusions were made from the crossover study of single doses in healthy volunteers:

- SF_6 T_{max} in blood occured 1 to 2 minutes after intravenous administration of SonoVue and after reaching the maximum peak, it declined rapidly.
- For 0.03 mL/kg dose, SF_6 concentrations were usually below quantifiable levels 12 minutes after injection.
- For 0.3 mL/kg, the elimination half-life of SF_6 in blood averaged 9.9 minutes in healthy volunteers.
- The pharmacokinetics of SF_6 appeared to be dose-linear over 0.03 mL/kg to 0.3 mL/kg doses as observed by similar values for dose-normalized SF_6 $AUC_{(0\text{-}60min)}$ in blood.
- Most or all of the SF_6 from a SonoVue dose is eliminated by the lungs. The cumulative recovery of SF_6 in expired air averaged 86% to 94% of the administered dose.
- The first-pass extraction of SF_6 from blood as it passes through the lungs is estimated to be 41% to 46%, based on the fractional recovery of SF_6 in expired air during the first minute following SonoVue injection.
- The calculated total body clearance of SF_6 in blood was very high.
- No consistent or clinically relevant differences in pharmacokinetic parameters have been observed between men and women.

The following conclusions were made from the single-dose 0.3 mL/kg pharmacokinetic study in patients with pulmonary impairment:

- SF_6 T_{max} in blood occurred 1 to 4 minutes after administration.
- The mean elimination half-life (11.6 minutes) for SF_6 in blood was not markedly different from that observed in healthy subjects.
- The recovery of SF_6 in expired air averaged 102%. This finding may indicate that the patients eliminate virtually all of the SF_6 via the lungs.
- The total exposure to SF_6 appeared to be lower in patients with impaired lung function as compared with healthy volunteers (AUC of 5.87 and 10.26 ng.min/mL, for patients and healthy volunteers, respectively).
- The calculated total body clearance of SF_6 from blood in patients with pulmonary impairment was higher than observed in healthy volunteers.

- Within the group of patients with impaired lung function, the calculated total body clearance of SF_6 decreases with increasing degree of pulmonary dysfunction. However, nearly 100% of the gas was collected over time, adjustment of SonoVue dosage in patients with pulmonary impairment may be not needed.

What are the overall comparison between healthy and pulmonary patients?

Overall Comparison

PHARMACOKINETIC PARAMETERS OF SF_6 IN BLOOD OF PATIENTS WITH PULMONARY IMPAIRMENT AND HEALTHY SUBJECTS FOLLOWING INTRAVENOUS ADMINISTRATION OF SONOVUE 0.3 ML/KG.

Parameter	Patients With Pulmonary Impairment[a] (Study BR1-036)	Healthy Subjects[b] (Study BR1-010)
C_{max} (ng/mL)	1.45 ± 1.03 [c] 1.25 (0.35-3.79)	3.53 ± 1.77 3.08 (1.29-7.79)
T_{max} (min)	2.2 ± 0.8 2.0 (1.0-4.0)	1.5 ± 0.5 1.5 (1.0-2.0)
AUC (ng.min/mL)[d]	5.87 ± 4.04 4.40 (1.49-15.08)	10.260 ± 3.354 9.896 (5.486-15.589)
CL/F (L/hr)	20,520 ± 14,232 19,002 (5,208-49,566)	8,298 ± 3,466 7,873 (3,514-15,699)
$T½_{\lambda z}$ (min)	11.64 ± 9.23 8.57 (1.54-29.09)	9.88 ± 8.73 7.49 (1.88-32.95)

Abbreviations: $AUC_{(0-\infty)}$ = area under the blood-concentration time curve, C_{max} = maximum blood concentration, T_{max} = time of maximum blood concentration, CL/F = apparent total body clearance, $t½_{\lambda z}$ = terminal elimination half-life, Vss/F = apparent volume of distr bution at steady-state.
[a] N=12 patients with pulmonary impairment.
[b] N=12 healthy subjects.
[c] Values presented are arithmetic mean ± standard deviation and median (minimum – maximum).
[d] AUC is from time 0 to infinity for Study BR1-036 and from time 0 to 60 minutes for Study BR1-010.

Higher mean C_{max} estimates and lower T_{max} estimates were observed in healthy volunteers as compared with patients with impaired pulmonary function. The Applicant's rationale was that this may be due to the differences in administration time of SonoVue between the two studies (approximately 15 seconds in Study BR1-010 versus approximately 20 seconds in Study BR1-036). Half-life estimates were similar between the two studies with means of 11.6 minutes and 9.9 minutes for patients and healthy volunteers, respectively. Comparison of SF_6 AUC estimates indicate that the extent of exposure to SonoVue in healthy volunteers is approximately double the exposure achieved in patients.

Study Number	Route of Administration	Dose (mL/kg)	Analyte	C_{max} (ng/mL)	T_{max} (min)	V/F or Vss/F[a] (L)	$AUC_{(0-60)}$ (ng.min/mL)	$AUC_{(0-\infty)}$ (ng.min/mL)	$t½_{\lambda z}$ (min)	CL/F (L/hr)	Percent of Dose Eliminated via the Lungs
BR1-010	IV bolus	SONOVUE: 0.03 mL/kg	SF_6 in blood	0.499 (0.216)	1.3 (0.5)	341 (185)	1.024 (0.335)	ND	NC	8,506 (4,648)	NA
		SONOVUE: 0.3 mL/kg	SF_6 in blood	3.531 (1.774)	1.5 (0.5)	710 (374)	10.260 (3,354)	ND	9.88 (8.73)	8,298 (3,466)	NA
		SONOVUE: 0.03 mL/kg	SF_6 in expired air	NA	NA	NA	NA	NA	NA	NA	85.7 (22.3)
		SONOVUE: 0.3 mL/kg	SF_6 in expired air	NA	NA	NA	NA	NA	NA	NA	93.9 (27.4)
BR1-036	IV bolus	SONOVUE: 0.3 mL/kg	SF_6 in blood	1.45 (1.03)	2.2 (0.8)	4,045 (3,268)	ND	5.87 (4.04)	11.64 (9.23)	20,520 (14,232)	NA
			SF_6 in expired air	NA	NA	NA	NA	NA	31.32 (16.80)	NA	102.2 (18.4)

Abbreviations: SF_6 = sulfur hexafluoride, $AUC_{(0-60min)}$ = area under the blood-concentration time curve from time 0 to 60 minutes, $AUC_{(0-\infty)}$ = area under the blood-concentration time curve from time 0 to infinity, C_{max} = maximum blood concentration, T_{max} = time of the maximum blood concentration, $t½_{\lambda z}$ = terminal elimination half-life, CL/F = apparent total body clearance, V/F = apparent volume of distribution, Vss/F = apparent volume of distribution at steady-state, NA = Not applicable, NC = Not calculable, ND = Not done, SD = standard deviation.
Values presented are arithmetic mean (SD).
[a] Volume of distribution estimates are Vss/F in Study BR1-010 and V/F in Study BR1-036.

Note that AUC 0-60min for BR1-010 and AUC 0-inf for BR1-036 studies.

What is the major route of elimination for SonoVue?

The SF_6 gas is eliminated via the pulmonary route:

ELIMINATION OF SF_6 IN EXPIRED AIR FOLLOWING INTRAVENOUS ADMINISTRATION OF SONOVUE TO HEALTHY VOLUNTEERS.		
Parameter (expired air)		
Recovery (% of dose)	**0.03 mL/kg** 85.7 (22.3)	**0.3 mL/kg** 93.9 (27.4)

ELIMINATION OF SF_6 IN EXPIRED AIR FOLLOWING ADMINISTRATION OF SONOVUE 0.3 ML/KG TO PATIENTS WITH IMPAIRED LUNG FUNCTION.	
Parameter (expired air)	
Recovery (% of dose)	102.2 ± 18.4
$t½_{\lambda z}$ (min)	31.3 ± 16.8

Are there any gender differences observed for SonoVue?

The data showed no consistent gender differences in pharmacokinetic parameters.

PHARMACOKINETIC PARAMETERS OF SF_6 BY GENDER FOLLOWING ADMINISTRATION OF A 0.03 ML/KG DOSE OF SONOVUE TO HEALTHY VOLUNTEERS.

Parameter	Males (n = 7)	Females (n = 5)
$AUC_{(0-60\ min)}$ (ng.min/mL)	0.955 ± 0.381[a] 0.951 (0.326-1.466)	1.121 ± 0.268 1.087 (0.742-1.472)
C_{max} (ng/mL)	0.436 ± 0.187 0.517 (0.145-0.632)	0.587 ± 0.243 0.643 (0.282-0.860)
T_{max} (min)	1.4 ± 0.5 1.0 (1.0-2.0)	1.2 ± 0.5 1.0 (1.0-2.0)
CL/F (L/hr)	9,929 ± 5,736 8,246 (6,072-22,737)	6,514 ± 1,250 7,057 (4,359-7,441)
Vss/F (L)	402 ± 222 358 (225-893)	256 ± 69 224 (193-368)

Abbreviations: $AUC_{(0-60\ min)}$ = area under the blood-concentration time curve from time 0 to time 60 minutes post-dose, C_{max} = maximum blood concentration, CL/F = apparent total body clearance, Vss/F = apparent volume of distribution at steady-state.
[a] Values presented are arithmetic mean ± standard deviation and median (minimum-maximum).

