

**Appendix A.**

**Table 1**

**QTc Prolongation in Patients Treated with DMP 115 and Saline Placebo**

	QTc increase							
	30-59 msec		60-89 msec		90-119 msec		> 120 msec	
	DMP	Saline	DMP	Saline	DMP	Saline	DMP	Saline
<b>DMP 115 - 004</b>	<b>{n=69}</b>	<b>{n =18}</b>						
over baseline	11	7	0	0	0	1	0	0
over preceding value	15	7	1	0	0	0	0	0
<hr/>								
<b>DMP 115 - 115 - 005</b>	<b>{n=80}</b>	<b>{n=19}</b>						
over baseline	6	1	2	0	3	1	0	0
over preceding value	9	2	2	0	1	1	1	0

**Medical Officer Review**

**NDA#** 21-064  
**M.O. Review #** 1

**Date of letter:** 12/8/98  
**Date FDA received:** 12/9/98  
**Date reviewer received:** 12/10/98  
**Date review completed:** 7/12/99

**1. General Information**

**Drug name:** DMP 115  
**Generic name:** Perflutren  
**Proposed trade name:** DEFINITY  
**Chemical name:** Phospholipid liposomes with perfluoropropane in saline

**Status:** Regular review

**Sponsor:** DuPont Pharmaceuticals  
331 Treble Cove Road  
North Billerica MA 01862

**Note:** All the statements made by Sponsor in the NDA submission, which appear in this review, are in *italics*. These were transferred from the submission ad verbatim. All the appendices are as they appeared in the submission.

**Pharmacologic Category:** Sonographic contrast agent  
**Proposed Indication(s):** *for contrast-enhanced ultrasound imaging of cardiac structures (ventricular chambers and endocardial borders) and function (regional wall motion)*

**Submissions During the Initial Review**

SUBMISSION/TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE	CONTENT
Original	9-Dec-98	10-Dec-98	10-Dec-98	
Amendment (BM)	21-Dec-98	22-Dec-98	22-Dec-98	Corrected QT data
Amendment (C)	11-Feb-99	12-Feb-99	16-Feb-99	Inspection information
Amendment (BM)	12-Mar-99	15-Mar-99	16-Mar-99	Minor efficacy corrections
Amendment (SU)	7-Apr-99	8-Apr-99	8-Apr-99	Safety Update
Amendment (NC)	14-Apr-99	15-Apr-99	15-Apr-99	Ox. sat., shift tables
Amendment (BM)	13-May-99	16-May-99	17-May-99	20% change in vital signs
Amendment (BM)	18-May-99	19-May-99	19-May-99	ADEs and lab values
Amendment (BM)	25-May-99	26-May-99	26-May-99	QTc and vital signs (±20%)
Amendment (BM)	18-May-99	19-May-99	19-May-99	Phase 1 + 2, Ox. Sat. + QTc
Amendment (BM)	23-Jun-99	24-Jun-99	24-Jun-99	Phase 1 + 2, Ox. Sat. + images
Amendment (BC)	24-Jun-99	25-Jun-99	25-Jun-99	Liposome physical chemistry

**Dosage Form(s) and Route(s) of Administration, Directions for Use:**

*... a single dose 10 uL/kg by slow I.V. bolus injection over 30-60 seconds, followed by a 10 ml-saline flush. A second 10 ul/kg dose may be administered to prolong optimal imaging. May also be administered via an I.V. infusion of 1.3 ml added to 50 ml of preservative free saline. The rate of infusion is suggested to be initiated at 4 ml/ minute, but should be titrated as necessary to achieve optimal image enhancement.*

**NA Drug Classification:**

**Related Approved Drugs:**

Albunex, Optison

**Related Reviews:**

Statistical Review, Biopharm Review, Chemistry Review, Pharmacology/Toxicology Review

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- Appendix B Complete set of tables on demographics from the Integrated Summary of Safety
- Appendix C ECG parameters in normal volunteers (complete database), a change from baseline, from the Safety Update (identical to that from Integrated Summary of Safety)
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- Appendix G Vital signs in patients in all pivotal trials, a change from baseline, from the Safety Update (identical to that from the Integrated Summary of Safety)
- Appendix H Adverse Drug Events (a complete set of summary tables from the Integrated Summary of Safety)
- Appendix I Protocol for Phase 1 trial, main section.
- Appendix J Reviewer's DBP Database and Results
- Appendix K Reviewer's ECG Database, QTc Results and Graphs

### 3. Material Reviewed

This submission was comprised of a total of 234 volumes of roughly 400 pages each. Clinical data appeared in volumes 42 - 118 and 196 - 234, a total of 116 volumes. The application was reviewed in full.

