SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Sonovue and has been updated until 1 October 2004. For information on changes after this date please refer to module 8B.

1. Introduction

The development of contrast agents for ultrasound examinations began during the 1980s. These contrast agents can be used diagnostically for several purposes:

- Better contrast resolution between normal and abnormal tissues;
- Better outlining of cavities, such as the cardiac cavities, and vessels;
- Enhancement of Doppler signals in blood flow measurements;
- Tissue characterization.

The basic principle of the first echo-enhancing agents was to produce microscopic gas bubbles by shaking the agent, which, when injected into a peripheral vein, gave rise to altered acoustic echoes from blood or tissue. The newer contrast agents are in the form of stabilised microbubble dispersions.

In the context of echocardiography, notwithstanding the availability of echo-enhancing agents there are still limitations in day-to-day clinical echocardiography, i.e. a reliable definition of the entire endocardial perimeter of the left ventricle which is based upon complete opacification and clear endocardial border delineation, and identification of the morphology of the left ventricle. Suboptimal detection of the endocardial border makes the assessment of segmental and global left ventricle kinetic function less reliable. Similar fluorocarbon-containing products that have been authorised via a centralised procedure in an echocardiography indication, provide a satisfactory duration of useful contrast for evaluation of wall motion (2.5 to 4.5 minutes, depending on the dose).

These contrast agents have been approved for use in B-mode echocardiography in patients with suspected or established cardiovascular disease to provide opacification of the left ventricle and cardiac chambers, and to improve left ventricular endocardial border delineation, with resulting improvement in wall motion visualisation. Patients should have had a previous examination without contrast enhancement, which turned out to be inconclusive.

In this submission, the applicant originally claimed a number of non-cardiac uses for SonoVue. In the context of non-cardiac Doppler Vascular Ultrasonography, it was claimed that echo-enhancing agents could in principle be used to improve signals from blood in:

- large vessels to enhance weak signals resulting from deep vessels or slow flow,
- small vessels to identify hyperaemia or ischaemia (such as in tumour detection),

However, in the opinion of the CPMP, the data as originally submitted were not sufficient to support all of these claims (See Section 5 of this report, overall conclusions on efficacy and benefit risk assessment.)

Sonovue is a stabilised microbubble preparation containing sulphur hexafluoride (SF_6), an echogenic, poorly soluble gas. The microbubbles of gas are stabilised in aqueous dispersion by a monolayer of phospholipids. After reconstitution of the 25 mg lyophilisate with 5 ml sodium chloride 0.9%w/v solution for injection, these SF_6-containing phospholipids are in dispersion in the aqueous vehicle. As with other dispersed systems used as ultrasound contrast media, the active part of the product (the echogenic system) is the interface between the gas and the liquid phase in the microbubble dispersion.

Two presentations are defined, each containing 8 microlitre/ml SF_6 after reconstitution, but differing in the medical device elements included in the pack to facilitate reconstitution and administration.

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2. Chemical, pharmaceutical and biological aspects

Composition

Each type I colourless glass vial (11.5ml) contains 25mg sterile pyrogen-free lyophilised white powder (dispersing and stabilising agents), and the head-space of the vial contains the active substance sulphur hexafluoride gas.

The lyophilisate is composed of macrogol 4000 and distearoyl-phosphatidylcholine (DSCP), dipalmitoylphosphatidylglycerol sodium (DPPG.Na), and palmitic acid as microbubble stabilisers.

The vials are closed with butyl rubber stoppers coated with a fluoropolymer film.

Two presentations are intended. Sealing is performed either with a Bio-Set system or an aluminium cap with a polypropylen disc (Flipcap system). The Bio-Set system is an integral plastic seal which holds the stopper and is also used as a spike for the delivery of the saline solution at the reconstitution. In case of vials sealed with Flipcap, they will be supplied with a separate commercial 'spike' (Mini Spike Plus 6/8 R) to assist reconstitution.

Following reconstitution, both presentations contain 8 microlitre/ml SF_6 as a lipid-stabilised dispersion in the aqueous phase.

Active substance

Sulphur hexafluoride is obtained from reaction of fluorine with sulphur vaporised in special furnaces and followed by purification. Fluorine is obtained from electrolysis of hydrofluoric acid.

$S + 3\ F_2 \rightarrow SF_6$

Impurities from the synthetic process may theoretically include- HF, CF_4, air, as well as sulphur containing impurities : SOF_2, SO_2F_2, SF_4, S_2F_{10}. In particular, S_2F_{10} is a toxic potential by-product of SF_6 , although never detected in practice.

The specification contains tests for purity (>99.9% SF_6) and relevant impurities.

Stability:

Samples from three commercial batches, in commercial 40 kg half-filled cylinders, were stored inverted in temperature and humidity controlled cabinets under ICH conditions. The proposed retest period of 36 months is supported by the results.

Other ingredients

Macrogol 4000, isopropyl alcohol, Water for Injections comply with the PhEur monographs.

The Non-compendial ingredients are tested according to internal monographs, and furthermore do not present a risk in the context of TSE.

Various device elements are included in each presentation to assist reconstitution; these meet the requirements of EU Medical Devices legislation.

Product development and finished product

Various formulae were used for clinical trials.

The other ingredients (used mainly to ensure good dispersibility of the gas) were varied but the differences between the formulations are considered non significant by the experts as it has been demonstrated that the addition of palmitic acid and the slight change in phospholipid content does not significantly change the acoustic parameters.

The dispersing agents used are Distearoylphosphatidylcholine (DSPC) and Dipalmitoylphosphatidylglycerol (DPPG.Na) as sodium salt. These emulsifying agents at the selected ratio are able to disperse the hydrophobic gas phase in the hydrophilic phase constituted by water and Macrogol 4000.

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Palmitic acid is used to stabilise the interfacial layer. It also ensures the good stability of the product in the freeze dried state for a sufficient shelf-life period and promotes a better particle size regulation of bubbles throughout the various batches manufactured during the development of the product.

The applicant declares that the phospholipids form a monolayer around the gas bubble.

By means of these agents, after activation a bubble size of average 2 to 8 µm and practically no bubbles over 15 µm is achieved. The volume of gas in the microbubbles (Coulter counter method) is 2-10µl/ml

To explain the choice of the quantitative composition, several development tests were used:

- bubble diameter by use of electronical particle size analyser (Coulter multisizer)
- the acoustic properties
- the resistance to pressure
- microbubble volume concentration.

Of the parameters studied (SF_6 content in the gas phase of the vial, % of phospholipids, macrogol/phospholipids ratio, volume of saline solution added for the reconstitution, etc.) few have little effective influence on the microbubble volume concentration, echogenicity and bubble size distribution in the product prior to administration. On the other hand, it has been shown that the microbubble volume concentration is a relevant parameter which reflects the echogenicity (by contrast there appears to be a poor correlation between bubble number and echogenicity)

Product Specification

The product specification contains relevant tests for identity of SF6, and the excipients are all specified and controlled quantitatively at batch release.

The microbubble volume concentration after reconstitution can vary widely without having an impact on efficacy or safety . The optimum value specified is 8 microlitres SF_6 per ml of reconstituted dispersion, derived from clinical studies. The size distribution of the resulting microbubbles is 90% < NMT 8µm; 99% < NMT 15 µm.

Additional tests include the usual requirements relating to sterility and pyrogens etc.

Batch analyses confirm satisfactory uniformity with the proposed specification.

Stability of the Product

Physicochemical stability of the product has been investigated in three batches, stored as follows: 25°C/60%RH : 30 months, 30°C/60%RH : 12 months, 40°C/75%RH : 6 months, including photostability studies. Particular attention has been paid to confirming the microbubble size distribution characteristics following reconstitution of stored product. The results support the unopened shelflife and storage conditions as defined in the SPC. The quality characteristics and stability of the solvent (0.9%w/v sodium chloride solution for injection) are satisfactory.

Discussion on chemical, pharmaceutical and biological aspects

The novel aspects of the formulation upon which evaluation has focussed, relate to the lipid stabilisation of a gas dispersion in a liquid, and the maintenance of satisfactory particle size characteristics following reconstitution to allow consistent and uniform echogenic properties from batch to batch without a danger of large bubbles which may in theory cause an embolism. The product is manufactured under satisfactory GMP conditions, and physicochemical aspects are considered to be satisfactory for a product of this type.

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3. Toxico-pharmacological aspects

Pharmacodynamics

All studies were performed with a formulation devoid of palmitic acid, in contrast to the formulation used in clinics, however the applicant has demonstrated comparative stability and rheology of both formulations.

Pharmacodynamics related to the proposed indications

- *In vitro* acoustic studies show that the most significant contribution is given by the bubble population between 2.5 and 5.5 µm diameter.
- The variations of the backscatter and of attenuation are negligible in the 2-10 MHz range.
- Sonovue produced a full left ventricular opacification in rat, minipig and rabbit with improved endocardial border delineation.
- In pigs and sheep, Sonovue produced an enhanced spectral color and power Doppler signal in peripheral vessels of several diameters (femoral, renal, mid-cerebral, carotid arteries, portal vein).

General pharmacology

- Sonovue was devoid of cardiovascular and respiratory effects in rat, rabbit minipig and sheep.
- In the dog, transient hypotension, thrombocytopenia and leucopenia were observed, probably due to histamine release.
- The formulation devoid of palmitic acid did not impair blood flow rate in major organs, did not modify blood gases, did not affect the cerebral circulation, did not alter *in vitro* platelet aggregation nor *in vitro* human blood coagulation at doses corresponding to 30 times the recommended dose in clinics, and 10 times the maximal authorised dose in clinics, assuming a maximum of three consecutive injections. Red blood cells morphology was modified at final 50% (v/v) concentrations of Sonovue (leptocytes and echinocytes).

Pharmacokinetics

One preclinical pharmacokinetic study was performed in the rabbit :

Two single doses, 0.3 ml/kg of Sonovue (formulation devoid of palmitic acid), corresponding to approximately 10 and 30 times respectively the recommended imaging dose in human, were administered intravenously.

SF_6 was assayed in plasma, urine and exhaled air by using a validated gas chromatography method coupled with electron capture detector. The limit of quantification of this technique was 0.02 ng/ml.

Results show that blood level decay of SF_6 is extremely fast :

- about 80% of the injected dose is cleared after the first minute following administration of Sonovue
- blood levels of SF_6 drop to background levels by 11 min (dose : 0.3ml/kg) and 20 min (dose : 1 ml/kg).
- Total blood clearance of SF_6 is rapid (218 to 231 ml/min/kg) and half-life elimination is less than 1 minute.

SF_6 is almost exclusively eliminated via the pulmonary route.
98 % of the injected dose was recovered in exhaled air within 2 minutes. Only negligible trace amounts of SF_6 (0.1 ng/g urine, i.e. < 0.001 % of the injected dose) were detected in urine contained in the bladder at 2 h.

Toxicology

Toxicological evaluation of Sonovue was performed with an initial formulation, devoid of palmitic acid. Only reproduction toxicological studies were performed using the final formulation. Palmitic acid (8 ppm) was added in the final formulation to improve the long-term stability of the lyophilisate.

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*Single Dose Studies*Sonovue administered at a dosage up to 20 ml/kg (500 fold more than the expected dose in humans) in rats and monkeys using the IV route did not induce any adverse effects or animal deaths. The acute toxicity potential of Sonovue is probably very low.

Repeated Dose Studies

In a 4 week study in SD rats (0; 0,2; 1; 5 ml/kg) caecitis, colitis, caecum erosion and ulcers were observed from 0,2 ml/kg (dose-related in females but not in males). The effects were reversible within 14 days after treatment cessation. Colon erosion was observed in one animal at the 5 ml/kg dose. These toxic effects were not observed in a confirmatory study in rats and in a similar study in cynomolgous monkeys. Nor was caecum inflammation observed in the fertility and general reproductive performance studies conducted in male and female rats. The mechanism of the toxicity to the gastrointestinal system observed in the one rat study was not elucidated. However, it must be noticed that it was observed only in one study and one species.

In summary, Sonovue up to 5 ml/kg administered for 28 days by the IV route to rats and monkeys showed a low toxicity potential in rats and monkeys.

Toxicokinetic measurements were not performed in these repeated-dose toxicity studies.

Reproduction Toxicity Studies

Reproduction toxicity studies included fertility and general reproductive performance in rats with a treatment of the females up to day 17 after mating, embryotoxicity in rabbits, peri- and post-natal toxicity in rats.

In the repeated-dose toxicity study conducted in rats Sonovue was devoid of testicular toxicity. Hence, it was possible to reduce the premating treatment of the males in fertility studies to 2-4 weeks.

Effects of Sonovue on reproductive functions:

Sonovue administered at dosages up to 5 ml/kg did not show any adverse effects on fertility, gestation and litters. Sonovue is not embryotoxic nor teratogenic and F1 development was normal. The NOAEL for both maternal and developmental toxicity is 5 ml/kg.

Mutagenicity Studies

Sonovuewas neither mutagenic in *Salmonella typhimurium* nor clastogenic in human lymphocytes in vitro or in the mouse bone marrow micronucleus assay

Carcinogenicity Studies

Carcinogenicity Studies were not performed due to the intended use of this product. This is acceptable and in agreement with current EU Guidelines on development of medicinal products for imaging .

Local Tolerance

SonoVue did not induce any effects in one study in rabbits either the IV route (ear vein, dose up to 10 ml/animal) or the S.C. route (ear vein, up to 0,5 ml/animal).

Special Toxicity Studies

Sonovue was tested for its compatibility with blood and serum elements. No effects on haematocrit or coagulation neither increased haemolysis were noticed.
However, a 50% solution of Sonovue altered erythrocytes morphology (leptocytes, echinocytes), whereas a 20% solution exhibited no effects.
Sonovue has no effect on ADP or collagen-induced platelets aggregation.

Sonovue appears to have a good compatibility with the blood but a 50% solution provokes modifications in erythrocytes morphology.

Toxicity of SF6

The applicant has provided an analysis of the toxicity of SF6 based on the literature.

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DL50 of SF6 is 5790 mg/kg (the recommended 0,02 ml/kg dose of Sonovue contains 2.1 µg/kg of SF6). Up to 1000 ppm (6070 mg/m3) are authorised in working places for long-term exposure (8-hour TWA reference period).

Toxicity of PEG 4000

A critical evaluation of the toxicity of PEGs based on the literature is provided by the Applicant.