PHARMACOKINETIC PARAMETERS OF SF_6 BY GENDER FOLLOWING ADMINISTRATION OF A 0.3 ML/KG DOSE OF SONOVUE TO HEALTHY VOLUNTEERS.

Parameter	Males (n = 7)	Females (n = 5)
$AUC_{(0-60\ min)}$ (ng.min/mL)	10.408 ± 3.368[a] 9.713 (5.862-14.881)	10.053 ± 3.718 10.080 (5.486-15.589)
C_{max} (ng/mL)	4.149 ± 1.898 3.660 (2.246-7.785)	2.666 ± 1.286 2.223 (1.292-4.691)
T_{max} (min)	1.3 ± 0.5 1.0 (1.0-2.0)	1.8 ± 0.5 2.0 (1.0-2.0)
CL/F (L/hr)	8,778 ± 4,149 7,633 (3,514-15,699)	7,626 ± 2,498 8,112 (4,644-10,693)
Vss/F (L)	635 ± 372 550 (322-1,365)	814 ± 393 808 (387-1,366)

Abbreviations: $AUC_{(0-60\ min)}$ = area under the blood-concentration time curve from time 0 to time 60 minutes post-dose, C_{max} = maximum blood concentration, CL/F = apparent total body clearance, Vss/F = apparent volume of distribution at steady-state.
[a] Values presented are arithmetic mean ± standard deviation and median (minimum-maximum).

Are there any differences due to age for SonoVue?

No, according to the overall comparison table above, there appears to be no age related differences.

What is the Applicant's position regarding pediatric population?

(b) (4)

Is dose adjustment needed in diffusion limited pulmonary patients?

No. See above, Elimination Route question.

What additional information is needed for SonoVue?

The Applicant should commit to conducting a study to measure the intact microbubbles, pending availability of the microbubble assay methodology. The currently submitted PK studies measured SF_6 gas, which is considered to be a biomarker for the intact microbubbles.

Are there any issues regarding assays?

With respect to SF_6 assay, since SF_6 does not undergo metabolism, the measurement of SF_6 by gas chromatography (GC) is appropriate.

The Applicant reported the following table regarding assay values.

Study Number	Matrix	Method	Analyte	Precision (%CV)	Linear Range[a]	Lower Limit of Quantification[a]
BR1-010	Blood	GC-ECD	SF_6	ND	0.024 to 0.23 ng/mL (low range) 0.23 to 1.3 ng/mL (high range)	0.024 ng/mL
BR1-010	Expired Air	GC-ECD	SF_6	ND	0.024 to 0.39 ng/mL (low range) 0.39 to 1.36 ng/mL (high range)	0.030 ng/mL
BR1-036	Blood	GC-ECD	SF_6	≤20.1% (concurrent QC samples)	0.024 – 1.4C5 ng/mL	0.024 ng/mL
BR1-036	Expired Air	GC-ECD	SF_6	≤17.7% (concurrent QC samples)	0.024 – 1.4C5 ng/mL	0.024 ng/mL

Abbreviations: GC-ECD = Gas Chromatography with Electron Capture Detection, SF_6 = sulfur hexafluoride, ND = Not Determinable.
[a] Both the linear range and the lower limit of quantification changed slightly on a daily basis depending on the temperature and the pressure in the laboratory.

IV. INDIVIDUAL STUDY REPORTS

Review of Pharmacokinetics in Healthy Volunteers: BR1-010

Summary:

The blood pharmacokinetic parameters observed for SF_6 were generally similar for males and females. Examination of dose-normalized parameters suggested dose-proportional pharmacokinetics for SF_6. The apparent route of elimination was via the lungs in expired air.

As the study BR1-010 measured the 'biomaker' SF_6 in order to evaluate the SonoVue, the Applicant should consider conducting a study to measure the intact microbubbles.

It should be noted that BR1-036 study obtained information from older subjects (mean 55.6 years of age) with pulmonary fibrosis condition.

Study Title: "Pharmacokinetic Study of SonoVue™ (BR-1) in Normal Healthy Volunteers"

Study objectives:

- To establish the blood kinetics and pulmonary elimination of the gas SF6 after i.v. administration of SonoVue (0.03 and 0.3 mL/kg body weight) in healthy subjects
- To provide additional safety information to that already collected for SonuVue.

Protocol synopsis/design: BR-1 010 was a single-center (Europe), Phase 1, randomized crossover safety and pharmacokinetic (PK) study. Twelve adult healthy volunteers received two intravenous bolus doses of SonoVue in random order. The washout period was at least 5 to 14 days between doses. No placebo was given. Expired air samples and 3 mL blood samples were taken at multiple time-points.

(b) (4). The 0.3 mL/kg was utilized to enhance the SF_6 concentration in blood and in expired air. It should be noted that the most of the clinical studies dosed by volume. However in this study the subjects were dosed by body weight.

Inclusion criteria

Healthy 18 to 40 years old volunteers; body weight of 55 kg to 100 kg; non-smokers.

Exclusion criteria

Pregnant or nursing; history of alcoholism or drug addiction; hypersensitive to any drug; smoked within 4 weeks preceding the study; history of cancer or clinically significant disorders; evidence of right to left shunt; infection or known inflammatory process; used prescription drugs, received any radiological drugs within 4 weeks preceding the study.

Dosing procedure

SonoVue was administered over 20 seconds using a 3-way stopcock followed by a saline flush (5 mL). A 20-gauge in-dwelling catheter was used. SonoVue is injected into either a large forearm or antecubital vein.

Pleases note that after the reconstitution of the lyophilized powder in 5 mL saline, the microbubble suspension is presumed to contained 10 microliter/mL of SF_6 (of which 5 microliter is in the microbubbles).

Pharmacokinetic (PK) sampling

Blood samples for determination of SF_6 concentrations:

Three (3) mL sample was collected at each of the following time-points (heparinized Vacutainer tubes): pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, 60 and 90* minutes post-dose.

*for Subjects 1 and 2 only; replaced by a sample at 16 minutes post-dose for Subjects 3 to 12.

Expired air samples for determination of SF_6 concentrations:

Expired air was collected through a respiratory mask into plastic bags. Samples were collected at the following time-points: Pre-dose (-2 to -1 minutes), 0 - 0.5, 0.5 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 6, 6 - 8, 8 - 11, 11 - 15, 15 - 20, 20 - 30 and 45 - 55 and 80 – 90 minutes post-dose (the first two patients enrolled had an additional sample collected at 80 to 90 minutes post-dose).

The volume of gas exhaled during each sampling period was monitored using the pulmonary monitoring system (Spirobank).

Sample assays

Blood and expired air samples were analyzed using a gas chromatograph electron-capture detector (GC-ECD), performed by the Department of Biopharmaceutical Analysis at Bracco Research SA. (The analytical validation report – Appen. 10.1.8) The limit of quantification ranged from 0.015 to 0.036 ng/mL (Listing 11).

Safety evaluation and adverse events monitoring

The safety evaluation included adverse events, vital signs, physical exam, local tolerability, labs (CBC, chemistries, urinalysis) and βHCG in females.

AE's were monitored from screening to 24 $\pm$ 2 hours post dosing. Physical examination was performed at screening and 24 hours post dosing. Vital signs (BP, heart rate and respirations) were recorded at 5 minutes pre-dose and 5 and 100 minutes post-dose. Clinical laboratory tests were taken $\leq$ 24 hours pre-dose and 24 hours post-dose.

Statistical methods

Descriptive information was obtained from the study. No formal statistical method was proposed by the Applicant. However, for the determination of sample size, SAS version 6.12 was used. A total of 10 subjects was estimated as sufficient to provide excretion parameters with MSD < 10 %.

Safety analysis

Descriptive safety data were obtained.