### 4. Chemistry/Manufacturing/ Controls

Please refer also to the Chemistry Review of this submission.

The drug mixture was described in the Investigator's Brochure by the Sponsor as

#### Emulsion Characteristics

The liposomes were prepared from the mixture by shaking the content. The shaking was done in a closed transport vial, described in the chemistry portion of the NDA. Vialmix, a modified dental amalgamator designed for preparing DEFINITY was used for shaking, which took 45 seconds.

In regard to the size of liposomes, the sponsor stated (Vol.1, p.105, par.2):

- *NMR techniques were used to characterize the drug substance:*

- *optical microscopy, with images showing the presence of spherical objects that are in the size range of the drug substance, 1 to 10 um in diameter;*
- *particle sizing, showing that for particles greater than or equal to 1 um, the majority of the particles in the drug product are in the size range of 1 to 10 um in diameter.*

In the first sentence of the suggested package insert for the drug, the following reference is made:

DRAFT

(Vol. 1, p.2, 1.1).

However, what the Sponsor meant by the latter designation cannot be found in this submission.

### **Liposome Size**

The size distribution outside of the range 1 um – 10 um in solution (in vitro) by scanning microscopy was not determined. Likewise, the size distribution of liposomes in various batches of the drug product and the **physical** (temperature, osmotic pressure, dilution, etc) **and/or chemical factors which can alter it were not adequately documented, or discussed in the original submission..** For example, the liposome size in four different size ranges as a function of shaking frequency was shown (Vol. 6, p.222), but what happens to the size of liposomes between the activation and injection, or upon agitation and multiple re-agitations is not described. It was said, in the same context, that the drug should be activated at **room** temperature, but the temperature is not otherwise specified. The effects of other physicochemical parameters have not been described.

In a series responses to specific questions by the reviewing chemist the Sponsor provided some clues in this respect on 6/24/1999. Unfortunately, the data provided is not informative enough. Thus, for the effect of temperature, it was concluded, that "The data obtained at 37 degrees Centigrade are within the range of measurements obtained at room temperature.". However, as apparent from Table 2, values for individual comparisons, or individual differences were not provided and conclusions are based on averages of absolute values. In addition, as seen under the column "Total particles" (Table 2) their number can differ ten fold from one application to another. For liposomes greater than 10um in size the difference from one use to another about 100 fold at 37 degrees Centigrade and about 600 fold at room temperature. Under these circumstances the use of averages and standard errors for any comparison seems inappropriate. I disagree with the review chemist (Chemistry Review, p.64, last paragraph) that from this data one can satisfactorily conclude that there is no effect of temperature on liposome size and numbers.

### **Method of Administration**

The Sponsor asserted that "*The product can be administered as a slow bolus injection (30-60 seconds), or as a slow infusion of 1.3 mL diluted in 50 mL of*

*preservative saline.*" (Vol.6, p.213, par 2) , but what is the effect of the dilution on the liposome and its size is not given. The suggested labeling calls for the drug to be used as a continuous infusion. However, most of the data about liposome size relates to simulated infusion and evaluation of the liposomes in infusion bags. Thus, the bulk of information about the liposome size is assessed as if used by infusion, while the majority of safety data on the drug was obtained during the pivotal studies which used the bolus injection, or bolus and infusion (the effects on safety cannot be separated) exclusively. Therefore, the chemistry data on the liposome size distribution with time in the original submission was not compatible with the crucial clinical data. The data submitted in the above mentioned submission of 6/24/99 (Table 1) appears to show no syringe difference effect on the drug's liposome composition, but whether this is real could once more be hidden in averaging out the results.

#### **Liposome Size Dynamics**

The Sponsor described the liposome size distribution over time using two different plastic bags by different manufacturers during infusion of the drug in the initial submission, but there was no comparable evaluation for the drug injected via a syringe. The data submitted in the above mentioned submission of 6/24/99 (Table 1) appears to show no syringe effect on the drug's liposome composition, but whether this is real could once more be hidden in averaging out the results.