It is well known that large doses of high molecular weight PEGs may induce acidosis, increase in osmolarity gap and renal failure. The intravenous LD50 of PEG 4000 is 7,5 g/kg in rats and 16 g/kg in mice. Sonovue contains 0.2 mg PEG 4000 per ml, and since the recommended dose of Sonovue in clinics is 2 to 2.4 ml (possibly twice), injection of PEG 4000 as part of Sonovue is not expected to result in the occurrence of toxicological manifestations.

SF6 impurities

The purity of SF_6 as stipulated by the applicant is >99,9%. A single dose of Sonovue is 2,4 ml (recommended human posology), containing 8 µl SF_6/ml = 19 µl SF_6. Total impurities administered to humans with Sonovue are thus 0, 1% x 19 µl = 0,019 µl (0.114 µg total equivalent to 0,0019 µg/kg). The most toxic expected impurity of SF_6 is sulphur pentafluoride (S_2F_{10}) with a 8-hour TWA of 0,025 ppm. Analysis of different batches of SF_6 has shown that quantities of S_2F_{10} were below 10 ppb which is the limit of quantification.

Environmental Risk Assessment

Due to the very small amounts, which are administered, no significant impact on the environment arising from use in accordance with the SPC is expected. SF_6 has negligible ozone-depleting properties compared to the chloro-fluorocarbons.

Discussion on toxico-pharmacological aspects

A complete set of toxicological studies was performed, including acute toxicology and repeated-dose toxicology studies in rats and monkeys, reproduction toxicology studies in rats and rabbits, in vitro and in vivo mutagenicity studies, local tolerance studies and blood compatibility studies. All the protocols were performed according to GLP.
Only toxicokinetic data are missing in the toxicological dossier, which has been justified by the applicant on the grounds that the active substance is inert, present in very small amounts and is eliminated rapidly. Moreover, there were justified methodological difficulties related to the collection of very small quantities of gas and analysis close to the limit of detection. This is considered acceptable.

Administration of Sonovue showed a low toxicity potential. Only in one study conducted in rats was observed an inflammation of the caecum which was dose-dependent in females but not in males. This effect was not repeated in a second study in rats and was not observed in monkeys.

Sonovue is not teratogenic nor embryotoxic.
Sonovue does not appear to be mutagenic.
Carcinogenicity studies have not been conducted which is acceptable in light of the intended use of the product. Sonovue didn't show any toxicity at the injection site.

The amounts of SF_6, S_2F_{10} and PEG present in the maximum recommended therapeutic dose are far below (respectively 1.3 µg, < 10 ppb and 1.16 mg) the currently permitted concentrations in working places for long-term exposure.

The NOEL of Sonovue is 5 ml/kg corresponding to >100-fold more than the intended posology in humans

Although, the toxicological profile was assessed only with the former formulation devoid of palmitic acid. (with the exception of reproduction studies), it can be assumed that palmitic acid would not have any significant effect on the toxicological profile of Sonovue.

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4. Clinical aspects

As originally submitted, the clinical dossier was composed of a cardiac programme (Echocardiography) and a non-cardiac programme (Doppler ultrasonography). The total number of subjects in the combined clinical programme was 1573, 1419 of whom received SonoVue and 154 received comparator. The studies can be further summarised as follows:

- 7 dynamic studies relating to the indications as originally applied for, i.e. B_mode echocardiography, Doppler echocardiography, Doppler Ultrasonography of the portal vein, renal arteries, cerebral arteries,
- 3 dynamic studies in special populations ; CHF, COPD, prolonged duration of enhancement, (
- 2 pharmacokinetic studies,
- 8 Phase II/III, 6 confirmatory and 2 supportive efficacy & safety studies, in:

- B-mode echocardiography,
- Doppler imaging of large vessels (cerebral arteries, carotid or peripheral arteries, abdominal or renal arteries)
- Doppler imaging of vasculature of focal parenchymal lesions (microvasaculature)

Pharmacodynamics

5 studies were performed in healthy volunteers : BR1-001, BR1-002, BR1-007, BR1-008, BR1-009

2 studies were performed in patients with coronary heart disease and valvular heart disease.

All studies were aimed at assessing safety and tolerability of Sonovue, and all were escalating dose studies except for BR1-007.

Clinical pharmacodynamics program

Study type	Study N°	Dosage	Nb of subjects	
			Sonovue	placebo
B-mode echocardiography in healthy volunteers				
4 single ascending doses	BR1-001a BR1-001b	0.003 to 0.12 ml/kg	24	12
4 repeated ascending cumulative doses	BR1-002	0.15 to 0.650 ml/kg	20	10
Repeated fixed doses	BR1-007	4 x 2 ml	10	0
B-mode echocardiography in patients volunteers				
Patients with coronary heart disease and suboptimal border delineation	BR1-005	0.5 to 4 ml (4 doses)	36	0
Doppler echocardiography in patients volunteers				
Patients with valvular heart disease and suboptimal border delineation	BR1-006	0.3 to 2.4 ml (4 doses)	43	0
Doppler ultrasonography in healthy volunteers				
Portal vein and renal artery	BR1-008	0.15 to 1.3 ml (4 doses)	11	0
Cerebral arteries	BR1-009	0.3 to 2.4 ml (4 doses)	12	0

Results:

Exploratory echocardiographic studies

- The most adequate mode of administration is an IV bolus followed by a 5-ml flush of saline.

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- Repetition intervals of Sonovue injections in cross-over trials should be of at least 10 minutes to avoid carry-over effects.
- Sonovue improves endocardial border delineation in left ventricular cavity when compared to placebo from the doses of 0.7 ml in healthy volunteers and 0.5 ml in patients.
- The best balance between contrast enhancement and attenuation, with the maximum duration of contrast enhancement, appeared from the 0.7 to the 4.2 ml dose (0.01 to 0.06 ml/kg).
- Good contrast enhancement was observed at all transducer frequencies between 2.5 and 5.0 MHz.

This justifies the choice of the 0.5 to 4 ml dose regimens to be tested in phase II and III studies conducted in echocardiography.

Exploratory Doppler ultrasonography of vessels

- Administration of Sonovue by power injection syringe required a 3/10 dilution
- The best repetition intervals between two injections in cross over trials should be longer than 10 minutes to avoid a carry-over effect.
- The most adequate method for ultrasonography of slow velocity flow vessels such as portal vein is the spectral Doppler.
- The most adequate method for ultrasonography of high velocity flow vessels such as renal artery is the power Doppler.
- Pulsed wave spectral Doppler and colour Doppler are effective in imaging cerebral arteries.
- Satisfactory improvement in Doppler images were obtained from the 0.3 ml dose.

This justifies the choice of the 0.3 to 2.4 ml dose regimens in efficacy studies conducted in Doppler imaging of vessels.

The pharmacodynamic dossier is complete and well-realised.

Pharmacokinetics

The information summarised in this section was derived from 12 healthy volunteers and 13 patients with compromised pulmonary function enrolled in 2 studies, 24 subjects were evaluated. A validated method of gas chromatography coupled with an electron capture detector was the method used for assay in blood and exhaled air. The lower limit of quantification was 0.02 ng/ml in plasma and in exhaled air.

Pharmacokinetics in healthy volunteers : study BR1-010

Five females and seven males were administered single intravenous doses of Sonovue (0.03 and 0.3 ml/kg, i.e. the intended dose level and 10 times this dose level, respectively) in a randomised cross-over design. Concentrations of SF_6 in both the blood and expired air were determined through 90 minutes post-injection.

Pharmacokinetics in at risk population : study BR1-016 in patients with compromised pulmonary function

Because Sonovue is eliminated entirely via the lungs, study BR1-036 was designed to assess the pharmacokinetic profile in patients with compromised pulmonary function.

Eight males and five females with known diagnosis of diffuse interstitial pulmonary fibrosis received a single intravenous 0.3 ml/kg dose of Sonovue.
Collection of blood and expired air samples for analysis was completed approximately two hours after Sonovue administration.

Results and Conclusions on the clinical pharmacokinetic studies

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After a single intravenous injection of 0.03 or 0.3 ml of Sonovue/kg (approximately 1 and 10 times the maximum clinical dose) to human volunteers, SF_6 was cleared rapidly via the lungs. The mean terminal half-life was 12 minutes (range : 2 to 33 minutes). More than 80 % of the administered dose was recovered in exhaled air within 2 minutes after injection and 100 % after 15 minutes. In patients with diffuse interstitial pulmonary fibrosis, the percent of dose recovered in expired air averaged 100 % and the terminal half-life was similar to that measured in healthy volunteers.

No dosage adjustment seems to be warranted in patients with diffuse interstitial pulmonary fibrosis. There are no pharmacokinetic data documenting the effect of chronic obstructive pulmonary disease (a rather common condition) on the pharmacokinetics of Sonovue. The applicant justified this on the basis that in DIFP the majority of the main elements of gas exchange are simultaneously impaired (diffusing capacity, ventilation, perfusion, ventilation-perfusion matching, total lung capacity) It therefore represents the 'worst case' likely to have an impact on elimination. Furthermore, pharmacodynamic/clinical trial data in patients with COPD do not indicate a problem of clearance.

Due to the lack of data on secretion in milk, caution should be exercised when Sonovue is administered to breast-feeding women.

Because SF_6 is an inert gas, i.e. chemically unreactive, administered in very small quantities, the CPMP considered that investigations on biotransformation and drug-drug interactions are not warranted.

Clinical Efficacy

8 Phase II/III, 6 confirmatory and 2 supportive efficacy studies (n >1300) were performed with the dosages determined during phase II pharmacodynamic studies aimed at demonstrating the efficacy of Sonovue in a number of indications originally proposed by the applicant, all related to Sonovue-induced enhancement of the echogenicity of blood, and the consequent improvement in signal to noise ratio : -

'Cardiac':

i. Evaluation of left ventricular morphology and function (visualisation of cardiac chambers and endocardial border delineation) during B-mode echocardiography.

   3 controlled trials and 2 uncontrolled trials

'Non-Cardiac':

ii. Detection of blood vessels abnormalities by enhancing the colour Doppler signal of the flow and improving quality of the flow velocity profile during Doppler examination of large vessels (cerebral arteries, carotid or peripheral arteries, abdominal or renal arteries, portal circulation)

   e.g the enhancement of weak signals resulting from deep vessels or slow flow.

iii. Assessment of vascularity of focal parenchymal lesions by enhancing the Doppler signal in the microvasculature (breast, liver and pancreas, kidney, ovary, prostate).

Some of the 'Non-cardiac' indications were subsequently withdrawn by the applicant, i.e. relating to diagnostic imaging of abdominal / renal arteries, and imaging of the pancreas, kidney, ovary and prostate.

The results of these non-cardiac trials contributed to the overall safety information, but the efficacy results are not discussed in any detail since they do not contribute to the indication as adopted by CPMP at the time of the opinion.

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I) B-MODE ECHOCARDIOGRAPHY, CONTROLLED TRIALS

Evaluation of efficacy of Sonovue for echocardiography: controlled trials						
Protocol number	**Nb of patients treated**	**Location**	**Study design**	**Sonovue doses (ml)[a]**	**Control**	
					Agent	**Dose[a]**
BR1-019A	143 (76/67)[b]	USA 11 centres	Blinded (off-site), parallel-comparative, crossover dose-response	0.5, 1.0, 2.0, 4.0	Active comparator 0.9% Saline	0.08 ml/kg 0.22 ml/kg 0.08 ml/kg 0.22 ml/kg
BR1-019B	121 (62/59)[b]	USA 10 centres	Blinded (off-site), parallel-comparative, crossover dose-response	0.5, 1.0, 2.0, 4.0	Active comparator 0.9% Saline	0.08 ml/kg 0.22 ml/kg 0.08 ml/kg 0.22 ml/kg
BR1-013	53	USA 6 centres	Blinded (off-site), 3-period crossover	1.0, 2.0	Active comparator	0.22 ml/kg
a All study agent doses administered as an iv bolus injection b Number of patients who received Sonovue / number of patients who received control						

Inclusion criteria :
In all three controlled efficacy trials, patients were included if :

- they were highly suspected of having cardiac disease and
- they had suboptimal endocardial border delineation in the left ventricle during unenhanced echocardiography at rest (left ventricular border delineation score ≤ 14/24).

Exclusion criteria :
Age less than 18 years, severe congestive heart failure (NYHA class IV), unstable angina, severe arrhythmia, recent myocardial infarction, severe pulmonary hypertension, pregnancy and lactation.

Dosages
In studies BR1-019A and B patients received either four doses of Sonovue or two doses of an active comparator (another microbubble dispersion authorised in the EU) and two equivalent volumes of saline vehicle. All doses of Sonovue were administered as bolus injection.

A centralised off-site evaluation by two blinded readers was used to evaluate the end-points.

The Comparator is an agent of the old generation, but more recently approved agents were not available when the studies were conducted.

Primary diagnostic efficacy endpoints:

- *Endocardial border delineation score*; i.e. change from baseline in the total left ventricle endocardial border delineation score.
  The apical 4-chamber and 2-chamber views of the left ventricle were divided into segments by using cardiac maps. A total delineation score (0-24) was obtained by adding the scores (0 = inadequate, i.e., border not visible; +1 = sufficient, i.e., border barely visible; +2 = good, i.e., border clearly visible) from the six individual segments in each of the two views. A suboptimal

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echocardiographic image was arbitrarily defined as a total border delineation score not greater than 14 out of 24 for the two-chamber views combined. Patients had to have at least 4 segments out of 12 with sufficient or good visibility (i.e., a delineation rating of +1 or +2).

- *Left ventricular opacification* on a 4-point scale, from non visible contrast to complete homogeneous and high intensity effect.
- *Duration of useful contrast.*
- Additionally, in study BR1-013 *ejection fraction* measurements obtained from echocardiographic images were compared to those obtained from radionuclide ventriculography. *Wall motion abnormalities* and *overall confidence in primary diagnosis* were also assessed in this study.

Secondary efficacy endpoints were defined for study BR1-013 :

- Ejection fraction measurements when compared to ejection fraction measurements obtained with radionuclide ventriculography.
- Wall motion abnormalities
- Overall confidence in primary diagnosis

Sample size was calculated based on the comparison of the maximum change from baseline in the total left ventricle endocardial border delineation score observed with Sonovue as compared to the maximum change observed with the comparator with α=5% and β=20% using a bilateral t-test. The study should have the power to detect a difference of 4, assuming a standard deviation of 8. With these assumptions, 52 subjects per arm were necessary and 56 scheduled. This number was increased to 62 when the protocol was changed from weight depending doses to fixed doses for patients randomised to Sonovue.