Pharmacokinetics:

The following PK parameters were computed and recorded:

- Pulmonary elimination of SF_6 (Ae) and percentage of dose eliminated (fe)

- Maximum observed blood concentration (C_{max})
- Time of maximum observed blood concentration (t_{max})
- Area under the blood concentration versus time curve (AUC) from time zero to 4 min post-dose (AUC(0-4 min))
- Area under the blood concentration versus time curve (AUC) from time zero to 60 min post-dose (AUC(0-60 min))
- Apparent blood distribution rate constant (λ_1) , using residuals, and associated half life ($t_{½}\lambda_1$); ($t_{½}\lambda_1$) = ln(2)/λ_1.
- Apparent blood terminal elimination rate constant (λ_z) and associated half life ($t_{½}$);$t_{½}$ = ln(2)/λ.
- Mean residence time (MRT); MRT = [AUMC 0-60min / AUC 0-60min.
- Apparent total body clearance (CL/F); CL/F = [injected dose of gas / AUC 0-60 min]. The **constant F ('bioavailability')** is the fraction of the dose reaching the blood sampling site: F ≥ 1 – [Ae(0-60 sec) / dose] due to the predicted rapid first pass elimination. The constant Ae (0-60 sec) is the quantity of gas eliminated during the first pass of the administered dose through the lungs.
- Apparent volume of distribution at steady-state (V_{ss}/F)

Results:

1. Dosing and Extent of Exposure

Twelve patients (all were white) were enrolled (n=7 males, n=5 females). All patients received one 0.3 ml/kg dose and one 0.03 mL dose of SonoVue. The mean age was 26 years; mean weight 66.8 kg, mean height 176 cm.

	Age (years)	BW kg	Height cm
Males	27 (4)	72.6 (5.19)	179 (5.1)
Females	23 (2.3)	58.8 (6.76)	172 (3.4)
Overall	26 (3.9)	66.8 (9.03)	176 (5.7)

Mean (SD)

Note that the BW between males and females are noticibly different.

2. Adverse Events

Following 0.03 mL/kg injection, Subjects 7 (male), 11 (female) and 12 (female) reported headache at approximately 5 days, 14 hours, and 6 days post administration, respectively.

Following 0.3 mL/kg injection, Subject 4 (male) reported headache at approximately 12 days post administration and rhinitis at approximately 13 days post administration. Subject 10 reported back pain at approximately 1 day 15 hours post administration.

3. Vital Signs

There were no clinically significant changes in vital signs for any subject during the study.

4. Clinical Laboratory Tests

Several subjects had individual laboratory values (hematology and chemistry as well as urine pH and specific gravity; hematocrit (1), total bilirubin (2), chloride (1) and urine pH (2)) outside the normal ranges at sometime during the study. However, none of these were considered by the Investigator to be of clinical significance.

5. SF_6 Concentration

The Applicant stated that the blood and expired air concentrations below the limits of quantification (0.015, 0.027, 0.028, 0.029, 0.030, 0.031, 0.032 and 0.036 ng/mL for blood concentrations, and, 0.023 and 0.024 ng/mL for expired air concentrations) were replaced with zero for the calculation of all PK parameters.

6. Calculation of percentage of the dose eliminated in the expired air

Pulmonary elimination (Ae) was calculated as:

(Expired air volume) x (expired air SF_6 concentration)

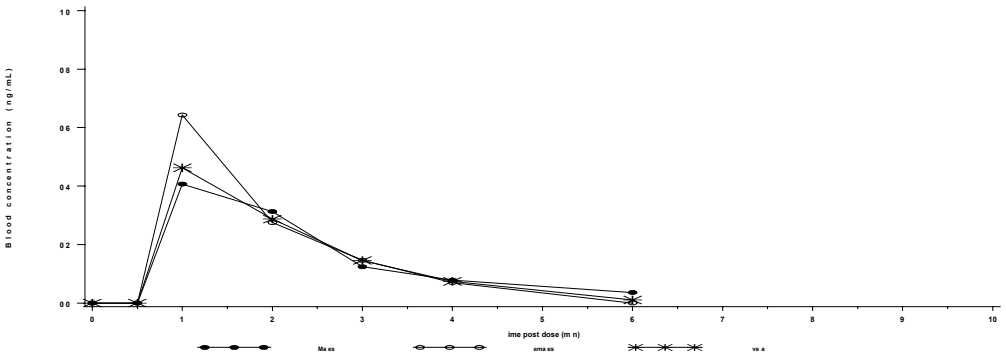
Percentage of the dose (fe) eliminated was calculated as:

[(Ae) / (injected dose of SF_6)] x 100

7. Pharmacokinetics Results

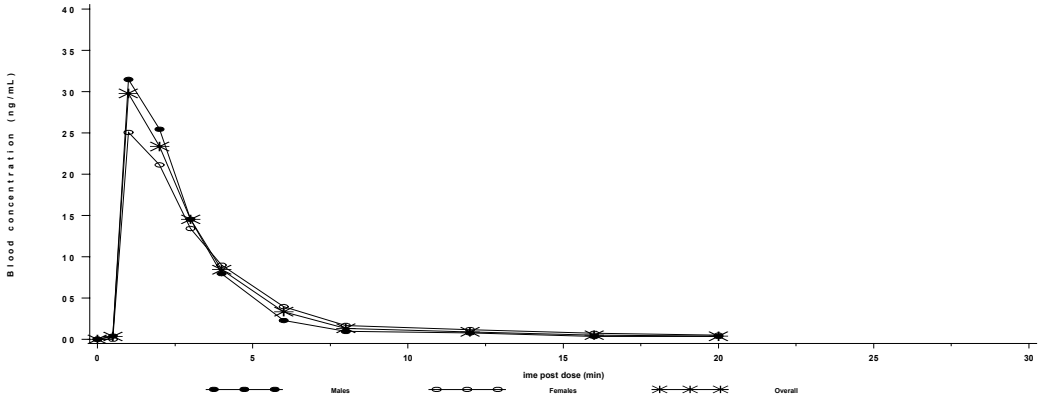
a. Blood concentration profiles for 0.03 and 0.3 mL/kg doses

Median Blood Concentrations of SF_6 Following Intravenous Administration of Sonovue in Healthy Volunteers: 0.03 mL/kg (Ref. p. 76, vol. 15)



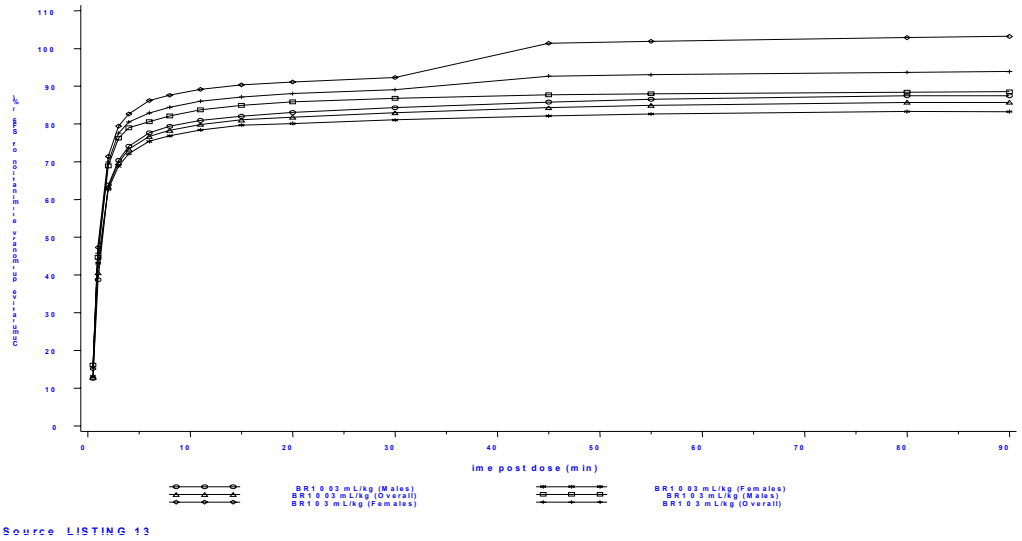
Source: LIS NG 11

Median blood concentrations of SF_6 following intravenous administration of SonoVue in Healthy Volunteers: 0.3 ml/kg (Ref. p. 78, vol. 15)



Source: LIS ING 11

b. **Figures: Mean Cumulative Elimination of SF_6 In Expired Air Following Intravenous Administration of Sonovue in Healthy Volunteers (Ref. Figure E, p. 82, vol. 15)**



Source LISTING 13

c. **The blood pharmacokinetic parameters of SF_6 are summarized in the following table (Blood Pharmacokinetic Parameters - Study BR1-010 Dose = 0.03 mL/kg and 0.3 mL/kg)**

MEAN BLOOD PHARMACOKINETIC PARAMETERS FOR SF_6 (STUDY BR1-010)			
Parameter[a]	Subjects	SONOVUE 0.03 mL/kg	SONOVUE 0.3 mL/kg
$AUC_{(0\text{-}60\ min)}$ (ng·min/mL)	Males Females Overall	0.955 ± 0.381 1.121 ± 0.268 1.024 ± 0.335	10.408 ± 3.368 10.053 ± 3.718 10.260 ± 3.354
C_{max} (ng/mL)	Males Females Overall	0.436 ± 0.187 0.587 ± 0.243 0.499 ± 0.216	***4.149 ± 1.898*** ***2.666 ± 1.286*** 3.531 ± 1.774
T_{max} (min)	Males Females Overall	1.4 ± 0.5 1.2 ± 0.5 1.3 ± 0.5	1.3 ± 0.5 1.8 ± 0.5 1.5 ± 0.5
$t½\lambda_1$ (min)	Males Females Overall	NC NC NC	1.01 ± 0.25 1.25 ± 0.38 1.11 ± 0.31
t½ (min)	Males Females Overall	NC NC NC	***7.94 ± 4.08*** ***12.80 ± 13.49*** 9.88 ± 8.73
MRT (min)	Males Females Overall	(b) (4)	

AUC (0-60 min) = Area under the blood concentration vs time curve from time zero to 60 min postdose;
C_{max} = Maximum observed blood concentration; T_{max} = Time of maximum observed blood concentration;

$t½\lambda_1$ = Distribution phase half-life; t½ = Elimination phase half-life; MRT = mean residence time;
NC = Not calculable

A All parameter values are reported as arithmetic mean ± standard deviation.

Table data derived from BR1-010 clinical trial report (*End-of-Text Tables 10.1, 10.5, 10.7, 10.9, 10.11, 10.12*).

0.03 mL/kg dose (min – max range for all subjects):

Parameter N=12	C_{max} (µg/L)	T_{max} (min)	$AUC_{0\text{-}60}$ (µg·min/L)	$t_{½}$ (min)	CL/F (L/hr)	V/F (L)
Mean	0.499	1.3	1.024	NC	8,506	341
SD	0.216	0.5	0.335	NC	4,648	185
Minimum	0.145	1.0	0.326	NC	4,359	193
Maximum	0.860	2.0	1.472	NC	22,737	893

0.3 mL/kg dose (min-max range for all subjects):

Parameter N=12	C_{max} (µg/L)	T_{max} (min)	$AUC_{0\text{-}60}$ (µg·min/L)	$t_{½}$ (min)	CL/F (L/hr)	V/F (L)
Mean	3.531	1.5	10.260	9.88	8,298	710
SD	1.774	0.5	3.354	8.73	3,466	374
Minimum	1.292	1.0	5.486	1.88	3,514	322
Maximum	7.785	2.0	15.589	32.95	15,699	1,366
NC = not calculable						

- Blood concentrations of SF_6 reached a maximum within 1 to 2 minutes for both doses and declined bi-exponentially.
- Maximum concentrations of SF_6 in blood (C_{max}) ranged from 0.145 to 0.860 µg/L for the 0.03 mL/kg dose and 1.292 to 7.785 µg/L for the 0.3 mL/kg dose.
- T_{max} occurred between 1 to 2 minutes after administration of both doses.

- Terminal half-life values ranged from 1.88 to 32.95 minutes, with a mean of 9.88 minutes for the 0.3 mL/kg dose; this was not calculable for the 0.03 mL/kg dose.

  The data showed that by 12 minutes after the 0.03 mL/kg dose, blood concentrations of SF_6 were below the limit of quantitation; the blood concentrations were not measurable beyond 60 minutes for the 0.3 mL/kg dose. For the 0.3 mL/kg dose, the terminal half-life was 9.88 minutes (mean).

- The AUC values for the 0.3 mL/kg dose were approximately 10 times that for the 0.03 mL/kg dose.

- There was a relatively large inter-subject variation in blood concentrations of SF_6. C_{max} values varied approximately 6-fold.

- Blood pharmacokinetic parameters were generally similar for males and females. Again, note that there is a relatively high variability in values. This may be due to the small sample size.

d. Regarding in the linearity, dose-normalized parameters suggests that the pharmacokinetics of SF_6 are dose linear. Dose-normalized parameters are summarized in the below.

SUMMARY OF DOSE-NORMALIZED AUC AND C_{MAX} FOR SF_6[a] STUDY BR1-010

Dose of SONOVUE	Subjects	$AUC_{(0\text{-}60\ min)}$[b]	C_{max}[b]
0.03 mL/kg	Males	0.955 ± 0.381	0.436 ± 0.187
	Females	1.121 ± 0.268	0.587 ± 0.243
	Overall	1.024 ± 0.335	0.499 ± 0.216
0.3 mL/kg	Males	1.041 ± 0.337	0.415 ± 0.190
	Females	1.005 ± 0.372	0.267 ± 0.129
	Overall	1.026 ± 0.335	0.353 ± 0.177

a Normalized for lowest dose (0.03 mL/kg); b Arithmetic mean ± standard deviation.
Table data derived from BR1-010 clinical trial report (*End-of-Text Tables 10.2* and *10.6*).

e. The following table presents the apparent total clearance, clearance, and volume of distribution.

TOTAL BODY CLEARANCE, VOLUME OF DISTRIBUTION, AND ESTIMATES OF F FOR SF_6[a] STUDY BR1-010

Dose of SONOVUE	Subjects	F[b]	CL/F (L/h)	CL (L/h) ≥	Vss/F (L)	Vss (L) ≥
0.03 mL/kg	Males	0.613 (0.248)	9929 (5736)	6258 (4775)	402 (222)	252 (187)
	Females	0.569 (0.091)	6514 (1250)	3687 (883)	256 (69)	141 (16)
	Overall	0.594 (0.192)	8506 (4648)	5187 (3805)	341 (185)	206 (150)
0.3 mL/kg	Males	0.554 (0.168)	8778 (4149)	5092 (3127)	635 (372)	372 (297)
	Females	0.527 (0.144)	7626 (2498)	3751 (496)	814 (393)	414 (188)
	Overall	0.543 (0.152)	8298 (3466)	4533 (2429)	710 (374)	389 (248)

F = 'bioavailability'; CL = total body clearance; CL/F = apparent total body clearance; Vss = volume of distribution at steady-state; Vss/F = apparent volume of distribution at steady-state;
a Arithmetic mean (SD)
b $F \geq 1 - \frac{Ae(0\text{-}60\ sec)}{Dose}$
Table data derived from BR1-010 clinical trial report (*End-of-Text Tables 10.13* through *10.17*).

- Total body clearance values were high (as well as apparent total body clearance (CL/F) values) were very high, reflecting the rapid elimination of SF_6. This may be due to the rapid first pass elimination through the lungs.

- Volume of distribution values (as well as apparent volume of distribution at steady state (Vss/F)) values were high.

f. Pulmonary elimination of SF_6

	0.03 mL/kg	0.3 mL/kg
Recovery % of dose		
Total	85.7 (22.3) (range 53.5 – 128.2)	93.9 (27.4) (range 61 – 153.4)
Males	87.5 (28.3) (range 53.5 – 128.2)	88.5 (24.5) (range 61 – 129)
Females	83.2 (12.17) (range 67.8 – 99.0)	103.2 (33.6) (range 83.4 – 153.4)

Mean (SD)

- Pulmonary elimination of SF_6 in expired air was rapid for both doses.

- For 0.03 mL/kg, the actual percentage recovered as SF_6 in the expired air ranged from 53.5% to 128.2% with a mean value of 85.7%.

- For 0.3 mL/kg, the percentage recovered as SF_6 in the expired air ranged from 61.0% to 153.4% with a mean value of 93.9%.

- Noted that there is high variability in % elimination. For values in excess of 100% for cumulative percent of SF_6 excreted should be taken with caution. This variability may be attributed to technical difficulties associated with collecting and analyzing expired air samples.

- Approximately 39% to 45% (males) and 43% to 47% (females) of the administered dose eliminated within the first minute postdose at both the 0.03 and 0.3 mL/kg dose levels. By 11 minutes postdose, 81% to 84% (males) and 78% to 89% (females) of the administered doses had been expired. Thus, it appears that elimination of SF_6 was similar for males and females and was independent of dose.

8. Discussion and Conclusion:

Safety data in BR-1 010 show that an intravenous dose of SonoVue approximately 10 times that recommended in the labeling for echocardiography was well tolerated.

The Applicant reported that no unusual safety concerns were raised during the course of this study. Of course the safety data should be examined by the Reviewing Medical Officer.

With respect to pharmacokinetics, it appears that PK parameters observed for SF_6 indicate rapid elimination via the lungs and were generally similar for males and females. Dose normalized PK parameters indicated that dose-linearity exists for 0.03 mL/kg and 0.3 mL/kg doses for SF_6.

As with the most microbubble drugs, it should be noted that the PK data showed high variability (as indicated by the large standard deviations).

Finally, there is no information of the pharmacokinetic disposition of the *phospholipid shell* components of SonoVue.

Therefore, as the study BR1-010 measured the 'biomaker,' $SF_{6,}$ in order to evaluate the SonoVue, the Applicant should consider conducting a study to measure the intact microbubbles, if feasible.

It should be noted that BR1-036 study obtained information from older subjects (mean 55.6 years of age) with pulmonary fibrosis condition since the major route of elimination for SF_6 is via the lungs.

Review of Pharmacokinetic Study BR1-036:

Study title: "Pharmacokinetic study of SonoVue in patients with known diffuse interstitial pulmonary fibrosis"

Summary:

Because SF_6 is eliminated entirely via the lungs, it was considered necessary to evaluate the pharmacokinetic profile of SonoVue in patients with compromised pulmonary function in order to demonstrate the safety of SonoVue in patients with possible impaired elimination pathways as well as to determine whether dosage adjustment would be necessary in such patients.