Analysis of efficacy endpoints was performed on the intent-to-treat (ITT) population, i.e., all patients who had received at least one dose of study agent and who had both baseline and at least one post-contrast evaluation.
Statistical significance was defined as $p<0.05$ (two tailed) for all studies. In study BR1-013, within each of the three separate reader analyses (i.e., on-site reader and two off-site readers), statistical significance for the endocardial border delineation analysis was defined as $p<0.025$ (two-tailed) to adjust for the multiple comparisons of each dose of Sonovue versus control.

Studies population: In study BR1-019A, conducted in the USA from September 1996 to April 1997, 148 subject were enrolled and 143 randomised and analysed (76 in the Sonovue Group and 67 in the comparator group), while in study 019B, conducted in the USA from October 1996 to April 1997, 121 were randomised (62 in the Sonovue Group and 59 in the comparator group) and 120 analysed.

Results

Primary endpoints

In each study, baseline scores for primary endpoints were similar across all treatment and control groups. In all three controlled studies, administration of Sonovue resulted in significantly greater increases in left ventricular endocardial border delineation score and opacification score relative to unenhanced (baseline) images than did administration of the comparator and/or saline ($p<0.001$).

In studies BR1-019 A/B, similar results were observed for the duration of useful contrast (which was not an endpoint in study BR1-013), with a definite dose-response relationship up to the 2 ml dose.
No useful contrast effect was observed with the saline vehicle.
The mean duration of useful contrast with the highest dose of the comparator was less than 15 seconds, when compared to 1.7 to 4 minutes with Sonovue.
In studies BR1-019 A/B, for all primary endpoints and for both readers, the maximum effect observed with Sonovue was significantly greater than the maximum effect observed with the comparator.
For reader A, the 2 ml dose and the 4 ml dose were having a larger effect than the best dose of the comparator, whereas for reader B all doses were having a larger effect than the best dose of the comparator.

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In study BR1-013, mean change from baseline endocardial delineation scores for Sonovue ranged from 3.6 - 4.6 across both readers and doses, when compared to a mean 0.4 - 0.7 change for the comparator.
Changes for both Sonovue doses (1.0 and 2.0 ml) were significantly higher ($p<0.001$) as compared to control (the comparator) for both primary endpoints (endocardial delineation score and opacification score).

Secondary endpoints

Regarding ejection fraction measurements in study BR1-013, no statistically significant differences were found between baseline and post-Sonovue assessments when compared to the reference method (scintigraphy).

Ventricular wall motion assessments performed during study BR1-013 showed a significantly greater number of segments rated for wall motion following Sonovue administration when compared to the comparator administration.

In all three pivotal controlled studies, the administration of Sonovue resulted in non statistically significant greater decrease in the percentage of patients with non diagnostic images when compared to the comparator administration.

In the three controlled studies, an increase from baseline of 4 points in left ventricular endocardial border delineation score, corresponding to improved visualisation of at least two additional segments, was considered clinically significant. Thus, clinically significant increases in left ventricular endocardial border delineation scores were observed for all off-site readers at all doses of Sonovue in all three pivotal studies, in the exception of reader A, study BR1-019A, 0.5 and 1.0 ml doses.
The differences between Sonovue and the comparator were also clinically significant according to these criteria.

In studies BR1-019A/B, the percentage of patients with inadequate endocardial border delineation in at least one segment (apical 4-chamber view) at baseline ranged from 61% to 95%. Following Sonovue administration, this percentage decreased dose-dependently up to 26-45 % at the 0.5 ml dose and 8-26% at the 4.0 ml dose.

Non-controlled studies

Two additional multicentre dose-response (0.5/1.0/2.0/4.0 ml) non-controlled studies (BR1-011 and –012) were conducted to confirm the efficacy of Sonovue in patients undergoing echocardiography at rest (n = 218) or during dobutamine or arbutamine-induced stress (n = 219).

Blinded off-site assessment provided similar results than pivotal controlled studies regarding left ventricular border delineation score.

Statistically significant Sonovue-induced dose-related increases in duration of useful contrast effect adjusted for shadowing were observed for both off-site readers, with durations > 4 minutes for the 2 and 4 ml doses, both at rest and during stress.

II) DOPPLER IMAGING OF LARGE BLOOD VESSELS (MACROVASCULATURE)

(cerebral arteries, extracranial carotid, peripheral arteries and portal vein.)

The efficacy of Sonovue in increasing the accuracy of detection or exclusion of blood vessel abnormalities by enhancement of Doppler flow signals during Doppler ultrasound investigation was studied in two large scale dose-response multicentre European clinical trials (BR1-014 and -017) involving 361 patients. Studies BR1-014 and BR1-017 were identical in design.

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Evaluation of efficacy of Sonovue for Doppler diagnostic assessment of macrovasculature: controlled trials				
Protocol number	**Nb of patients. treated**	**Location**	**Study design**	**SONOVUE doses (ml)[a]**
BR1-014	196	Europe 10 centres	Controlled, blinded off-site read, 4-period crossover, dose response	0.3, 0.6, 1.2, 2.4
BR1-017	165	Europe 13 centres	Controlled, blinded off-site read, 4-period crossover, dose response	0.3, 0.6, 1.2, 2.4
total	361			

a All study agent doses were administered as an iv bolus injection

Inclusion criteria

Patients participating in the trials were those referred for Doppler ultrasound investigation to assess vessel haemodynamics and presence of abnormalities, i.e., stenosis and degree of stenosis, occlusion, malformations, accessory arteries, collaterals etc… of the abdominal, cerebral, extracranial carotid, iliac, femoral or peripheral arteries and portal circulation.

Exclusion criteria

Severe chronic heart failure (NYHA class IV), unstable angina, severe arrhythmia, recent myocardial infarction, pregnancy and lactation, recent organ transplant, ongoing chemotherapy or radiation therapy.

Comparator

The study was conducted versus gold standards, i.e., angiography, RMI angiography or Computerised Tomography angiography, depending on the concerned anatomical area: the following different anatomical areas were analysed separately : cerebral (a), extracranial carotid or peripheral arteries (b), portal vein (c).

No approved reference product exists, and placebo was not used because it was thought that unenhanced Doppler provided an equivalent control when assessment is performed in a random and blinded way.

Dosages

The doses of Sonovue were 0.3, 0.6, 1.2 and 2.4 ml.

These doses are based on the results of pharmacodynamic studies (BR1-008 and BR1-009).

Blinded off-site assessment (2 blinded independent readers):

- Separate assessments of randomised baseline and post-contrast images, and
- Assessment of paired baseline and corresponding post-contrast sets for each patients

Primary efficacy criterion

Initially the primary efficacy criterion was the global quality of Doppler investigation i.e. change from baseline in global quality score graded on a 4-point scale (very poor, poor, adequate, excellent).

Diagnostic accuracy versus gold standards (percentage of agreement with gold standard diagnosis) was a secondary endpoint, except for Doppler investigations of the portal vein, where no reference method exists. After discussion with the FDA, diagnostic accuracy was raised to a primary endpoint and sample size calculated for accuracy by anatomical area.

Sample sizes were calculated for global quality score by anatomical area for each study, with $\alpha = 5\%$ and $\beta = 5\%$, with an expected difference in quality scores of at least 1 and a standard deviation of 1.5, leading to a sample size of 30 patients by anatomical area.

Analysis of efficacy endpoints was performed on the ITT population.

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The changes from baseline in global quality scores were evaluated by a 7-point scale (-3, -2, -1, 0, 1, 2, 3) and treated as a continuous variable. An analysis of variance appropriate for a cross-over design was performed, with patient, dose and injection number as factors for the change from baseline.

Diagnostic accuracy was assessed by an independent panel of 3 experts which was asked to compare the diagnoses made by the off-site readers from the Doppler investigations with the diagnosis obtained from a reference imaging modality on a 4-point scale (full agreement, basic agreement, partial agreement and disagreement). The 4-point scale was then reduced to 2 (full and basic versus partial and disagreement) and comparison of the percentages of agreement performed using McNemar's test on 2x2 tables of agreement/disagreement for pre- versus post-contrast examinations.

Results

Studies populations

Number of patients evaluable for efficacy criteria broken down by anatomical area

Anatomical area	**Number of patients included**			**Number of patients assessed for quality score**			**Number of patients assessed for diagnostic accuracy**		
	BR1-014	***BR1-017***	***Total***	***BR1-014***	***BR1-017***	***Total***	***BR1-014***	***BR1-017***	***Total***
Cerebral arteries	73	40	113	73	40	113	61	17	78
Extracranial carotid or peripheral arteries	44	39	83	42	39	81	32	27	59
Abdominal or renal arteries*	43	38	81	42	38	80	36	19	55
Portal vein	37	48	85	36	48	84	-	-	-
Total	197	165							

**Original claims for an indication relating to these structures were withdrawn by the applicant.*

Cerebral arteries

- **Technical efficacy:** In the initial dossier, the global quality score was significantly improved after administration of Sonovue ($p \leq 0.004$).
- **Diagnostic accuracy:** Cut-off values to assess sensitivity and specificity were determined a posteriori.

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Agreement statistics

	Baseline	**Post Sonovue (2.4 ml)**
Off site assessment		
Gold standard		
Study 014 (n=61)		
Reader 1	8%	67% *
Reader 2	25%	67% *
Study 017 (n=17)		
Reader 1	12%	41% *
Reader 2	12%	47% *
* P< 0.05		

- **Diagnostic performance :**

  In Study BR1-014 the sensitivity in the cerebral vessels increased from 0% pre-contrast to 15% at the 2.4 ml dose for Reader 1 and from 0% to 20% for Reader 2. In Study BR1-017 the sensitivity in the cerebral vessels was unchanged from 33% pre-contrast to 33% at the 2.4 ml dose for Reader 1 and increased from 50% to 83% for Reader 2. In Study BR1-014, specificity increased from pre-contrast values of 14% to 86 % at 2.4 ml for Reader 1 and from 29% to 89% for Reader 2. In study BR1-017, specificity increased from pre-contrast values of 17% to 67 % at 2.4 ml for Reader 1 and from 0% to 58% for Reader 2. In both studies, the positive and negative predictive values increased from pre-contrast to post-contrast for both readers. The likelihood ratio also showed an increase from pre-contrast to post-contrast in this population.

Extracranial carotid or peripheral arteries

Across both studies there was a statistically significant increase from baseline in global quality of Doppler investigation score.

Agreement statistics

	Baseline	**Post Sonovue (2.4 ml)**
Off site assessment		
Gold standard		
Study 014 (n=32)		
Reader 3	6%	72% *
Reader 4	31%	60% *
Study 017 (n=27)		
Reader 3	56%	70%
Reader 4	30%	74% *
* P< 0.05		

In Study BR1-014 the sensitivity in the carotid and peripheral vessels increased from 13% pre-contrast to 75% at the 2.4 ml dose for Reader 3 and from 50% to 75% for Reader 4. In Study BR1-017 the sensitivity in the carotid and peripheral vessels changed from 89% pre-contrast to 100% at the 2.4 ml dose for Reader 3 and increased from 22% to 67% for Reader 4. In Study BR1-014, specificity increased from pre-contrast values of 0% to 85 % at 2.4 ml for Reader 3 and from 31% to 75% for Reader 4. In study BR1-017, specificity increased from pre-contrast values of 23% to 46 % at 2.4 ml for Reader 3 and from 8% to 85% for Reader 2. In both studies, the positive and negative predictive values increased from pre-contrast to post-contrast for both readers. The likelihood ratio also showed an increase from pre-contrast to post-contrast in this population.

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Portal vein

- **Technical efficacy**: Global quality was significantly improved, with a dose dependency, after administration of Sonovue in both studies.

- Diagnostic accuracy: No standard reference exists, and diagnostic accuracy when compared to the reference method cannot be determined. However, clinical usefulness of SonoVue in the portal vein was claimed and supported by a literature review and a post-hoc analysis of the existing data according to specific patient pathology. From the literature data, incomplete Doppler examinations and impaired visualisation of vessels and blood flow can lessen accuracy and lead to erroneous results in: 10% of patients to be evaluated for portal vein thrombosis, 32% of patients with liver cirrhosis, 7% of patients where no flow is visible after TIPS, and - in those cases investigated for portal vein involvement by liver tumours, in which no flow is visualised.

From the post-hoc analysis, the use of SonoVue in Doppler imaging of the portal vein increased the proportion of diagnostic scans in previously inconclusive Doppler investigations of the portal vein in:34% cirrhotic patients with suspected portal vein thrombosis, 40% patients with portal hypertension, 25 to 50% patients with portal involvement due to tumours, and in 82 to 91% patients for follow-up investigations of TIPS insertion.

It was considered that a technical efficacy in terms of improvement in the global quality when compared to precontrast Doppler examination was not demonstrated for Doppler imaging of abdominal arteries.

III DOPPLER IMAGING EVALUATION OF VASCULARITY OF PARENCHYMAL LESIONS (MICROVASCULATURE). (i.e. vascularity of breast lesions ; liver lesions)

One European, blinded off-site reading, 4-period cross over, dose-response study was conducted from November 1996 to January 1998 in 217 patients (BR1-018).
This study also included investigations into focal lesions of the pancreas, kidney, ovary and prostate. Original claims for indications relating to diagnostic imaging in these areas were later withdrawn by the applicant and are not discussed in detail as they do not appear in the SPC as adopted by CPMP.

Study methodology and endpoints

The doses of Sonovue were 0.3, 0.6, 1.2 and 2.4 ml as for Doppler studies of macrovasculature. No approved active comparator existed; saline vehicle was not used as non active control because it was felt that unenhanced Doppler provides an equivalent control when assessed in a random and blinded way.

- Inclusion criteria : at least one focal lesion in the breast, liver, pancreas, kidney, ovary or prostate identified during unenhanced-B mode Doppler investigation.
- Exclusion criteria were similar to those in the large vessels Doppler studies.
- Primary endpoint : change from baseline in the global quality score of the Doppler investigation (4-point scale), as assessed by 2 off-site blind readers with the same protocol as for Doppler studies of macrovasculature.
- Secondary endpoint : diagnostic accuracy, as assessed by an on-site unblinded reading, when compared to a reference diagnostic modality (cytology or histology or pathology).
- Sample size calculation : at least 30 patients by anatomic site.