Blood concentrations of SF_6 declined bi-exponentially following administration of SonoVue 0.3 mL/kg. Mean C_{max} estimates were lower and T_{max} estimates were higher than those observed in healthy subjects (study BR1-010), while half-life estimates were similar for the two populations. Linear regression analysis demonstrated a statistically significant decrease in apparent blood clearance of SF_6 as the severity of pulmonary impairment increased ($p<0.05$); however, the results were no longer significant when clearance estimates were normalized to body weight. Recovery of SF_6 in expired air was similar among patients with varying degrees of pulmonary impairment and similar to that observed in healthy subjects. These results suggest that administration of SonoVue does not pose any increased risk for patients with pulmonary impairment and no adjustment in SonoVue dose would appear to be necessary in such patients.

Study Design and Methods

Objectives:

- To evaluate the PK profile of SonoVue in pulmonary impaired patients at a dose of 0.3 mL/kg
- To evaluate the safety profile of SonoVue in pulmonary impaired patients at a dose of 0.3 mL/kg

Design

Study BR1-036 was a Phase I, open-label study conducted in patients with a known diagnosis of diffuse interstitial pulmonary fibrosis associated with any autoimmune, industrial, occupational, infectious, or connective tissue disease.

Subjects

A total of 12 patients, including at least five males and five females, were to be included in the study. Degree of pulmonary compromise was recorded on a 5-point scale as follows: 1 = mild; 2 = mild/moderate; 3 = moderate; 4 = moderate/severe; 5 = severe. All patients received a single intravenous bolus injection of 0.3 mL/kg S SonoVue .

Amount of SF6 in 1 mL of reconstituted SonoVue

Approximately (b) (4) microliters SF6/mL.

Pharmacokinetic Sampling

Blood samples 2.5 mL for determination of SF_6 concentrations were obtained from an indwelling catheter placed in a large forearm vein of the patient at the following time points (anaerobically into individually marked and tared heparinized Vacutainer® tubes): 1 minute pre-dosing, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, 30, 45, and 60 minutes postdose.

Expired air was collected through a respiratory mask and a pulmonary monitoring system (Spirobank™) into plastic bags at the following time-points: -2 to -1 minute (pre-dosing), and 0 - 0.5, 0.5 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 6, 6 - 8, 8 - 11, 11 - 15, 15 - 20, 20 - 30, 30 - 40, 40 - 50, and 50 - 60 minutes postdose. The first two patients enrolled had additional samples collected at 80 to 90 and 110 to 120 minutes postdose.

Assay

Blood samples were sonicated for 1 minute in an ultrasonic bath directly in their sealed Vacutainer. Head space samples (25 to 100 μL) were injected directly into a gas chromatograph with electron capture detector (GC-ECD) for SF_6 analysis.

Exhaled air samples (25 to 100 μL) were directly injected in the GC-ECD. The first five postdose samples (ie, obtained from 0 to 4 minutes postdose) were diluted with a measured amount of air in (b) (4)-sealed vials before measurement. The volume of gas exhaled during each sampling period was monitored using the pulmonary monitoring system.

Safety evaluation and adverse events monitoring

The safety evaluation included adverse events, vital signs, physical exam, local tolerability, labs (CBC, chemistries, urinalysis) and βHCG in females.

AE's were monitored from screening to 24 ± 2 hours post dosing. Physical examination was performed at screening and 24 hours post dosing. Vital signs (BP, heart rate and respirations) were recorded at predose, 0.5, 2,4, 6,10,30, 45, 60 90 and 120 minutes post-dose. Clinical laboratory tests were taken ≤ 24 hours pre-dose and 24 hours post-dose.

Oxygen saturation was evaluated at 6 and 2 minutes pre-dose, 0.5, 2, 4, 6, 10, 30, 45, 60, 90 and 120 minutes post dose.

Statistical methods

Descriptive information was obtained from the study. No formal statistical method was proposed by the Applicant. However, for the determination of sample size, SAS version 6.12 was used. A total of 10 subjects was estimated as sufficient to provide excretion parameters with MSD < 10 %. Additionally, the relationship between individual patient blood clearance values, both absolute and corrected for body weight, and degree of pulmonary compromise was examined.

Safety analysis

Descriptive safety data were obtained.

Pharmacokinetics:

The following PK parameters were computed and recorded:

- Pulmonary elimination of SF_6 (Ae) and percentage of dose eliminated (fe)
- Maximum observed blood concentration (C_{max})
- Time of maximum observed blood concentration (t_{max})

- Area under the blood concentration versus time curve (AUC) from time zero to infinity post-dose (AUC(0-inf min))
- Apparent blood distribution rate constant (λ_1) , using residuals, and associated half life ($t_{½}\lambda_1$); ($t_{½}\lambda_1$) = ln(2)/λ_1.
- Apparent blood terminal elimination rate constant (λ_z) and associated half life ($t_{½}$);$t_{½}$ = ln(2)/λ.
- Mean residence time (MRT); MRT = [AUMC 0-60min / AUC 0-60min.
- Apparent total body clearance (CL/F); CL/F = [injected dose of gas / AUC 0-60 min]. The **constant F ('bioavailability')** is the fraction of the dose reaching the blood sampling site: F ≥ 1 – [Ae(0-60 sec) / dose] due to the predicted rapid first pass elimination. The constant Ae (0-60 sec) is the quantity of gas eliminated during the first pass of the administered dose through the lungs.
- Apparent volume of distribution at steady-state (V_{ss}/F)

Results

1. Patient Characteristics

Thirteen (13) patients were enrolled and completed study; 12 patients were included in the analysis of pharmacokinetics. One patient was excluded from the pharmacokinetic analysis because the exact dose administered could not be determined due to an error in obtaining the predose weight of the syringe.

Of the 13 patients who were enrolled, 8 (61.5%) were male and 5 (38.5%) were female. The majority of patients (84.6%) were white. Ages ranged from 36 to 80 years (mean age, 55.6 years). The majority of patients (84.7%) had mild to moderate pulmonary impairment.

2. Adverse events and vital signs

Two patients experienced 3 AEs. One patient experienced mild chest pain and pharyngitis which were considered unrelated to SonoVue. There were some changes, however, none of these were considered by the clinical investigator to be of clinical importance.

3. Pharmacokinetic resuts

a. Pharmacokinetic parameters following 0.3 mL/kg dose

BLOOD PHARMACOKINETIC PARAMETERS OF SF_6 FOLLOWING ADMINISTRATION OF SONOVUE 0.3 ML/KG IN PATIENTS WITH DIFFUSE INTERSTITIAL PULMONARY FIBROSIS STUDY BR1-036

Parameter	C_{max} (µg/L)	T_{max} (min)	$AUC_{0-\infty}$ (µg·min/L)	λ_z (min^{-1})	$t_{½}$ (min)	$t_{½,\lambda 1}$ (min)	CL/F (L/min)	V/F (L)
N	12	12	12	12	12	12	12	12
Mean	1.45	2.19	5.87	0.1257	11.64	0.86	342.0	4045
SE	0.30	0.24	1.17	0.0358	2.66	0.12	68.5	943
SD	1.03	0.82	4.04	0.1241	9.23	0.40	237.2	3268
Median	1.25	2.03	4.40	0.0871	8.57	0.80	316.7	3190
Minimum	0.35	0.97	1.49	0.0238	1.54	0.37	86.8	1438
Maximum	3.79	4.00	15.08	0.4495	29.09	1.71	826.1	12761

C_{max} = maximum observed blood concentration; T_{max} = time of maximum observed blood concentration; $AUC_{0-\infty}$ = area under the blood concentration versus time curve from time zero to infinity; λ_z = elimination rate constant; $t_{½}$ = terminal half-life; $t_{½,\lambda 1}$ = distribution half-life; CL/F = apparent total body clearance; V/F = apparent volume of distribution. Table data derived from BR1-036 clinical trial report.

- It should be noted that there was a high degree of variability observed in all parameter estimates.

- Maximum concentrations of SF_6 in blood (C_{max}) ranged 10-fold from 0.35 to 3.79 µg/L and occurred between 0.97 and 4.0 minutes after administration of SonoVue. Note that the mean C_{max} estimate of 1.45 µg/L (ng/mL) was lower than that observed in healthy subjects who received the 0.3 mL/kg dose (overall mean = 3.53 ng/mL).

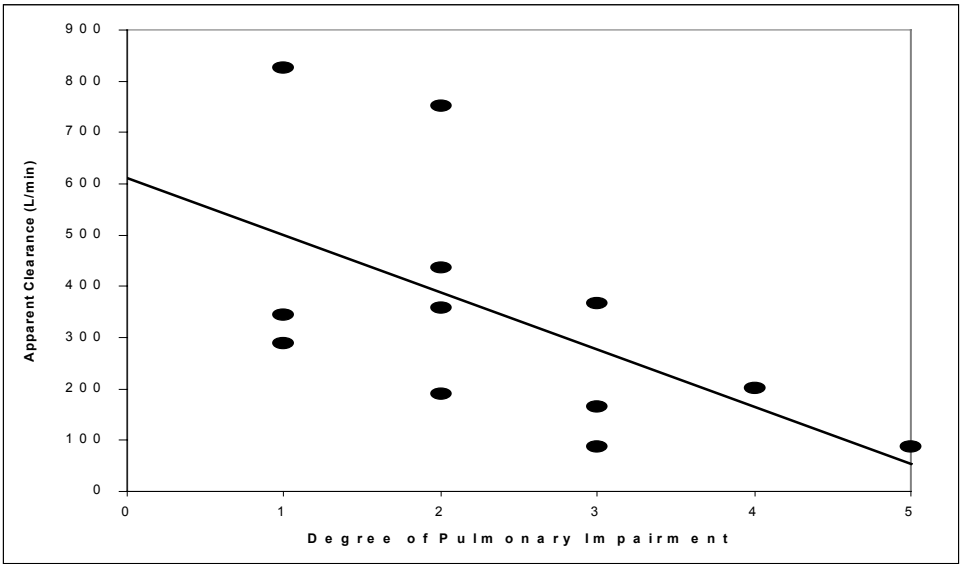
- The mean T_{max} (2.19 min) was higher than that observed in healthy subjects (1.5 min).

- The mean terminal half-life of 11.6 minutes was similar to that observed in healthy subjects (9.88 min). Eight of 12 patients had terminal half-life values less than 15 minutes.

- The patient with the greatest degree of lung impairment (score of 5 = severe) had the longest T_{max}, the longest terminal half-life, the greatest $AUC_{0-\infty}$, and the next to lowest clearance value (87.4 L/min).

b. The relationship between individual patient blood clearance values, both absolute and corrected for body weight, and degree of pulmonary compromise was examined. Linear regression analysis demonstrated a statistically significant decrease in apparent total blood clearance as the severity of pulmonary impairment increased (p=0.0469; see figure below). The strength of the relationship diminished when clearance estimates were normalized to body weight (p=0.0831).

RELATIONSHIP BETWEEN THE BLOOD CLEARANCE OF SF_6 AND THE DEGREE OF PULMONARY IMPAIRMENT FOLLOWING INTRAVENOUS ADMINISTRATION OF SONOVUE 0.3 ML/KG TO PATIENTS WITH DIFFUSE INTERSTITIAL PULMONARY FIBROSIS STUDY BR1-036



Expired air parameters

PHARMACOKINETIC PARAMETERS OF SF_6 FROM EXPIRED AIR DATA FOLLOWING ADMINISTRATION OF SONOVUE 0.3 ML/KG IN PATIENTS WITH DIFFUSE INTERSTITIAL PULMONARY FIBROSIS NON-PARAMETRIC ANALYSIS STUDY BR1-036

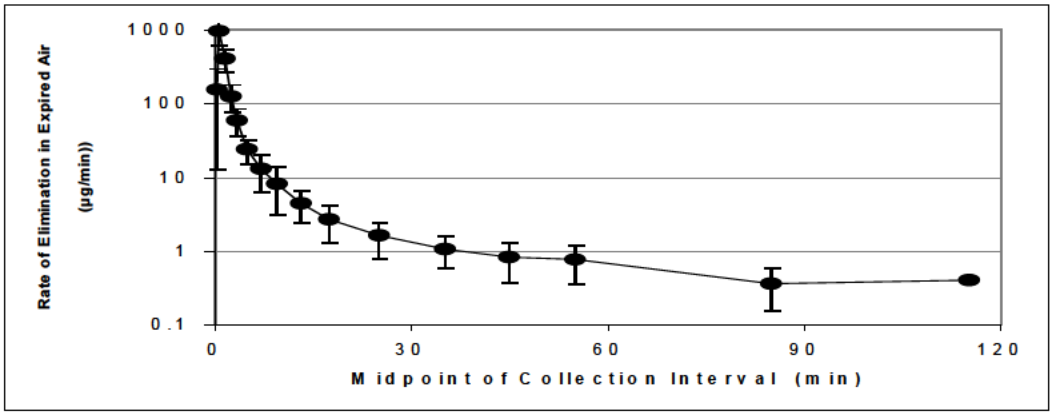
Parameter	Amount (SF_6) excreted to infinity (µg)	**Percent of dose recovered in expired air (%)**	λ_z (min^{-1})	$t_{1/2,z}$ (min)
N	12	**12**	12	12
Mean	1407.9	**102.2**	0.0261	31.32
SD	266.8	**18.4**	0.0090	16.80
Median	1452.1	**108.2**	0.0260	26.68
Minimum	885.0	**69.7**	0.0092	17.06
Maximum	1827.7	**128.7**	0.0406	75.57

λ_z = elimination rate constant; $t_{1/2,z}$ = elimination half-life. Table data derived from BR1-036 clinical trial report.

- Elimination half-lives ranged from 17.1 to 75.6 minutes with a mean of 31.3 minutes.
- The actual percentage of the dose recovered as SF_6 in the expired air ranged from 69.7% to 128.7% with an overall mean value of 102.2%. This is similar to the range observed in healthy volunteers who received the same dose of SonoVue (61% to 153%, study BR1-010).

d. The mean (SD) rate of elimination of SF_6 in expired air

MEAN (SD) RATE OF ELIMINATION OF SF_6 IN EXPIRED AIR FOLLOWING INTRAVENOUS ADMINISTRATION OF SONOVUE 0.3 ML/KG IN PATIENTS WITH DIFFUSE INTERSTITIAL PULMONARY FIBROSIS
STUDY BR1-036



Note: Rate of elimination collected over each sampling interval is plotted at the midpoint of that interval.

4. Discussion and Conclusion

Blood concentrations of SF_6 declined bi-exponentially following administration of S SonoVue 0.3 mL/kg. Mean C_{max} estimates were lower than those observed in healthy subjects. T_{max} estimates were higher than those observed in healthy subjects (study BR1-010). The half-life estimates were similar for the two populations.

Linear regression analysis demonstrated a statistically significant decrease in apparent blood clearance of SF_6 as the severity of pulmonary impairment increased ($p<0.05$); however, the results were no longer significant when clearance estimates were normalized to body weight.

Recovery of SF_6 in expired air was similar among patients with varying degrees of pulmonary impairment and similar to that observed in healthy subjects. These results suggest that administration of SonoVue does not pose any increased risk for patients with the patient population studied in this study and suggest no adjustment in SonoVue dose would appear to be necessary in these patients.

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/s/

David Lee
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BIOPHARMACEUTICS

John P. Hunt
9/24/01 10:01:53 AM
BIOPHARMACEUTICS

Appendix 4.2. Clinical Pharmacology Review of re-submission of NDA 21-315

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA:	**21-315**
Submission Date:	**June 30, 2003**
Brand Name:	**SonoVue (sulfur hexafluoride microbubbles)**
Route of Administration:	**Intravenous (bolus) administration**
Proposed Dosing Regimen:	**For echocardiography 2.0 mL (8 µL microbubbles/mL) by intravenous bolus, with second injecteion of 2.0 mL if deemed necessary by physician, followed by 5 mL saline flush**  (b) (4)
Indication:	**Endocardial Border Delineation**
Sponsor:	**Bracco Diagnostic Inc. Princeton, NJ**
Type of Submission:	**Resubmission (Response to the Action Letter January 4, 2002)**
Reviewer:	**Christy S. John, Ph.D.**
Team Leader:	**Young Moon Choi, Ph.D.**
Dates of Review:	
	Received for Review: July 30, 2003
	Review Date: October 21, 2003

I. INTRODUCTION

The sponsor resubmitted New Drug Application (21-315) on June 30, 2003. The sponsor seeks the approval of SonoVue as an ultrasound contrast agent for use in echocardiography in patients with suspected or established cardiovascular disease to improve visualization of cardiac chambers and endocardial border delineation. The Human Pharmacokinetics and Bioavailability Section of original NDA was reviewed by Dr. David Lee.

The following key findings were noted in review by Dr. Lee:

1) **SF_6 Tmax in blood occurred 1 to 2 minutes after intravenous administration of SonoVue and after reaching the maximum peak, it declined rapidly.**
2) **For 0.03 mL/kg dose, SF_6 concentrations were usually below quantifiable levels 12 minutes after injection.**
3) **For 0.03 mL/kg, elimination half-life of SF_6 in blood averaged 9.9 minutes in healthy volunteers.**
4) **The pharmacokinetics (PK) of SF_6 appeared to be dose-linear over 0.03 ml/kg to 0.3 ml/kg doses as observed by similar values for dose-normalized SF_6 $AUC_{(0\text{-}60\ min)}$ in blood.**
5) **Almost all of the SF_6 from SonoVue dose is eliminated by the lungs. The cumulative recovery of SF_6 in expired air averaged 86% to 94% of the administered dose.**

PK of SF6 gas was fully described (refer to the original review by Dr. Lee). However, PK of intact microspheres, metabolism of its shell components, and the data for bioanalytical assays were not satisfactory in the original NDA. Therefore, three questions/comments were sent to the sponsor from PK reviewer (Dr. Lee). The comments and sponsor's response to comments/questions and this reviewer's evaluations are addressed.