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Results

Study population :

Table 1. Study BR1-018 : study population			
Anatomical area	**Nb of patients included**	**Nb of patients evaluable for on-site analysis of diagnostic accuracy**	**Nb of patients evaluable for off-site analysis of global quality**
Total	**220**		**212**
Breast	40	38	40
Liver	69	24	68
Pancreas	2	2	2
Kidney	36	24	34
ovary	26	23	26
prostate	44	37	42

- **Focal lesions of the breast**

- **Technical efficacy:** In the initial dossier, the global quality score was significantly improved with Sonovue in breast lesions.

On pre-contrast inconclusive examinations in Doppler studies of breast issue, the applicant's response shows that the majority of subjects included in Doppler studies of breast experienced a pre-contrast inconclusive examination (nearly 60%).

Furthermore the sample size calculation based on diagnostic accuracy as primary endpoint provided as requested shows that the evaluation of 41 patients was required in order to detect a change in agreement from unenhanced to Sonovue-enhanced Doppler. In fact 38 patients were evaluated for diagnostic accuracy which is acceptable.

- **Diagnostic accuracy:** Cut-off values to assess sensitivity and specificity were determined a posteriori.

Agreement statistics

	|Baseline	**Post Sonovue (2.4 ml)**
On site assessment		
Gold standard (n=38)	16%	40% *
Final |diagnosis (n=40)	35%	63% *
Off site assessment		
Gold standard (n=38)		
Reader 3	48%	58% *
Reader 4	71%	68%
Final diagnosis (n=40)		
Reader 3	53%	65% *
Reader 4	70%	73%
* P< 0.05		

- **Diagnostic performance :** Both sensitivity and specificity increased from pre- to post-contrast. This increase was statistically significant for specificity (p=0.025). Although it did not reach statistical significance (p=0.102) for sensitivity, the increase in sensitivity of 0.19 was substantial as the expected change from pre- to post contrast is reached. The positive and negative predictive values were both increased. The likelihood ratio also showed a marked increase from pre-contrast to post-contrast. In the Gold standard and final diagnosis off-site analysis only the sensitivity increased significantly for one reader out of two.

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- **Focal lesions of the liver**

- **Technical efficacy:** A technical efficacy of Sonovue in the Doppler of liver lesions was clearly demonstrated in the initial dossier.

- **Diagnostic accuracy:** The same methodology as for breast lesions was used (i.e. cut-off for sensitivity and specificity was determined a posteriori).

Agreement statistics

	Baseline	**Post Sonovue (2.4 ml)**
On site assessment		
Gold standard (n=24)	54%	67%
Final diagnosis (n=69)	55%	68% *
Off site assessment		
Gold standard (n=23)		
Reader 1	70%	65%
Reader 2	30%	65% *
Final diagnosis (n=64)		
Reader 1	61%	56%
Reader 2	36%	62% *
* P< 0.05		

- **Diagnostic performance:** Sensitivity changed very little from pre- to post-contrast, since it was already very high before Sonovue administration. However, specificity did increase from pre- to post-contrast in both analyses gold standard and final diagnosis. This increase was statistically significant for the final diagnosis (p=0.0016). The positive and negative predictive values both increased for patients with a final diagnosis. The likelihood ratio also showed a marked increase from pre-contrast to post-contrast in this population. In the Gold standard off-site analysis specificity and not sensitivity increased significantly for one reader out of two. In the Final diagnosis off-site analysis specificity and sensitivity increased significantly for one reader out of two.

Clinical Safety

20 clinical studies have been conducted to assess the pharmacokinetic profiles, the efficacy, the safety and tolerability of Sonovue, arising from use in cardiac and non-cardiac studies.
1573 subjects were enrolled, 1419 subjects received either Sonovue or Sonovue + active comparator or Sonovue + placebo (saline).

The safety of Sonovue was evaluated in

- healthy volunteers (89 non patients subjects),
- in at risk populations : patients with a pulmonary fibrosis (13 patients), or with a chronic obstructive pulmonary disease (12 patients),
- in patients with suspected or known heart disease (726 patients), and
- in patients who required investigation of large vessels or parenchymal focal lesions (578 patients).

In patients, mean age was about 60 years. There are no more details about the repartition of patients according to age. No study was performed in children.

Different dose regimens of Sonovue were assessed with regard to safety and efficacy.

4 different formulations of Sonovue were used in these studies. In the first and second formulations, there was no palmitic acid. In the third and fourth formulations, there was palmitic acid. The fourth formulation was the one proposed for the market. The studies BR1-010, BR1-011, BR1-012, BR1-013,

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BR1-014, BR1-016, BR1-017, BR1-018, BR1-019 A/B, BR1-022, BR1-026, BR1-036 were performed with the final formulation.

Adverse events and serious adverse event/deaths :-

Most commonly reported adverse events

The overall incidence of adverse events "related" to Sonovue is 18.8 % (267 related adverse events /1419 patients who received Sonovue). There has been no “related” serious adverse event. One serious adverse event was considered as "unknown related" to Sonovue by the investigator (sensory motor paresis).

The most commonly reported adverse events were : reactions at the injection site (pain, heat…), headache, paresthesia, nausea, and sensations of warmth (vasodilatation, flushing, hot sensation).

Serious adverse events and deaths

Over the 20 clinical studies, 10 serious adverse events were reported : 9 with Sonovue and 1 with the comparator (congestive heart failure with fever : unrelated).

Among the 9 serious adverse events reported in Sonovue-administered patients, only one sensory motor paresis was considered as related to Sonovue by the investigator.

The other SAE were 2 chest pains, one congestive heart failure and 5 deaths (1 hepatocellular carcinoma, two heart failures, one liver and renal failure secondary to alcoholic liver disease and one disseminated adenocarcinoma), but none was considered as related to Sonovue by the investigator.

There is no difference between Sonovue and the comparator in terms of related serious adverse events.

Discontinuations due to adverse events
3 patients discontinued due to adverse events in 3 studies : one for mild superficial pain at the site of ultrasound probe, one for diarrhoea and vomiting and one for mild restlessness.

Study BR1-005 prematurely discontinued, apparently due to technical problems.
The incidence of adverse events in this study was high when compared to other studies: 22.2 % (8/36 patients experienced 10 adverse events, all deemed related to Sonovue administration)

Adverse events in healthy volunteers studies
6 studies were conducted in 89 healthy volunteers. 43 adverse events were reported with Sonovue, of which 33 were considered drug-related

In studies BR1-001 and 002 conducted versus placebo, 24 adverse events occurred in the Sonovue group (n = 44) versus 13 in the placebo group (n = 12).

No serious adverse events were reported in these studies.

Adverse events in echocardiography studies
720 patients received Sonovue A total of 211 adverse events were experienced by 137 patients receiving Sonovue. 121 adverse events experienced by 82 patients were considered to be related to Sonovue by the investigator.

The overall incidence of patients who experienced at least one adverse event was 19.02 % (137/720). The incidence of patients who experienced adverse events "related" to Sonovue, was 11.4 % (82/720).

3 studies, BR1-013, BR1-019A and BR1-019B, are comparative studies versus the comparator and BR1-016 was a comparative study versus placebo (saline vehicle).
No serious adverse events related to the study agents were reported in these 3 trials

Concerning the Phase II & III studies in particular, these may be summarised as below:-

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Adverse events during phase II and III efficacy studies in echocardiography

N° of the study	Clinical Phase Indication comparator	Number of Subjects	Inclusion criteria	Dosage	Safety and tolerability
BR1-016	II Echocardiography Sonovue vs placebo	19	Patients with a NYHA functional class II-IV congestive heart failure	2 IV doses Sonovue (2 ml and 4 ml) + 2 IV doses placebo (2 ml and 4 ml) in 13 patients 2 doses placebo in 6 patients	**Total AE** 5 AE with (Sonovue + placebo) + 1 AE with placebo **related AE** 4 AE unknown related to (Sonovue + placebo) (increase of creatinin, increase of urea, increase of uric acid, hypotension) 1 AE probably related to placebo : paint at injection site
BR1-006	II Echodoppler Echocardiography	43	Patients with valvular heart disease	4 doses of Sonovue order randomised 0,3-0,6-1,2-2,4 ml	**Total and related AE** 4 AE 1 sensation of warmth : possibly related 1 pain at injection site : possibly related 1 vasodilatation : doubtfully related 1 hypersalivation : doubtfully related
BR1-012	II-III Echocardiography	219	Patients with known or suspected ischemic heart disease	2 identical doses of Sonovue per person : one at rest and the other after a pharmacological stress 0,5 ml in 54 patients, 1 ml in 55, 2 ml in 56, and 4 ml in 54. Pharmacological agents : arbutamine or dobutamine	**Total AE** 88 AE **related AE** 34 AE (the most frequent « related » AE : 4 nausea, 4 injections site reaction, 3 paresthesia, 2 asthenia, 2 headache, 2 dizinness,2 hyperesthesia, 2 taste perversion). 1 atrial fibrillation : possibly related, but non serious.
BR1-011	II-III echocardiography	218	Patients with known or suspected ischemic heart disease	4 doses of Sonovue order randomised 0,5-1-2-4 ml	**Total AE** 54 AE **related AE** 40 AE (the most frequent « related » AE : headache, nausea, injection site reaction) 1 serious : 1 chest pain : unrelated
BR1-005	II Echocardiography	36	Patients with highly suspected cardiac disease	4 doses of Sonovue order randomised 0,5-1-2-4 ml	**Total and related AE** 10 AE 1 reaction at injection site : definitely related 1 pain at injection site : probably related 1 ventricular extrasystole : probably related 1 increase of bilirubin : probably related 1 hyperuricemia : probably related 1 ventricular extrasystole : possibly related 1 increase of bilirubin : possibly related 1 hyperuricemia : possibly related 1 exanthema : doubtfully related 1 hyperglycaemia : doubtfully related

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N° of the study	Clinical Phase Indication comparator	Number of Subjects	Inclusion criteria	Dosage	Safety and tolerability
BR1-013	II-III Echocardiography Sonovue vs the comparator	53	Patients with highly suspected cardiac disease	2 doses of Sonovue : 2 ml and 1 ml and 1 dose of the comparator : 0,22 ml/kg order randomised	**Total AE** 5 AE « related » AE 2 AE : probably related to Sonovue + the comparator 1 nausea and 1 taste perversion 1 serious : 1 congestive heart failure : unrelated to Sonovue + the comparator
BR1-019A	II-III Echocardiography Sonovue vs the comparator	143 (76 Sonovue) (67 control)	Patients with highly suspected cardiac disease	4 doses of Sonovue in randomised order : 0,5-1-2-4 ml OR 2 doses of the comparator in randomised order : 0,08 or 0,22 ml/kg + 2 doses of saline	**Total AE** 66 AE : 34 AE with the comparator /saline « related » AE 20 AE related Sonovue (the most frequent related AE : paresthesia, taste perversion) 17 AE related to the comparator /saline (the most frequent related AE : injection site reaction, vasodilatation) 2 serious : 1 chest pain : unrelated to Sonovue 1 congestive heart failure with fever : unrelated to the comparator
BR1-019B	II-III Echocardiography Sonovue vs the comparator	121	Patients with highly suspected cardiac disease	4 doses of Sonovue in randomised order : 0,5-1-2-4 ml OR 2 doses of the comparator in randomised order : 0,08 or 0,22 ml/kg + 2 doses of saline	**Total AE** 29 AE : 11 AE with Sonovue and 18 AE with the comparator /saline **related AE** 7 AE related to Sonovue (headache, injection site reaction, pain, nausea, hyperglycaemia, and respiratory disorders) 11 AE related to the comparator (3 injection site reaction, 2 headache, paresthesia, dry mouth, partial transitory deafness, taste perversion)

The results of the comparative studies BR1-013 and BR1-016 did not allow for the separation between Sonovue-related, the comparator -related and placebo-related adverse events (the number of administered injections was not the same in the Sonovue and the control group). Similarly, BR1-012 study assessing efficacy of Sonovue in echocardiography examination during pharmacological stress agent did not allow for the separation between Sonovue-related or pharmacological stress agent-related adverse events.
Based on the results of pivotal BR1-019A/B studies, Sonovue appeared to be as safe as the comparator.

Adverse events in Doppler studies

- 3 studies (BR1-014, -017 and –018) enabled to enrol 578 patients to assess Sonovue efficacy for Doppler examination of macro or microvasculature.
  All 578 patients received Sonovue.
  A total of 153 adverse events were experienced by 90 patients :

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Adverse events during phase II and III efficacy studies in Doppler investigations :

N° of the study	Clinical Phase Indication	Number of Subjects	Demographic design	Dosage	Dosage
BR1-014	II-III Doppler	196	Patients who required Doppler investigation of large vessels	4 doses of Sonovue order randomised 0,3-0,6-1,2-2,4 ml	**Total AE** 74 AE **related AE** 53 AE (the most frequent « related » AE : injection site reaction, injection site pain, paresthesia) 2 serious AE : 1 death : hepatocellular carcinoma : unrelated 1 sensory motor paresis : unknown related
BR1-017	II-III Doppler	165	Patients who required Doppler investigation of large vessels	4 doses of Sonovue order randomised 0,3-0,6-1,2-2,4 ml	**Total AE** 41 AE **related AE** 27 AE (the most frequent « related » AE : hypertension, vasodilatation, injection site reaction, injection site pain) 2 serious AE : 1 death : heart failure : unrelated 1 death : hepatic and renal failures secondary to alcoholic liver disease : unrelated
BR1-018	II-III Doppler	217	Patients who required Doppler investigation of parenchymal focal lesions	4 doses of Sonovue order randomised 0,3-0,6-1,2-2,4 ml and patients investigated for ovary and prostate were given 2 of 4 doses	**Total AE** 38 AE **related AE** 21 AE (the most frequent « related » AE : nausea, vasodilatation, injection site reaction) 2 serious AE : 1 death : heart failure : unrelated 1 death : disseminated adenocarcinoma : unrelated

105 AE experimented by 57 patients were deemed related to Sonovue by the investigator. The overall incidence of patients who have experienced at least one adverse event was 15.6 % (90/578). The incidence of patients who have experienced an adverse events deemed related to Sonovue by the investigator was 9.9 % (57/578).

In addition,

Study BR1-036 was a PK study conducted in 13 at risk patients with pulmonary fibrosis.
3 adverse events were reported (1 chest pain and 1 pharyngitis were reported related).