II. REVIEW

1) <u>Comment #1:</u>

This application lacks adequate information on the pharmacokinetics of intact microspheres. To address the deficiency, provide the following:
Pharmacokinetic data that describes the fate of SonoVue bubbles, i.e., the intact SF6 filled microsphere. This pharmacokinetic information is needed for the Dosage and Administration section of the proposed package insert.

SPONSOR'S RESPONSE:

The pharmacokinetics of the "intact" microbubbles is best characterized using echography. Since only intact microbubbles that contain gas can be imaged, the disappearance of image coincides with disintegration of intact microbubble and release of the encapsulated gas. However, the fate of smaller bubbles (<2 μm) which are present in high concentration in SonoVue cannot be followed by echography since these bubbles are non-echogenic and therefore non-imageable. In order to follow the fate of this particular category of bubbles and to obtain information on the stability and fragility of SonoVue microbubbles in plasma (independently of their size), an in vitro study BRG 014 entitled "Kinetics of SonoVue microbubbles disappearance in plasma at 37 °C" was performed. The report of this study is summarized:

The changes in concentration and size distribution of the microbubbles as a function of incubation time were followed by light microscopy. All bubbles disappear within 20 minutes. The initial disappearance rate (from 0 to 10 minutes) follows a mono-exponential decay curve with a half-life of 2.2 minutes and is independent of bubble sizes. The good stability of SonoVue microbubbles in saline at 22 °C is lost when temperature is raised to 37 °C. Similarly, incubation in plasma even at 22 °C leads to a rapid disappearance of SonoVue microbubbles. This study shows that a prolonged presence of SonoVue in the blood stream or of a subpopulation of SonoVue bubbles is unlikely to occur beyond 20 minutes after injection.

In addition a retrospective analysis of the videotaped recordings of echocardiographic images from a clinical study BR1-001 was performed to determine the kinetics of contrast intensity over a range of SonoVue doses.

Method: A total of 36 healthy adult male subjects were divided into 6 groups of 6 subjects each, with 4 subjects in each group randomized to receive SonoVue and 2 subjects randomized to receive matched volumes of placebo. The six dose groups were 0.003, 0.01, 0.03, 0.06, 0.09 and 0.12 mL/kg of SonoVue (5 mg/mL). Two dimensional echocardiographic images (apical 4-chamber view) were recorded starting 2 minutes prior to each injection of SonoVue or placebo and continuing until 5 minutes after injection.

Areas of interest were determined in each ventricle and mean pixel video intensity (0 to 255) was calculated. The mean video intensity before contrast injection was subtracted from the mean value obtained after contrast injection. Time-intensity curves were obtained for each injection by plotting the mean video intensity levels obtained frame by frame (20 frames/injection).

Results: Left ventricle peak contrast intensity increased from a value of 57 at the 0.003 mL/kg dose to 138 at the 0.01 mL/kg dose and did not increase further at the higher SonoVue doses (saturation effect due to nonlinear transformation of the echocardiographic signal). The time elapsing from injection of SonoVue to left ventricle peak contrast effect was relatively constant, about 20 seconds, at doses of 0.003 to 0.03 mL/kg, and increased to 50 seconds at the two highest doses. This increase could be due to shadowing which precludes observation of an earlier peak contrast time. Left ventricle contrast half-life increased with SonoVue dose possibly due to saturation and/or shadowing effects. The time elapsing from the first appearance of contrast in the left chamber to ½ peak intensity (contrast persistence) was dose related at doses of 0.03 mL/kg (28 seconds) to 0.09 mL/kg (142 seconds), but did not increase further at 0.12 mL/kg dose.

EVALUATION:

The sponsor has demonstrated the fate of SonoVue bubbles (>2 µm) using echography. The disappearnace of image coincides with disintigration of large (>2 µm) intact microbubbles and release of encapsulated gas. However, the fate of

smaller bubbles (<2 µm) could not be determined using echography as these bubbles are nonechogenic and therefore non-imageable. The changes in concentration and size distribution of microbubbles (less than 2 µm) as a function of incubation time were studied using light microscopy. It was demonstrated that as the temperature is raised to 37 °C the microbubbles disappear within a minute in plasma. Although the direct measurement of intact small microbubbles in-vivo is difficult to be quantitated, the sponsor has provided in-vitro incubation evidence that suggest that the bubbles disintegrate within two minutes in blood at 37 °C. Therefore, response of the sponsor is acceptable.

<u>COMMENT # 2:</u>

This application lacks adequate information on the pharmacokinetics of the microsphere shell. To address this deficiency, provide the following:

Data on the pharmacokinetics of the lipid components of the microsphere shell. Regarding metabolism, it is requested that supportive information, e.g., in vitro metabolism regarding lipids (Distearoylphosphatidylcholine (DSPC), Dipalmitoylphosphatidylglycerol sodium (DPPG.Na)) be submitted.

Although the individual shell components are naturally metabolized, the shell itself is a (b) (4) of these components. The application lacks data to document the dissolution of the (b) (4) into individual components for metabolism. Therefore, either document the dissolution or provide details on how the (b) (4) is handled by the body.

<u>SPONSOR'S RESPONSE</u>:

The sponsor describes the metabolic fate of the lipid components of microbubbles citing literature.

The majority of phospholipids in plasma are bound to lipoproteins. The total concentration of phosphatidylcholine in human plasma is 1432±180 µmol/L (Schwarz et al. Clin. Chem. 23, 1548-1550, 1977). In a 2.4 mL of SonoVue there is 0.235 µmol of phosphatidylcholine. Given that, in a 60 kg individual there is between 1.2 and 1.8 L of plasma. SonoVue causes an increase in total plasma concentration of between 0.131 and 0.195 µmol/L. Thus SonoVue increases the total concentration of total plasma phosphatidylcholine by only 91 to 136 ppm. The natural variation in plasma concentrations of phosphatidylcholine by far exceeds the increase caused by SonoVue.

The total concentration of phosphatidylglycerol in human is 30 µmol/L. In 2.4 mL of SonoVue there is 0.251 µmol phosphatidylglycerol. Given that in a 60 kg individual there is between 1.2 and 1.8 L of plasma. SonoVue causes an increase in total plasma concentration between 0.131 and 0.195 µmol/L. Thus SonoVue increases the total concentration of total plasma phosphatidylglycerol by only 0.43% to 0.65%.

The natural variation in plasma concentrations of phosphatidylglycerol by far exceeds the increase caused by SonoVue.

SonoVue contains phospholipids as part of the stabilized gas bubbles (15-23%) and as gas-free particles (77% to 85%).

Phospholipids in SonoVue particles, both bubbles and gas-free particles, can disappear from circulation by two different kinds of paths, a particular-uptake path and a lipoprotein-path. Both these paths lead to eventual catabolism of the particle constituents.

The Particulate-uptake Pathway: This pathway involves uptake of the particles by the reticulo-endothelial system, foremost the Kupffer cells in the liver. It is well known that liposomes containing negatively charged phospholipids are cleared much faster from the blood than those containing only neutral phospholipids (Huong et. al. Int. J. Pharmaceut. 215, 197-205, 2001). Their major destiny is macrophages in the liver, spleen, and bone marrow.

Liposomes containing egg diacyl-phosphatidyl glycerol, but not those containing distearoyl-phosphatidyl glycerol, can bind to low-density lipoprotein (LDL). In this way liposomes that do bind to LDL can enter cells possessing the LDL receptors as particles (Amin et. al. Pharm. Res. 18, 914-921, 2001). Apparently for binding to LDL the phospholipids must contain unsaturated fatty acids. Since SonoVue does not contain unsaturated fatty acids, the pathway involving particulate uptake with the help of LDL can be expected to be negligible.

The Lipoprotein Pathway: This route involves the transfer of phospholipids from particles to lipoproteins, mostly apolipoprotein A-1. Monomeric phospholipids in solution can equilibrate with phospholipids bound on lipoproteins in the blood. As the monomeric phospholipids become bound, additional phospholipid from the particle are dissolved. Thereby phospholipids can transfer from particles to lipoproteins. However, this uncatalyzed process is slow, in part because the critical (b) (4) **concentrations are low.**

Plasma contains a protein called phospholipid transfer protein, which catalyzes the transfer of phospholipids from their natural source, i.e. cells that catabolize low-density lipoprotein, to apolipoprotein A-1 and the partially formed pre beta-high density lipoprotein that contains it. Under the influence of lipoprotein-cholesterol-acyltranferase, fatty acids residue of phosphatidylcholine are transferred to cholesterol to form cholesterol esters, leaving lysophosphatidylcholine in lipoprotein in the lipoprotein. High density lipoprotein (HDL) is the product. The major destination of HDL is hepatocyte where lipoprotein is catabolized.