- Study BR1-022 was a pharmacodynamic study conducted in 12 at risk patients with moderate to severe chronic obstructive pulmonary disease. Each patient received one dose Sonovue and one dose placebo.
  9 patients experienced 20 adverse events.

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Adverse events of special interest

ECG changes

Most patients having had ECG changes as adverse events exhibited ECG changes or abnormalities *before* echocardiography. Moreover, those patients had antecedents of myocardial infarction or had a coronary disease, or a heart failure. So, it is difficult to determine if the ECG changes observed are really related to Sonovue or to the pathology of the patients

Changes in arterial blood pressure :

- in study BR1-019A, the percentages of patients with an increase of at least 2 mm in systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in patients of the Sonovue group when compared to the comparator group (10.5% versus 6% for SBP and 22.4% versus 11% for DBP). 6 patients of the Sonovue group experienced such disturbances in blood pressure, 1/6 after the first injection and after each following injection, 4/6 after the third injection and the sixth after the fourth injection.
- in study BR1-019B, the percentages of patients with an increase of at least 2 mm in DBP were higher in patients of the Sonovue group when compared to the comparator group (10% versus 3.4%). 3 patients of the Sonovue group are concerned, and experienced blood pressure disturbances following the fourth injection.
- In study BR1-013, no comparison can be established between blood pressure changes related to Sonovue or to the placebo, due to the design of the study.
- In study BR1-006, the investigator compared BP changes after one injection of placebo and after one injection of Sonovue in order to assess a possible effect of Sonovue on blood pressure. No relevant difference was observed.
- In study BR1-016, in the group who received Sonovue + placebo (N=13), 1 patient had an increase of the systolic blood pressure and 2 patients had an increase of the diastolic blood pressure. In the placebo group (N=6), 1 patient had an increase of the systolic blood pressure and 1 patient had an increase of the diastolic blood pressure.

5. Overall Conclusion and benefit risk assessment

- **Quality**

Pharmaceutical development has focussed on the presentation of a product giving consistently uniform microbubble size distribution characteristics following reconstitution. In turn this should allow consistent and uniform echogenic properties from batch to batch without a danger of large bubbles which may in theory cause an embolism. The product is manufactured under satisfactory GMP conditions, and physicochemical aspects are considered to be satisfactory for a product of this type.

- **Preclinical pharmacology and toxicology**

A complete set of toxicological studies has been performed according to GLP.
The absence of toxicokinetic data and carcinogenicity studies is considered acceptable in this particular case. Sonovue is not teratogenic nor embryotoxic, nor does it appear to be mutagenic. In general, administration of Sonovue showed a low toxicity potential. It should be noted that although the toxicological profile was assessed only with a former formulation devoid of palmitic acid. (with the exception of reproduction studies), it can be assumed that palmitic acid would not have any significant effect on the toxicological profile of Sonovue.

- **Efficacy**

1. Echocardiography:

The technical efficacy of Sonovue in echocardiography is demonstrated insofar as Sonovue administration enabled the visualisation of significantly more left endocardial border delineation than

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the comparator, with a significantly higher contrast duration. So, clinical usefulness has been indirectly demonstrated by a significantly reduction in the number of patients with inadequate delineation in at least one segment.

- Concerning the reliability of these results, the use of independent readers allows assessment of inter-observer variability and calculation of Kappa coefficient. Reported analyses show that the inter off-site readers difference is moderate (slightly better with Sonovue than without Sonovue). This discrepancy between readers is more related to subjectivity in the image interpretation than to the compound.
- Since no gold standard is currently easily available, diagnostic performance (yield of correct and incorrect diagnoses based on information provided by Sonovue) and efficacy variables such as sensitivity, specificity, likelihood ratios and predictive values cannot be determined.
- The CPMP accepted that the characteristics of the patients who participated in the three pivotal studies in support of the echocardiographic application of SonoVue are typical of those presenting with functional cardiac disease. It may be concluded that the study results are representative of the usual abnormalities and the full range of disease severity that are usually present in the population in which SonoVue is intended to be used, and the results indicate the clinical usefulness of SonoVue.
- In particular, concerning this patient population for which there is a clinical usefulness of the product in echocardiography, it is that population in which a first echocardiography without contrast agent was inconclusive with regards to diagnostic or prognostic issues

and which retrieves a benefit from having a longer left ventricular endocardial border delineation visualised. Improvement in image quality and longer duration of useful contrast obtained with Sonovue-enhanced echocardiography increases the level of delineation of the endocardial border and of visualisation of cardiac chambers.
Hence, the target population who retrieves a benefit from administration is the population of patients with known or suspected segmentary kinetic abnormalities, i.e. patients with myocardial ischaemia (coronary heart disease including history of myocardial infarction and some cardiopathies).

- Other patient populations do not retrieve a demonstrated benefit from Sonovue administration, and the benefit to risk ratio is therefore unfavourable in these cases.
- Dosage: The doses recommended in the SPC are considered to be justified. A second injection may be given when deemed necessary by the physician.
- During pharmacological stress, a situation in which patients will be become less echogenic a potential benefit of Sonovue could be expected.

2. Macrovasculature

The submitted studies demonstrated that Sonovue enhanced Doppler signals in general. However, there is no benefit (clinical usefulness) in patients with pre-contrast conclusive Doppler images. The main question has been whether Sonovue could enhance Doppler signals up to clinically useful levels in patients with pre-contrast inconclusive Doppler examination.

Cerebral Arteries, extracranial Carotid or Peripheral Arteries

The quality of the precontrast examination was often suboptimal which is expressed by the high number of of inconclusive examinations. This number is reduced after administration of Sonovue, to a clinically significant degree. Diagnostic accuracy shows an important increase in total agreement with gold standard after Sonovue administration. A gain in the majority of diagnostic performance characteristics was demonstrated.

Portal Vein

Also in this area the quality of the precontrast examination was often suboptimal, which has been supported by literature data submitted by the applicant in the responses to the consolidated list of questions. The assessment of diagnostic accuracy was not possible because Doppler evaluation itself is

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the gold standard. However, administration of Sonovue showed also for this indication a significant improvement in the number of evaluable evaluations.

3. Microvasculature

In an on-site assessment, the quality of Doppler imaging and the median duration of clinical useful contrast enhancement of the microvasculature (breast and liver lesions) were improved after administration of Sonovue compared with the pre-injection images. An increase in the percentage of patients with a possible diagnosis was observed.

However, the CPMP had some concerns over on-site evaluation, and requested an off-site blinded assessment of the clinical data. (See Benefit/Risk section of this report, below).

- **Safety**

The safety assessment of Sonovue has been hampered by the fact that no study was conducted in a double-blind manner, which makes a definitive conclusion difficult. No study has been conducted in children, nor in pregnant women. However, based on submitted data, Sonovue administration appeared to be safe, even in patient populations potentially at risk. Sonovue appears to be generally well tolerated at the recommended dosage. Based on the submitted studies, it seems difficult to conclude that Sonovue caused changes in ECG or blood pressure. However, no definite conclusion can be drawn, and ECG and blood pressure should be carefully monitored during a Sonovue enhanced echocardiography with a pharmacological stress (e.g. with dobutamine). A maximum of two consecutive injections is also recommended in the SPC.

Benefit/Risk Assessment

Concerning the indication relating to echocardiography, and considering the results summarised above, the CPMP considered that the benefit/risk balance was acceptable and the data presented could support an indication in line with the other ultrasound contrast agents evaluated by CPMP through the centralised procedure, except for wall motion investigations.

Following the evaluation of the macrovasculature and microvasculature studies by CPMP, a list of unresolved issues was defined and sent to the applicant , with a request to discuss them at an oral explanation in order to clarify the benefit/ risk balance for this product, in these indications, i.e:-

"...

Doppler imaging of microvasculature

- The applicant should discuss the claimed increase in accuracy in terms of diagnostic test criteria.
- For portal vein studies only, the applicant should provide a justification for the clinical usefulness of SonoVue in a defined patient population.

Doppler imaging of breast and liver lesions :

- The results of an off-site blinded assessment should be presented.
- Statistical analysis of diagnostic accuracy should be provided as a function of dose administered as it was done for cerebral arteries and extracranial or peripheral arteries. The relation of a 2.4 ml dose with the efficacy should be clearly established..."

These issues were addressed by the applicant. Diagnostic accuracy regarding macrovasculature has been subsequently accepted by the CPMP but for the microvasculature the presented information was not sufficient to establish diagnostic accuracy and performance but only improved quality of imaging leading to more specific lesion characterization, and this is reflected in the wording of the indication in the SPC.

Concerning safety, SonoVue administration appears to be safe, even in patient populations potentially at risk - however it should be noted that that the numbers of high-risk patient subgroups are relatively

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small. No significant differences were observed between Sonovue and the authorised comparator product, but also in this case the numbers are small.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of SonoVue was favourable in the following diagnostic indication :

"SonoVue is for use with ultrasound imaging to enhance the echogenicity of the blood, which results in an improved signal to noise ratio. SonoVue should only be used in patients where study without contrast enhancement is inconclusive.

Echocardiography

SonoVue is a transpulmonary echocardiographic contrast agent for use in patients with suspected or established cardiovascular disease to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation.

Doppler of macrovasculature

SonoVue increases the accuracy in detection or exclusion of abnormalities in cerebral arteries and extracranial carotid or peripheral arteries by improving the Doppler signal to noise ratio.

SonoVue increases the quality of the Doppler flow image and the duration of clinically-useful signal enhancement in portal vein assessment.

Doppler of microvasculature

SonoVue improves display of the vascularity of liver and breast lesions during Doppler sonography, leading to more specific lesion characterisation."

and therefore the CPMP recommended the granting of the marketing authorisation.

6. Post-Marketing

A number of changes have been implemented in section 4.8 of the SPC following the assessment of the 1st and 2nd Periodic Safety Update Reports. Following reports of severe hypersensitivity reactions, the following text has been added to section 4.8 of the SPC: "In post marketing surveillance, rare cases suggestive of hypersensitivity, including anaphylactic shock, have been reported." Further to an in-depth review of the clinical trial data, the following adverse events have been deleted as they are considered unrelated to Sonovue: "peripheral oedema", "nervousness", "personality disorder", "dry mouth", "respiratory disorder" and "ecchymosis". The description of some terms already included in section 4.8 has been change: "sinusitis" to "sinus pain", "abnormal vision" to "blurred vision", "non-specific pain" to "pain NOS" and "altered sensation at the injection site" to "injection site reactions including bruising, heat and paraesthesia at the injection site". The frequency of taste perversion, paresthesia and flushing (changed to vasodilation) has been updated to "uncommon". Finally, several adverse events from clinical trials listed in section 4.8 have been re-coded according to MedDRA terms.

6.1 Reintroduction of the echocardiography indication and addition of contraindications and warnings following the Urgent safety Restriction of May 2004

6.1.1 Introduction

On 4 May 2004 the MAH informed the Rapporteur and the EMEA that the FDA had placed ongoing studies of SonoVue in liver imaging and myocardial perfusion imaging on clinical hold based on safety data arising from spontaneous reporting in Europe. Three reported deaths were of particular concern, as all 3 patients died within minutes/a few hours of SonoVue administration. Furthermore, it was noted that post marketing data showed serious cardiac events occurring within seconds to minutes after SonoVue administration in cardiac patients.

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As a result of these findings, an Urgent Safety Restriction (USR) was triggered on 17 May. The following changes in the SPC were implemented as a result of the USR:

- Suppresion of the echocardiography indication (section 4.1)
- Addition of a contra-indication in patients with known coronary artery disease, myocardial infarction (MI), unstable angina, acute cardiac failure, class III/IV cardiac failure, severe rhythm disorders, acute endocarditis and prosthetic valves; (section 4.3)
- Recommendations to closely monitor patients during and for at least 30 min following the administration of SonoVue and to have emergency equipment and personnel readily available (section 4.4)
- Update of the "Undesirable effects" reported in post-marketing (section 4.8)

This variation follows the above-mentioned USR procedure. The MAH proposes to reverse some of the changes implemented during the USR, namely to remove the suspension of the echocardiographic indication, and to change some of the contra-indications.

6.1.2 Clinical aspects

The assessment of this variation has majored on the safety data presented by the MAH and on the clinical benefits of SonoVue in the suppressed indication in echocardiography, in order to reach an overall conclusion of the benefit: risk for the product.

6.1.2.1 Safety

Post-marketing data

The 3 fatal cases

The following 3 cases with a fatal outcome have been reported.

Case BRO-005943 (described in the 4th PSUR) was reported in Germany in a 69-year-old male patient admitted to hospital for coronary angiography performed 1 day prior to the contrast echocardiography examination. The patient's cardiovascular history included anterior and posterior myocardial infarctions (MI), and percutaneous transluminal coronary angioplasty (PTCA) and stent-implementation to the left anterior descending (LAD) artery and the diagonal branch (approximately 6 months prior to SonoVue exposure). One day before contrast echocardiography examination the patient was premedicated with antihistamines (8 mg dimetindenmaleate, 100 mg ranitidine) and 250 mg methylprednisolone due to known hypersensitivity to iodinated contrast agents. A 3-vessel coronary artery disease (CAD) was documented with a previously known occlusion of the right coronary artery, moderate stenosis in the circumflex artery and a high degree re-stenosis in the distal area of the LAD-stent, which was successfully treated by PTCA on the same day.

The following day the second contrast echocardiography was performed. The patient had received no further prophylactic treatment. Prior to Sonovue injection the patient was well (no angina, no dyspnoea), except for an unusual restlessness. The patient's heart rate was around 90 beats per minute (bpm) and his blood pressure (BP) was not measured. Following an initial dose of 2.0 ml SonoVue the patient experienced no ADR. A second 2.0 ml SonoVue injection was administered about 2 minutes later. Contrast echocardiography showed that the global left ventricular systolic function was reportedly normal with slight concentric hypertrophy and regional akinesia (apical, inferoseptal and inferior). Approximately 4-5 minutes after the second injection, the patient's restlessness increased and his heart rate (50 bpm) and BP decreased, which were accompanied by paleness and cold sweat. The patient was immediately treated with 250 mg methylprednisolone, 8 mg dimetindenmaleate, 100 mg ranitidine, and 1 mg atropine because of suspected anaphylactic shock, but no improvement was observed. Within minutes, the patient lost consciousness and no pulse was palpable. Following repeated administrations of adrenaline, sodium bicarbonate and atropine, the patient developed ventricular fibrillation and was defibrillated several times. An emergency coronary angiography was then performed under mechanical reanimation which showed a re-occlusion of the LAD-stent in the previously dilated area, which was successfully re-treated by PTCA.

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Following the intervention the patient's systolic BP was reported as 100 mmHg, and further treatment in the intensive care unit (ICU) included mechanical ventilation and mechanical circulatory support with an intra-aortic balloon pump. Later on the same day, the patient developed bradycardia and a ventricular escape rhythm. Despite treatment with high doses of adrenaline, the situation could not be stabilised and the patient died of cardiogenic shock approximately 4 hours after the administration of SonoVue. It should be noted that elevations of serum creatine kinase (25.48 micromol/L or 1500 U/L), LDH (42.98 micromol/L or approx 3000 U/L) and troponin levels (7.05 Iu/L) were observed 2.5 hours after the echocardiographic examination. The autopsy confirmed the presence of MI and did not show any signs of an allergic reaction.
The MAH considers that the patient was clinically unstable at the moment of receiving SonoVue on the basis of :
-the clinical description before SonoVue administration i.e restlessness, which is consistent with an ongoing myocardial infarction, and the patient's heart rate of 90 bpm despite betablocker treatment, indicating an ongoing cardiovascular problem;
-the pre-contrast echographic findings of total antero-septal akinesia, which is indicative of ischaemia in the territory of the LAD coronary artery and consistent with the angiographic finding of complete occlusion of the LAD stent which had been reopened the day before;
-the slow reduction of BP and heart rate consistent with an ongoing acute MI;
-the results of laboratory data which showed a marked increase on CK, troponin and LDH 2.5 hours post SonoVue administration with values consistent with an acute coronary event occurring 6-8 hours before the echographic examination.
-a low serum potassium level (3.4 mmol/l) which constitutes a potential increased risk of cardiac arrhythmias
-an angiographically documented occlusion of a stent in the LAD artery in a patient with a severe 3-vessel CAD.

Case BCM-000767 reported from Italy describes a serious AE in a 51-year-old male patient with history of recent anterior MI (November 2003) and PTCA of the LAD artery during which he was reported to have a 40% stenosis of the left main coronary artery, moderate left ventricular dysfunction (ejection fraction 41%) with akinesia in the anterior apex. The patient had previously received SonoVue without any adverse events.

On 16 December 2003, 5 ml of SonoVue was administered during an echocardiographic examination with high mechanical index (MI=1.3) ultrasound imaging. Approximately 2 minutes after SonoVue administration, the patient complained of throat burning and back pain and this was followed by cardiac arrest (asystole). Cardiopulmonary reanimation measures, including administration of adrenaline, cortisone and fibrinolytic agents, failed and the patient died. No signs of an anaphylactic reaction were observed on autopsy. Cardiac examination showed diffuse lesions of the coronary arteries and a severe (95%) stenosis of the left main coronary artery. The histopathology investigation of that vessel showed that the 95% stenosis was caused by an eccentric fibrohyaline plaque. No plaque rupture nor embolic phenomena were described and only low periadvential inflammatory activity was present with maintenance of the media.
According to the MAH, the cause of death was the underlying 95% stenosis of the main stem of the LAD found upon autopsy. After reviewing all the data available on this case, it was noted in the videoclips from the echographic examination of the day of the event that there was ECG evidence of a newly developed bundle branch block suggestive of electrophysiological instability. Therefore the MAH considers that the patient was already in an unstable condition before receiving SonoVue. The discrepancy between the preceding coronary angiography result (approx. 1 month before the event) of only a 40% luminal narrowing within the same vessel compared with the autopsy finding of a 95% stenosis could be explained by a fast progression of the LM plaque possibly due to plaque injury caused by the guiding catheter during the previous PTCA which accompanied the rapid ECG deterioration (development of bundle branch block). In these conditions, the spontaneous risk of death is very high and not readily quantifiable - no systemic reviews are available in the literature as patients with high grade main stem stenosis are candidates for immediate revascularisation.

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Case BRO-006772 reported from Germany describes a SAE in a 49-year-old male patient with a severe 3-vessel CAD, multiple MIs and a severely impaired left ventricular function (ejection fraction 31%) who experienced an acute anterior MI on 11 January 2003 and underwent coronary angiography. Multiple PTCA (3) and stent-implantation (1) were performed on multiple total (100%) and sub-total (99%) occlusions in the LAD artery and its branches. Additionally, severe coronary artery stenoses were described within the right coronary artery (90%) and left circumflex artery (80%). The patient was treated with clopidogrel, metoprolol, ramipril, hydrochlorothiazide, and simvastatin.

The patient was asymptomatic (denying angina or dyspnoea) and haemodynamically stable when admitted to hospital in February 2003. He underwent an echocardiographic examination on 17 February, during which 1 ml of SonoVue was administered for improvement of endocardial border definition. No stressor agents were used. During and after SonoVue injection, the patient remained asymptomatic (normal BP, heart rate and oxygen saturation). Upon attempting to get up from the examination table at the end of the examination, he lost conciousness. Pulse and BP were not measurable. Immediate resuscitation including electric defibrillation was performed for approximately 40 minutes but was unsuccessful. An ECG was not available at the onset of the event. During the event and resuscitation, asystole was recorded on the ECG, followed by pulseless electrical activity and ventricular fibrillation. No autopsy was performed.

The MAH considers that there is insufficient information available to clarify the aetiology of the event and thus exclude a possible role of SonoVue, but concludes that the patient was in an unstable condition and at risk of a sudden coronary event since :
-he had severe coronary lesions still present in the left circumflex and right coronary arteries;
-he had a severely impaired left ventricular function
-he was readmitted to hospital
-he had a high heart rate (92 bpm) before SonoVue administration despite betablocker treatment;
-he experienced hypoxemia (SaO2 = 92%) and required oxygen saturation monitoring during echocardiography, which is usually not performed during normal routine echocardiographic examination.

On the basis of the available data, the CHMP considers that a number of conclusions on the 3 fatalities can be drawn from the data provided by the MAH:
i) All 3 patients had advanced CAD with at least 1 of the following features: left main coronary stenosis, three vessel-disease, past history of myocardial infarction, PCI, left ventricular akinesia and low EF. They obviously represent a high risk group for acute coronary events and sudden death.

ii) An evolving coronary ischaemia at the time of Sonovue administration may confound the clinical interpretation of the clinical outcome. In case BRO 005943, an early stent occlusion is considered by the MAH as the cause of the cardiac arrest 5 minutes after Sonovue administration. However, the reverse hypothesis can also be discussed, i.e. the stent occlusion being the consequence of the circulatory arrest. The MAH's arguments to demonstrate an evolving myocardial infarction prior to the contrast echocardiograpy are questionable since:

- A significant rise in serum troponin and CK levels was found 2.5 hours after the contrast echo procedure. According to the MAH, this enzymatic rise reflected an evolving asymptomatic myocardial infarction 6-8 hours prior to Sonovue administration. However, the range of times to initial elevation of molecular markers of cardiac or muscle damage is 1-6 hours (Braunwald, Text Book of Cardiovascular Medicine, 2001). In addition, cardiopulmonary resuscitation (CPR) and defibrillation are known to increase cardiac/muscle markers, and this patient had several defibrillation attempts. Therefore, the elevation of molecular markers at the 2.5 hour time point is more likely related to the CPR and defibrillation.
- It is good clinical practice to perform ECG recordings after a PCI procedure and the following day prior to hospital discharge. There is no information on the ECG recordings of this patient, who should have had at least one ECG prior to the contrast echo. An evolving early stent occlusion, even clinically asymptomatic, would have induced ECG abnormalities. This information is critical to document the evolving myocardial ischaemia. Potentially useful

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information on the PCI procedure i.e. success of deployment and post-stent angiographic image is not available.

- There is a discrepancy between the first narrative of this case and the additional information provided by the MAH with regard to the echo findings. A normal left ventricular function with regional akinesia (apical, anteroseptal and inferior) was found in the first echo and mentioned in the first narrative. In the additional information, a total anteroseptal akinesia is stated. This point remains unclear.

In case BCM 000767, the evolving myocardial ischaemia is documented by a newly developed bundle branch block. The timing of this event is unclear. In case BRO 006772, there are no clinically relevant arguments for an evolving myocardial ischaemia. Sudden death occurred immediately after the contrast echo procedure in an apparently clinically stable patient with however advanced CAD.

iii) Besides a possible evolving coronary ischaemia, the strong temporal relationship between Sonovue administration and the fatal events suggests a triggering factor in this high coronary risk patient population. The experimental findings should be put into this context. In animal models microbubbles are destroyed by high energy ultrasound and cause microvessel rupture with myocardial ischaemia . Contrast echo with Sonovue might represent an ischaemic trigger. The level of acoustic power output is also a critical factor in the destruction of microbubbles. The level of ultrasound energy (MI) has not been provided for 2 of the deaths and for several of the other reported cardiac SAEs, nor has the echo technique (i.e. continuous or trigger mode). Hence, the specific role of contrast echo diagnostic technique in the occurrence of SAEs in the cardiac indication is still unclear.

Other serious cases reported post-marketing
Nineteen other serious ADRs have been reported, 18 of which were anaphylactoid or vasovagal reactions as defined by the reporting physician. Of these other 19 SAE, 11 correspond to patients who received SonoVue for the echocardiography indication. The cardiac events seen were part of (bradycardia (n=4), tachycardia (n=2)) or caused (myocardial ischaemia, transient ST depression or rise of troponin (n=3)) by the hypersensitivity reactions. Resuscitation, where needed, was always successful. There is no clear evidence of direct cardiac toxicity in any of the serious cases.

Based on the exposure data provided by the MAH (157,838 patients exposed to SonoVue, 67,832 in the cardiac and 90,000 in the non-cardiac indications), the reporting rate of serious ADRs is higher in the cardiac indication (13/67,832; 1/5,200) compared to the non-cardiac indication (7/90,000; 1/12,800). The reporting rate of cardiac events is higher in cardiac imaging (1/9,000) than in non-cardiac imaging (1/20,000), and the reporting rate of "allergy-like reactions" is also higher in cardiac imaging (8/67,832; 1/8,500) than in non-cardiac imaging (5/90,000; 1/18,000).

Safety data from clinical trials (as of 31 March 2004)
Further to a request from CHMP, the MAH has provided a comprehensive analysis of safety data from the 51 completed studies and the SAEs in the 17 ongoing studies, with a cut-off date of 31 March 2004. The 51 completed studies included 3,374 subjects, of which 3,212 (122 healthy volunteers and 3,090 patients) received SonoVue and 162 (30 healthy volunteers and 132 patients) received control agents. The 17 ongoing studies include a total of 1109 subjects who have received SonoVue.

Incidence of AE within completed and ongoing studies

The incidence of total AE (12.8%) and AE related to SonoVue (7.7%), and the incidence of SAE within completed studies has been investigated by the MAH. Overall 16 SAE were reported in completed trials in a total of 15 patients, of which 15 were considered unrelated (7 had a fatal outcome) and 1 case ("sensory motor paresis of the right arm") was considered to be of unknown relationship to SonoVue. Regarding the 17 ongoing trials, 7 SAEs have been reported in 6 patients, of which 6 were considered as unrelated and 1 (a cutaneous eruption) was considered to be probably

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related to SonoVue. The incidence of SAE related to SonoVue is 0.09% and the incidence of SAE related or with unknown relationship in all CT (ongoing and completed) is 0.04%.

A separate analysis of AE in completed trials according to the imaging indication has been performed for all SonoVue-treated and for healthy volunteers. The results of this analysis are shown in the table below

Table 1 Analysis of AE in different imaging indications in completed CT

	Number of studies	**Number of subjects receiving SonoVue**	**Incidence of total AE (n° of patients)**	**Incidence of AE related to SonoVue (n° of patients)**	**Number of serious AE**
Cardiac CT	18	SonoVue n=1266 Control n=126	19.6% (248) 26.2% (33)	**11.2% (142)** **12.7% (16)**	8 unrelated of which 1 death 1 unrelated
Macrovasculature CT	9	555	13.7% (76)	8.5% (47)	4 of which 3 death unrelated and 1 paresis of right arm with unk relationship
Microvasculature CT	14	1231	4.7% (58)	3.2% (40)	3 unrelated (1 death)
Special Patients Population CT	3	38	0	0	NA
Healthy volunteers	7	SonoVue n=122 Control n=30	27% (33)	23.7% (29)	0 0

As shown in the table above, the highest rate of AE is in cardiac studies, followed by macrovascular studies and microvascular studies. The MAH considers this finding most likely reflects differences in the frequency of study-protocol specified safety assessments, based upon the fact that the frequency and extent of safety assessment in Phase II/IIIA studies is higher than in Phase IIIB studies and the proportion of patients from Phase II/IIIA studies is larger in the cardiac and macrovascular groups than in the microvascular group. This is further underlined by the higher incidence of AE in the Comparator/Control Group within the cardiac studies, the fact that the higher AE-rates affect both related and unrelated AE, and the highest AE-rates were reported in the healthy volunteer studies (total rate of AE 32.6%, related 30.3%) representing the studies with the most extensive safety monitoring. The rate of total AE in the cardiac indication is slightly higher than that reported for Optison (19.6% and 16.8, respectively), although the incidence is lower (15.6%) when only considering the studies “at rest”.

The most frequently reported SonoVue-related AE in cardiac studies were: headache (2.1%), nausea (1.3%), chest pain (1.3%), taste perversion (0.9%), hyperglycaemia (0.6%), injection site reaction (0.6%), paresthesia (0.6%), and vasodilation (0.6%), and injection site pain (0.5%). All other AEs had an incidence of less than 0.5%. The incidence of chest pain was relatively higher in the cardiac studies (1.3% compared to 0.6% in All Patient Studies). This can be expected because the cardiac studies include 4 studies with a total of 338 patients undergoing pharmacological stress, with the majority of reported chest pain occurring in these 4 studies during stress testing.