In plasma there also exists a cholesterol-ester/phospholipids exchange protein. This protein catalyzes the exchange of cholesterol esters and triglycerides as well as phospholipids between different classes of lipoproteins.

The catabolic pathway for phosphatidylcholine in mammalian cells, including macrophages and hepatocytes, begins with phospholipase A2-mediated hydrolysis of fatty acid ester bond in 2 position.

DSPC and DPPG are both used in commercial liposomal products, in particular Ambisome, DaunoXome, and Doxil for DSPC and DepoCyte for DPPG. In addition, it has been shown in an in vitro study (BRG 017) (Pharm/Tox Section, Vol 45, page 102), that the phospholipids present in SonoVue, DSPC and DPPG, are easily cleavable by phospholipase A2.

EVALUATION:

The sponsor has satisfactorily addressed the reviewer's concerns about the metabolism of the lipid components of the micro-sphere shell. An extensive citation of literature about the metabolism of phospholipids, their concentration in plasma has been made. It is obvious from the discussion that the small additional quantity of phospholipids provided to plasma does not change the endogenous lipid profile and are also impossible to be quantified given that the change in concentration is so small. Furthermore, the other constituents such as DSPC and DPPG are found in FDA approved commercial liposomal products. Therefore, the sponsor's response is acceptable.

<u>COMMENT # 3</u>:

The application lacks adequate information on the assay of the SF_6 gas moiety. To address this deficiency, provide the following:
For the BRI-010 study (healthy subject pharmacokinetic study), assay information, e.g., standard curves, QC runs, etc., biological samples for the days the samples were actually analyzed. Additionally, submit individual subject SF_6 profiles for blood and expired.

SPNOSOR'S RESPONSE:

The sponsor has provided in Analytical Report BR1-010 (Volume 46-49) the analysis in human expired air and human EDTA whole blood for sulfur hexafluoride in support of the overall clinical study objectives. The dosing solutions were also analyzed for sulfur hexafluoride concentration.

Methodology: Before and after the administration of the study agent (SonoVue) as a bolus, expired air and blood were sampled. While patients breathed normally, expired air was collected through a respiratory mask and a pulmonary monitoring system (spirometer) into plastic bags. The volume of gas exhaled was monitored during each sampling period using spirometer. The expired air samples were

immediately assayed at the end of the testing phase of the study for each volunteer. Exhaled air samples (20 to 100 µl) were directly injected in the Gas Chromatography (Electron Capture Detector) (GC-ECD). When necessary, the samples were diluted with air in (b) (4) sealed vials before measurement.

Blood (2.5 ml) was collected anaerobically into Vacutainer tubes at predose and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 60 minutes following the dose. Head space analysis was done by injecting headspace gas directly into GC-ECD with or without dilution. The dosing solution were quantified using a single-point standard by headspace analysis and injected directly into GC. No sample processing was necessary for expired air. The blood tubes were sonicated 1 minute in an ultrasound bath.

Sulfur Hexafluoride Primary Stock Standard:

The stock solution was a gaseous solution of pure SF6. Depending on pressure and temperature, the concentration (or density) was determined with Gay-Lussac Law (PV=nRT). The concentration of primary stock solutions (n=5) varied from 0.024 to 1.333 ng/mL).

The following chromatographic conditions were used:

GC:	**Hewlett Packard, HP 5890 series II**
Column:	**Chrompack Poraplot Q 10 µm, 25mx0.32 mm I.D.**
Detector:	**Thermic conductivity (TCD)**
Gas:	**He**
Flow:	**Column 2.5 ml/min**
	Reference 7.5 ml/min
Temp:	**Owen 50 °C isotherm**
	Injector 50 °C
	Detector 300 °C
Headspace sampler:	**Perkin Elmer, HS 40**
Retention time:	**O_2+N_2** (b) (4)
	SF_6
	H_2O

A total of 336 human expired air samples were analyzed. A total of 288 human EDTA whole blood samples were analyzed.

The results of analysis for a volunteer are given below:

SF6 concentrations in dosing solution (µg/ml): 56.269 or 0.03 ml/kg
SF6 in expired air

Bag #	Conc. SF6 (ng/mL)
1	ND
2	0.216

3	**4.682**
4	**2.606**
5	**1.176**
6	**0.566**
7	**0.245**
8	**0.102**
9	**0.044**

SF6 concentration in blood:

Nominal Time	Conc SF6 (ng/ml)
-1 min	**ND**
30 sec	**ND**
1 min	**0.407**
2 min	**0.321**
3 min	**0.162**
4 min	**0.078**
6 min	**0.036**
8 min	**NQ**
12 min	**NQ**

ND = Not Detectable
NQ = Not Quantifiable (less than 0.032 ng/ml)

EVALUATION:

The sponsor has provided the in depth details on assay methodology. The linearity of method was validated with standard solutions and reconstituted dosage forms and analysis repeated three times. The area under the peaks was found linearly related to the SF_6 concentrations and the method was proportional. The accuracy was determined on 15 reconstituted dosage forms using the same raw data as used for linearity. The mean recovery was 102%. Precision was determined at 3 concentrations of lyophilisate powder in saline. Precision was investigated in terms of repeatability (same day, same operator) and reproducibility (different day, different operator). Repeatability was estimated with a mean of variance of the results on each series. It was expressed as a coefficient of variation (CV). The coefficient of variation of repeatability and reproducibility were 8.6%. In conclusion, the sponsor demonstrated that the analytical method was specific, linear, and was validated with satisfactory accuracy and precision for total SF6.

III. RECOMMENDATION:

The Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II has reviewed the resubmitted NDA 21-315 and has found that the sponsor has adequately addressed the previous reviewer's concerns. This application has been found to be acceptable from a PK perspective.

Christy S. John, Ph.D.
Clinical Pharmacology Reviewer
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

Concurrence

Young Moon Choi, Ph.D.
Clinical Pharmacology Team Leader
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

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Christy John
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Young-Moon Choi
11/17/03 03:44:00 PM
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Appendix 4.3. Applicant's Proposed Package Insert (original, annotated)

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Appendix 4.4. Office of Clinical Pharmacology New Drug Application Filing and Review Form

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	203-684	**Brand Name**	SonoVue™
OCP Division V	V	**Generic Name**	Sulfur hexafluoride microbubbles inj.
Medical Division	Division of Medical Imaging Products	**Drug Class**	Microbubble
OCP Reviewer	Christy S. John, Ph.D.	**Indication(s)**	SonoVue™ is indicated for use in echocardiography in patients with suboptimal echocardiograms to obtain left ventricular opacification and improve endocardial border delineation.
OCP Team Leader	Gene Williams, Ph.D.	**Dosage Form**	Lyophilized powder for reconstitution
		Dosing Regimen	2 mL intravenous bolus injection, a second injection of 2 mL may be administered when deemed necessary
Date of Submission	12/21/2011	**Route of Administration**	intravenous injection
Estimated Due Date of OCP Review	9/20/2012	**Sponsor**	Bracco Diagnostic, Inc.
PDUFA Due Date	10/21/2012	**Priority Classification**	S
Division Due Date	9/20/2012		

Clin. Pharm. Information

	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			

Reference Bioanalytical and Analytical Methods				
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	3		
multiple dose:	X			
Patients-				
single dose:	X	2		
multiple dose:	X			
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment: Diffuse pulmonary fibrosis		1		
hepatic impairment:				
PD:				
Phase 2:	**X**	1		
Phase 3:	**X**			
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	4		
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				

alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		11		

Filability and QBR comments

	"X" if yes	Comments
Application fileable ?	**X**	A previous NDA (#21-315) was submitted to the FDA on January 21, 2001 in support of a similar indication (use in visualization of the endocardial border) and was withdrawn without prejudice on December 20, 2007. (b) (4) [redacted] There were no outstanding clinical pharmacology issues with NDA 21-315. There are no new clinical pharmacology studies in the current resubmission.
Comments sent to firm ?	None	
QBR questions (key issues to be considered)	There were no outstanding clinical pharmacology issues at the time of last submission. There are no new clinical pharmacology studies in this resubmission. The primary item to be addressed in the review of the current submission is package insert language.	

Other comments or information not included above	
Primary reviewer signature	**Christy S. John, Ph.D.**
Secondary reviewer Signature and date	**Gene Williams, Ph.D.**

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/s/

CHRISTY S JOHN
01/31/2012

GENE M WILLIAMS
01/31/2012

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CHRISTY S JOHN
08/24/2012

GENE M WILLIAMS
08/24/2012

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CHRISTY S JOHN
10/31/2013

GENE M WILLIAMS
10/31/2013