Analysis of cardiac AE

Cardiac events were defined as signs and symptoms suggestive for myocardial ischemia (such as angina pectoris, retrosternal pain, chest pain, chest-pain-recurrent episode, burning feeling in chest, chest pressure, epigastric/chest pain, jaw cramp, jaw pain, throat tightness, chest discomfort (no angina), smart behind sternum at injection, heat and discomfort in abdomen/chest) and/or bradycardia and/or tachycardia. The total number of subjects reporting cardiac AE was 44 out of 3,212 subjects (1.4%;) and in the majority the AE were of mild intensity (30 out of 44 patients). Of the 44 cardiac events:

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- 4 were classified as SAE. Onset times ranged between 6 hours and 1 day and none were regarded as related to SonoVue

- 40 were classified as non-serious events of which 30 were of mild intensity and 10 were regarded as of moderate intensity. Of the 14 AE regarded as of unknown or possible relationship to study agent, 10 were classified as mild intensity and 4 events as of moderate intensity.

- 26 patients (59%) experienced cardiac AE (majority chest pain) during administration of pharmacological stressors.

- The rate of cardiac AE within SonoVue stress-echo studies was **7.7%** (26 out of 338 patients). The MAH considers that this rate corresponds to the lower range of published rates of non serious cardiac AE during stress-echocardiography. Seven AE (2%), all non-serious, were considered related to SonoVue and 1 AE with a causal relationship unknown.

Table 2 summarises the 14 AE considered as of unknown or possible relationship to SonoVue

Study	**Patient Gender /age Medical history**	**AE**	**Onset times**	**Total dose of SonoVue**	**Outcome**	**Relationship to SonoVue**
BR1-011 *Card Study*	*M/73 y* *Cor. Art. bypass graft*	*Mild chest disconfort*	*6 hours 39 mns*	*7.5 ml*	*Unknown*	*Unknown*
BR1-036 *Spe Patient Pop Study*	*F/45 y* *unknown*	*Burning feeling in the chest mild intensity*	*6 hours*	*18.7 ml*	*Resolved*	*Unknown*
BR1-012 *Card Study*	*F/60 y* *1-vessel CAD (>70%)*	*« smart behind sternum »*	*31 min after 1st inj*	*2 ml (2 injections)*	*Resolved quickly*	*Possible*
BR1-017 *Non-card study*	*F/ 60 y* *MI*	*Chest pressure*	*Immediately after 1st inj*	*4.5 ml*	*Resolved Within few minutes*	*Possible*
BR1-018 *Non-card study*	*F/ 36 y* *unknown*	*Heat and discomfort in the abdomen/ chest*	*42 minutes*	*4.5 ml*	*Resolved after 8 mns*	*Probable*
BR1-021 *Card Study*	*M / 60 y* *MI + Cor. Art. bypass graft*	*Hypotension + sinus bradycardia moderate*	*48 minutes*	*?* *4 infusions*	*Resolved With atropine*	*Possible*
BR1-063 Stress-echo	*M / 54 y* *Angina + hypertension*	*Mild chest pain*	*3 minutes*	*91.8 ml*	*Resolved* *Within 2 mns*	*Possible*
BR1-063 Stress-echo	*M / 57 y* *Angina + hypertension*	*Mild chest pain during stress test*	*92 min after 1st injection and 6 min after 2nd inj during stress test*	*?*	*Resolved*	*Possible*
BR1-063 Stress-echo	*F / 60 y* *Angina + hypertension*	*Moderate chest pain*	*87 min after 1st inj and 2 min before 2nd inj during stress test*	*111.3 ml*	*Resolved immediately with aminophylline*	*Possible*

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BR1-063 Stress-echo	*F / 60 y* *Angina + hypertension* *Stent implantation*	*Moderate chest pain*	*69 min after 1st inj and 6 min before 2nd inj during stress test*	*119.4 ml*	*Resolved within 12 min aminophylline*	*Possible*
BR1-063 Stress-echo	*M / 73 y* *Angina during exercice*	*Mild chest pain*	*87 min after 1st inj and 2 min before 2nd inj during stress test*	*124.9 ml*	*Resolved within 2 mns spontaneously*	*Possible*
BR1-063 Stress-echo	*F / 59 y* *Congestive heart failure + hypertension*	*Mild chest pain*	*77 min after 1st inj and 4 min before 2nd inj during stress test*	*97.9 ml*	*Resolved within 3 mns spontaneously*	*Possible*
BR1-063 Stress-echo	*F / 54 y* *Angina during exercice + stent implantation*	*Moderate chest pain* *+ new incomplete bundle branch block*	*89 min after 1st inj and 3 min before 2nd inj during stress test*	*84.0 ml*	*Resolved spontaneously within 13 min IBBB still noted at the 24-hour FU*	*Unknown*
BR1-063 Stress-echo	*M / 48 y* *Hypertension + stent LAD, NYHA class I*	*Vasodilatation and mild tachycardia*	*1 hour after 1st inj and 9 min before 2nd inj during stress test*	*50.36 ml*	*Resolved spontaneously within 20 min and 23 min*	*Probable*

Of the 14 cardiac "related" AE, 11 occurred in cardiac studies and 3 in non cardiac studies. Thus, the percentage of patients with related cardiac AE is slightly higher in cardiac imaging studies (11/1266, 0.87%) compared to non-cardiac imaging studies (3/1824, 0.16%), and the severity of the events is higher in the cardiac studies.

A subgroup analysis was performed in Study BR1-012, where 84 out of the 219 patients enrolled with known or suspected ischemic heart disease had also a documented coronary angiography. Nearly half (41) of the patients had coronary artery stenosis ≥ 90%. SonoVue was administered at two time points (during rest and at peak pharmacological stress - dobutamine or arbutamine) using one of four different doses (0.5, 1.0, 2.0 or 4.0 ml) in parallel-group comparison. Contrast echocardiography was performed under moderate mechanical index (0.6-0.8). Five cardiac AE were reported in 5 patients: 1 was observed in a patient with no detectable stenosis, 1 in a patient with less than 50% stenosis, 1 in a patient with > 70% 1-vessel disease, and 2 in patients with 3-vessel disease. The majority of AE possibly related to ischaemia occurred during stress 7-38 minutes after the 1st injection of SonoVue. All but one event were considered by the investigators not to be related to study drug. All events were mild in intensity and resolved spontaneously without sequelae. No meaningful causal relationship was observed between cardiac AE and the severity or extent of CAD, the dose SonoVue or the MI setting.

The CHMP agrees that the safety data on the use of low doses of SonoVue with low MI in patients with severe CAD are reassuring but quantitatively very limited (41 patients only).

Analysis of "allergy-like" AE in CT

Symptoms/findings considered as potentially indicative for "allergy-like" or anaphylactoid reactions were the following: allergic reaction, flu syndrome, flu-like symptoms, chills, fever, cough/dyspnoea, pruritus, rash, hypotension, paresthesia/tingling. After reviewing all the relevant AE the incidence of allergy-like reaction appears higher in patients in cardiac studies (1%) compared to non-cardiac studies (0.16%).

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The CHMP acknowledges the comprehensive analysis of the safety data collected in clinical trials with SonoVue carrried out by the MAH. The main findings of this analysis are:
i) an increased rate of total and related AE in cardiac imaging studies compared to non-cardiac imaging studies;
ii) The higher incidence of "allergy-like" reactions and cardiac ADRs reported in cardiac imaging patients during post-marketing surveillance is a reflection of the already observed higher risk of AE in cardiac imaging compared to non-cardiac imaging clinical trials. Indeed, the rate of "related" AE suggestive of "allergy-like" is respectively 1% and 0.16%, respectively, and the rate of "related" cardiac AE is 0.87% and 0.16%, respectively. There is currently no explanation for this difference, such as a possible difference in the dose, the speed of infusion, etc.
iii) a considerably higher frequency of cardiac AE was observed during stress-echocardiography (7.7% vs 1.6% in all patients). However, pharmacological stress alone has a significant rate of AE and published data report a rate of non-serious AE (chest pain) of 6-26% with dobutamine, 53% with adenosine and 30% with dipyramidole. Therefore, the cardiac AE rate of 7.7% with SonoVue in studies BR1-012 using dobutamine/arbutamine and BR1-027, -041, -063 and –068 using vasodilatator stress is not remarkable. The cardiac AE observed in CT during stress test are mainly chest pain, and therefore not unexpected.
Nonetheless, it is recommended to reinforce the warnings in section 4.4 as follows: "Stress echocardiography, which can mimic an ischaemic episode, could potentially increase the risk of SonoVue utilisation".

iv) in the same indications i.e. cardiac imaging, the incidence of total AE is somewhat higher with SonoVue (19.6%) compared to that with Optison (16.8%), although it would appear that the AE incidences are more comparable when the at rest cardiac imaging populations are compared.

The effect of the imaging technique

During contrast enhanced ultrasound examination, the microbubbles interact with the scanning process and this interaction depends on the scanning parameters, basically the peak negative pressure and the scanning frequency. The relationship of these two parameters is expressed by the Mechanical Index (MI). Further to a request from CHMP the MAH has investigated the possible impact of the mechanical index (MI) and degree of microbubble destruction according to echocardiographic techniques, the bubble stability, the impact of shell stiffness and other elements which could have an impact on the safety of SonoVue used for cardiac imaging.

Analyses by Mechanical Index
As can be seen in the table below, the incidence of AE generally increased for both groups of studies as the MI settings increased from < 0.7 to ≥ 1.0. At all MI setting intervals, most AE were of mild intensity; no event was of severe intensity. In the Cardiac Studies population, there was an increased incidence of AE with increasing MI values (30.8% of patients experienced AE with MI ≥1.0 vs 13.5% with MI<0.7). However, the ≥ 1.0 MI group (n=13 patients) is very limited to draw any conclusion of a MI-related effect and the higher incidence of AE considered to be related to study drug was observed in the < 0.7 MI group.

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	Mechanical Index		
	< 0.7	0.7 to < 1.0	≥ 1.0
ALL STUDIES WITH *MI*			
No. (%) of Patients Dosed	130 (100)	255 (100)	97 (100).
No of AE	23	60	30
No. (%) of Patients with AE	18 (13.8)	37 (14.5)	18 (18.6)
No. (%) of Patients with Study Agent-related AEs	13 (10.0)	14 (5.5)	11 (11.3)
No. (%) of Patients with Non-serious AE by Intensity Mild Moderate Severe	18 (13.8) 17 (13.1) 1 (0.8) 0	37 (14.5) 32 (12.5) 5 (2.0) 0	15 (15.5) 13 (13.4) 2 (2.1) 0
No. (%) of Patients with Serious AE	0	1 (0.4)	3 (3.1)
No. (%) of Deaths	0	0	3 (3.1)
CARDIAC STUDIES WITH *MI*			
No. (%) of Patients Dosed	104 (100)	165 (100)	13 (100)
No. of AE	18	53	8
No. (%) of Patients with AE	14 (13.5)	33 (20.0)	4 (30.8)
No. (%) of Patients with Study Agent related AEs	10 (9.6)	12 (7.3)	1 (7.7)
No. (%) of Patients with Non-serious AE by Intensity[b] Mild Moderate Severe	14 (13.5) 13 (12.5) 1 (1.0) 0	33 (20.0) 28 (17.0) 5 (3.0) 0	4 (30.8) 3 (23.1) 1 (7.7) 0
No. (%) of Patients with Serious AE	0	1 (0.6)	0
No. (%) of Deaths	0	0	0

It should be noted that in current clinical practice, the majority of ultrasound examinations are performed using low MI (< 0.4).

Further to a request from CHMP, information was obtained for 8 of the 14 patients with a SAE (including the 3 fatal cases) undergoing cardiac imaging. High MI (1.3) was used in one of the fatal cases (case BCM-0767) and low MI (≤ 0.3) was used in the 7 other cases. Despite the use of high MI in the case BCM-00767, no cardiac or vascular signs have been observed: no premature ventricular contractions were observed during insonation and the pathology assessment of the plaque did not show any signs of endothelial damage, periadvential inflammation or plaque rupture. For the 8 patients with a SAE undergoing non-cardiac imaging, information on the MI was obtained for only 1 patient and a low MI was used. Therefore, the MAH believes that the high MI did not play any role in the genesis of the event.
Moreover, there is no evidence of any biological interactions of the gas-filled SonoVue microbubbles with ultrasound field and there is no evidence of any potentiation of the biomechanical effects of ultrasound either from animal studies or clinical data.

The main issue under discussion is the combination of both Sonovue administration and the echocardiography technique, in particular the MI of the ultrasound beam. Recent experimental data indicate that ultrasound-induced destruction of contrast microbubbles can cause immediate rupture of the microvessels in which these microbubbles are located. The number of microbubble destruction events and the magnitude of bioeffects is proportional to the MI applied. The bioeffects consist of a decrease in left ventricular contractile performance, an increase in coronary perfusion pressure, an increase in lactate production and the occurrence of microvascular damage. They are probably ischaemic in origin, appear to be related to the MI, to be independent of the type of contrast agent and

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to require a threshold MI to occur. The clinical relevance of these experimental findings is debatable, since the human heart, which is much larger than isolated animal hearts, is exposed to far less acoustic pressure. In fact, only occurrence of premature ventricular contractions have been reported during triggered imaging with ultrasound contrast.

Experimental studies in rats and dogs with Sonovue at high MI have not shown tissue damage. Study BR1-113, single blinded, placebo controlled, looked at the cardiac effect (QT interval and PVCs) in 53 patients with CAD. No difference compared to placebo was found. A soft definition of CAD was used to include patients and the study report does not provide any information on the severity of CAD. This patient population does not seem to reflect the CAD severity of the 3 fatal cases. A retrospective analysis of the clinical database to assess the effect of MI was also performed. The information provided in this analysis is diluted by the non-cardiac studies which have little clinical relevance in this setting. For the cardiac studies, information on only 13 patients with high MI (>1.0) is available. There is an increased incidence of reported AEs with increasing MI values. However, the limited number of patients at high MI does not allow to draw any definitive conclusion .

Further to all the information assessed, the fact remains that the specific role of imaging technique in the occurrence of these SAEs is still unclear. As a precaution, a recommendation for the use of a low MI in the light of some of the experimental data above mentioned has been added to the SPC (section 4.4), in line with existing recommendations for similar products.

6.1.2.2. Clinical utility and diagnostic benefit of SonoVue in different patient populations

Cardiac imaging

Tissue Harmonic Imaging (THI) Mode has significantly improved the image quality in echocardiography. When used alone, it is superior to Fundamental Imaging for delineating left ventricular endocardial border. The percentage of patients who require contrast despite using THI is around 10-15%. In conjunction with SonoVue, the optimal display of the contrast effect is achieved with low transmit energy (MI <0.6).
In a recent European multicentre study with 120 patients (Hoffman R. and al, 2004), with evenly distributed ejection fraction groups (>55%, 35-55%, <35%), unenhanced THI and SonoVue-enhanced echocardiography at low MI were performed. For the assessment of ejection fraction (EF), unenhanced and SonoVue-enhanced echocardiography were compared to invasive cardioangiography (in all patients) and cardiac MRI (in a subset of 55 patients). Unenhanced THI echocardiography resulted in an underestimation of ejection fraction with only moderate correlation to the data obtained by cineventriculography or MRI, while SonoVue-enhanced echocardiography resulted in more accurate EF calculation.
An additional study performed on 110 patients using SonoVue and Definity (Malm S. *et al* 2004), each in approximately 50% of the patients, in conjunction with the latest stage ultrasound technology, confirms that the evaluation of left ventricle volumes and EF is more accurate when performed with contrast enhancement. Similar experience is reported by Nahar (Nahar T *et al*, Am. J. Cardiol. 2000), who compared left ventricular EF obtained by 3 different echocardiographic modalities (Fundamental B-mode Imaging, THI and Harmonic with contrast imaging) in 50 patients with technically difficult echocardiograms in comparison with radionuclide ventriculography (RNV).

Rest-Echocardiography

The clinical utility of SonoVue in rest echocardiography through the evaluation of the left ventricle opacification and evaluation of the endocardial border (EBD) and the conversion of non-interpretable to diagnostic images has been demonstrated. It constitutes a primary non-invasive method of evaluating regional and global left ventricular function. Conversion rates of 80% (59% - 97% dependent on off-site reader) have been observed within the pivotal SonoVue clinical trials.

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Stress-echocardiography

Excessive chest wall motion during hyperventilation and cardiac translational movement during tachycardia alter image quality during stress, and non-diagnostic echocardiograms have been reported in up to 30% of patients. In study BR1-012 a total of 219 patients with suspected or known coronary heart disease were investigated using the positive inotropic stressors dobutamine or arbutamine (which has near to identical pharmacodynamic effect). SonoVue was shown to significantly improve the endocardial border delineation during stress echocardiography.

6.1.2.3 Safe use of Sonovue contrast echocardiography consistent with the observed clinical events and proposed contraindications in the context of Stress echocardiography

The controlled induction of cardiac ischaemia during diagnostic stress testing in an *a priori* stable cardiac patient is not expected to put the patient into an unstable cardiac or haemodynamic state. Ischaemic reactions induced during stress testing are the objective and quantifiable representation of exertional symptoms which the patient experiences during everyday life. The typical lumen-narrowing coronary artery plaque aspect present in patients with positive stress-echo should not be altered by or during the ischaemic response. The continuous monitoring of patients undergoing stress testing is expected to lead to the timely detection of an ischaemic response, which is the diagnostic endpoint. Stress testing is then discontinued at an early stage of ischaemia.
In unstable patients or during acute coronary syndromes, the underlying mechanisms causing cardiac ischaemia are likely to be fundamentally different e.g. ruptured plaque with thrombotic occlusion and distal embolizations.
The proposed contra-indications do not represent any significant limitation for the use of SonoVue during stress-echocardiography, as they are practically superimposable to contraindications applicable for exercise testing and for pharmacological stress agents, which are all excluding patients with acute coronary syndromes and with clinically unstable conditions.
The frequency, type and seriousness of AE in Clinical Trials during SonoVue use in stress-echocardiography appear to be similar to those observed during unenhanced stress echocardiography. To date, no serious AE have been reported in patients undergoing stress-echo exams in post-marketing.
A retrospective analysis of 694 consecutive stress echocardiograms carried out in a single centre, 319 with no contrast, 299 with SonoVue and 76 with Optison, performed from January 2002 to May 2004 has been provided by the MAH. A low powered imaging modality with an MI of 0.1-0.2 was used throughout the imaging. The incidence of adverse reactions was similar in the 3 groups and no deaths were reported. The Optison group had a slightly lower peak diastolic BP compared to the non-contrast group ($p<0.005$) and the SonoVue group had a slightly higher peak HR compared to the non-contrast group ($p<0.001$). Patients receiving Optison had more supra-ventricular events compared to the SonoVue group ($p<0.05$) but there was no difference in the incidence of ventricular tachycardia, supraventricular tachycardia or vagal responses. Triggered imaging was performed only at the end of the study and did not lead to any AEs. In his conclusion the author states that in this series dobutamine combined with contrast (both SonoVue and Optison) appears safe with similar side effects to non-contrast studies. But based on the results of experimental animal studies (Taniyel A. et al, 2001), he does not exclude the fact that microbubbles contrast could be destroyed in humans by high MI ultrasound and lead to direct myocardial damage.

Safe use and benefit/risk of Sonovue in the context of compliance with practice guidelines for the adequate use of echocardiography in the management of stable angina.
Taking into account the new proposed contra-indications the target population for the use of Sonovue in the cardiac indication consists of patients with known or suspected chronic stable angina, which represents a large patient population. Stress-echocardiography in this population allows the detection, assessment of extent and follow-up of regional and global left ventricular dysfunction. The accurate determination of EF is important for choosing appropriate medical or surgical therapies, in making recommendations about activity levels and for the long-term prognosis for these patients.

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The use of SonoVue during stress echocardiography could avoid prolonged or extensive stress-induced ischaemia, which may potentially affect the patient's condition and result in side effects.
The benefit of image quality improvement of echocardiography by using ultrasound contrast agent has been acknowledged in the guidelines.

The CHMP acknowledges that left ventricular systolic function is an important prognostic variable in patients with CAD and can be safely assessed by conventional echocardiography at rest or under stress conditions. When identified, underlying left ventricular dysfunction is important both for prognostic assessment and clinical management. The administration of Sonovue allows a better delineation of endocardial border and thus improves the accuracy/reliability of echocardiography to evaluate left ventricular wall kinetics and function, particularly during stress conditions.

Conventional rest echocardiography is widely performed in the emergency setting of unstable angina and acute myocardial infarction. The use of Sonovue in this setting is definitely contraindicated.

Echocardiography also plays an important role in the clinical management of chronic CAD. The ACC/AHA 2002 guidelines on management of stable angina support the use of echocardiography to assess the extent or severity of ischaemia (left ventricular wall motion abnormalities) when the procedure could be obtained during pain or shortly after chest pain (within 30 minutes after its abatement). The guidelines recommend dobutamine stress echo as the initial test for diagnosis and risk stratification in patients with chronic stable angina who are unable to exercise. In patients who are able to exercise, the guidelines recommend rest or exercise echo in patients with history or ECG evidence of prior myocardial infarction, signs of congestive heart failure, baseline ECG abnormalities (preexcitation syndrome, more than 1 mm ST depression at rest), complex ventricular arrhythmias, prior coronary revascularization procedures (PCI or CABG). In addition, the guidelines support assessment of left ventricular function in patients with new or worsening CHF, evidence of intervening myocardial infarction by history or ECG. Echocardiography is not useful for assessment of left ventricular function in patients with normal ECG, no history of myocardial infarction, no evidence of CHF, no change in clinical status. However it is acknowledged that there is a patient population with known or suspected chronic stable angina, who may be suitable for the use of Sonovue, especially during stress where the assessment of the extent of regional left ventricular dysfunction is sensible to triage patients for further evaluation or treatment.

Suggested investigations to identify the underlying mechanisms of "allergy-like" reactions.
The MAH was asked to provide a programme of active investigations in order to identify the possible underlying mechanisms of "allergy-like" reactions (i.e. allergy, anaphylaxis, anaphylactoid reactions, histamine liberation) to SonoVue. The severe hypersensitivity adverse reactions reported during the post-marketing of SonoVue probably correspond to anaphylactoid reactions and not to type I anaphylactic reaction. These "pseudoallergic" reactions can be the result of complement activation similarly to the effects of the liposomal compounds injected intravenously in humans or be due to a direct effect on basophils and mast cells with release of histamine as observed in dogs after administration of Cremophor. Therefore an experimental programme has been defined by the MAH to evaluate the possible underlying mechanism of complement activation induced by SonoVue. This programme includes *in vivo* studies in pigs since the pig is the animal species which is most sensitive and closest to man for evaluating the cardio-vascular effects of complement activation by contrast media, liposomes and particles. In parallel to the pig model, an *in vitro* programme was recently started on complement activation of human serum.

The programme of animal studies proposed by the MAH is acceptable to the CHMP, but the role of the MI and complement activation induced by SonoVue should also be evaluated. It is well known that the final stages of the complement cascade lead to the formation of membrane attack complex (MAC) in cell membranes. Nevertheless, complement activation and its consequences could also occur via the two complement pathways (classical or alternative), without the terminal formation of MAC. These activations could lead to the production of several components such as C3a or C5a, which display

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anaphylatoxin properties. In this context, a full evaluation of the potential complement activation induced by Sonovue should be undertaken.

As a follow-up measure, the CHMP has asked the MAH to undertake to provide the results of the above investigations.

6.1.2.4. Discussion

The risk of SonoVue
The very close temporal association between Sonovue administration and the 3 deaths has caused concern, leading to the initial USR. Such a precautionary measure was taken as it cannot be ruled out that SonoVue played a role in the occurrence of these fatalities by inducing in patients with severe CAD serious AE with haemodynamic consequences, such as bradycardia and a drop of BP eventually leading to a myocardial infarction.

Overall 14 patients undergoing cardiac imaging reported SAEs (including the 3 fatal cases) in the post marketing phase. Most of these 14 cases suffered reactions categorised as anaphylactoid or vasovagal. All 3 fatal cases had advanced CAD as a common denominator and at least 2 of the 3 patients could be considered as "clinically unstable", representing a high risk group for acute coronary events and sudden death. However, according to the clinical practice guidelines, this is the patient population for whom echocardiographic assessment is the most valuable. It is recognised that the "clinically unstable" status falls within the contra-indication proposed by the MAH. However, besides a possible evolving coronary ischaemia, the strong temporal relationship between the SonoVue contrast echocardiography and the fatal events suggests a triggering factor with SonoVue administration in this high coronary risk patient population.

There is an increased incidence of reported AEs with increasing mechanical index values, although information on only 13 patients with high MI(>1.0) is available for the cardiac studies. This limited number of patients does not allow to draw any definitive conclusions. MI information was available for 8 of the 14 cardiac imaging patients reporting SAEs in the post marketing phase. A high MI was used in only 1 of the 8 patients (death report BCM-000767), and a low MI was used in all other 7 cases. In addition, it has to be acknowledged that there is limited evidence of cardiac toxicity of Sonovue in clinical studies at high doses of the agent and/or during insonation at high MI. The results of two placebo-controlled studies, albeit with a limited number of patients, did not show any differences between placebo and Sonovue. The retrospective analysis conducted in 675 patients did not evidence any significant effect of either high MI and/or intermittent insonation on the general safety profile of Sonovue. Despite the unclear role of the MI a recommendation to use a low MI has been added to the SPC (section 4.4), in line with existing recommendations for similar products.

Concerning stress echocardiography, it is recognised that the induction of cardiac ischaemia during stress testing in a stable cardiac patient does not put the patient in an "unstable" cardiac or haemodynamic condition (i.e the proposed contraindication). Moreover a clinical study (BR1-012) conducted in 219 patients and a retrospective analysis in 299 patients with Sonovue vs 76 patients with Optison did not raise any particular concern. Nonetheless, the fact remains that there is a potential risk of Sonovue administered during stress. Therefore appropriate precautions for use in this condition have been included in the SPC.

In order to continue to closely monitor the safety of SonoVue, the MAH has undertaken to reduce the periodicity of the PSURs to every 6 months. This is endorsed by CHMP.

The benefit of SonoVue
The invention of second harmonic imaging has rendered the clinical usefulness of SonoVue in rest echocardiography relatively limited. However, there is a patient population with known or suspected chronic stable angina, who may benefit from the use of Sonovue, especially during stress imaging

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where the assessment of the extent of regional left ventricular dysfunction is sensible to triage patients for further evaluation or treatment.

Benefit/Risk of SonoVue

On the basis of spontaneous reporting, SonoVue has been associated with a higher number of cardiovascular ADRs, including 3 fatal cases, than other available agents (mainly Optison in the echocardiography indication, where no death has been observed). The CHMP recognises, however, that the basis for maintenance of the revocation of the "echocardiography" indication for Sonovue may be currently insufficient. Therefore, the benefit risk balance of SonoVue in echocardiography could be maintained provided that reinforced contraindications (in acute coronary syndromes and clinically unstable patients) and warnings (in stress echography) are implemented in the SPC, as follows:

- **Section 4.1 "Indications"**

Echocardiography

SonoVue is a transpulmonary echocardiographic contrast agent for use in patients with suspected or established cardiovascular disease to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation.

- **Section 4.3 "Contra-Indications"**

"SonoVue is contraindicated for use in patients with *recent acute coronary syndrome or clinically unstable ischaemic cardiac disease, including: evolving or ongoing myocardial infarction, typical angina at rest within last 7 days, significant worsening of cardiac symptoms within last 7 days, recent coronary artery intervention or other factors suggesting clinical instability (for example, recent deterioration of ECG, laboratory or clinical findings)* acute cardiac failure, Class III/IV cardiac failure, or severe rhythm disorders*."*

- **Section 4.4 "Warnings and precautions for use":**

It should be emphasised that stress echocardiography, which can mimic an ischaemic episode, could potentially increase the risk of SonoVue utilisation. Therefore, if SonoVue is to be used in conjunction with stress echocardiography patients must have a stable condition verified by absence of chest pain or ECG modification during the two preceding days. Moreover, ECG and blood pressure monitoring should be performed during SonoVue-enhanced echocardiography with a pharmacological stress (e.g. with dobutamine). ECG monitoring should be performed in high-risk patients as clinically indicated.

Care should be taken in patients with ischaemic cardiac disease because in these patients allergy-like and/or vasodilatory reactions may lead to life-threatening conditions. Emergency equipment and personnel trained in its use must be readily available.

It is recommended to keep the patient under close medical supervision during and for at least 30 minutes following the administration of SonoVue.

In animal studies, the application of echo-contrast agents revealed biological side effects (e.g. endothelial cell injury, capillary rupture) by interaction with the ultrasound beam. Although these biological side effects have not been reported in humans, the use of a low mechanical index is recommended.

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