There was no evaluation of the effect of time from activation to injection, hand agitation to injection, or re-agitation to injection in the original submission. The data submitted subsequently (6/24/99) show about a 5 fold decrease in liposomes of 10um or more 3 minutes without hand suspension (Table 3, 6/24/99 submission). In this interpretation, I am in a partial agreement with the review chemist (Chemistry Review, p.68, par.2) who concluded that no growth of larger liposomes occurred, but did not comment on the decrease. Furthermore, and more importantly, resuspension (reagitation) caused an increase in the 10um liposomes 4x, 7x and 4x when resuspended once, twice, or three times respectively (Table 6, 6/24/99 submission). This amendment also revealed statistically significant differences for several liposome size categories at 12 hours post-activation, as compared to the baseline. It became apparent that the content of liposomes in a particular size category may vary from one injection to another by as much as 10 fold, or 100 fold, and for the 10-um or more category even more than a million fold (Table 7, last column, 6/24/99 submission). There was no attempt to assess the size of liposomes in vivo, either preclinically, or clinically.

#### **Effect of Plastic Bags on Liposome Size**

A substantial time effect on size of liposomes was found in the drug tested for infusion. There was a **decrease in the liposomes of relatively larger size** (6 um and larger), which differed depending on whether the bags were made by one or another

manufacturer (Vol.1, p.230, Fig.5.11 and 5.12 and p.236, Fig.5.16 and Fig. 5.17, related information in Figs.5.19 – 5.24).

#### **Drug Appearance**

In the specifications, the Sponsor described the drug as a clear solution. Upon activation in the presence of perfluoropropane, which had been in headspace, the resulting vial content appeared as a foamy, **milky white emulsion** (Vol.6, p 215).

According to the specifications, the drug formulation may contain more than a billion liposomes per dose (Vol.6, p.130). This may have safety concerns as the lungs are increasingly challenged by particle size and number increase.

#### **Drug Manufacturing**

Finally, it is noted that the drug was originally manufactured and packaged by \_\_\_\_\_ and later by DuPont in Manati, PR. There is at least one instance where the evidence shows that the Sponsor was making large quantities of the drug \_\_\_\_\_ at both sites at one time (Vol. 6., p.116 and p.129).

### **5. Animal Pharmacology/Toxicology**

Please refer also to the Pharmacology/ Toxicology Review of this submission.

#### **Drug Components**

The Sponsor described the lipid components of the drug mixture as endogenous. Whether the concentration of these components in blood will be altered by the drug administration is, of course, an altogether different, but not an illegitimate question. Likewise, the altered concentration of these lipids may have effects, extending beyond acute post-injection impact. These would be observable only during an observation period beyond several hours post-injection.

The difference between the drug mixture administered as liposomes and that administered clinically as a nonactivated solution was not studied in this submission.

#### **Drug Effects**

The preclinical studies with the drug in doses larger than those proposed for the clinical use demonstrated:

- 1) effects in the lungs at clinical level; increase in pulmonary pressure in pig model at clinical doses.
- 2) degenerative changes in the liver, in centrilobular areas in rats at higher than clinical doses;
- 3) effects in the spleen, CNS and bone marrow at higher than clinical doses.

All these organs could be potentially affected by the variations in the size of the liposomes as well as changes in the dose of the drug.

**Conflicting Preclinical Safety Reports Explained by Time Effect**

Approximately 3 months before the NDA submission, the Sponsor related to the Agency that a **repeat** of a preclinical trial in rats (T97-9-15, 1998) using the DuPont preparation had to be **discontinued** because of excessive mortality. Accompanying signs included abnormal respiration, ataxia, decreased motor activity and loss of righting reflex. The dose was 1 mL/kg/day and was given for about 2 weeks prior to termination of the experiment. The Sponsor emphasized that the injection was given within 30 min following activation and that an additional trial with rats established the effect of time interval from activation to injection as a factor affecting toxicity (Vol.1, p. 132, par.2, l.6). The deaths of animals may be prevented if the injection is administered more than 30 min after activation. This finding was discussed in a broader context on p.87, last paragraph, of PT review.

**Safety Ramifications of the Failure to Control for a Potentially Critical Safety Factor (which may include, but is not limited to, liposome size, liposome rigidity, liposome changes upon iv administration, which concern not only size but also coalescing, modification or alteration of properties after injection)**

It is noteworthy that:

- 1) No deaths of animals were reported with this same dose in similar earlier trials with product from \_\_\_\_\_ used by ImaRx.
- 2) The deaths of animals were reported using the to-be-marketed formulation produced by DuPont.
- 3) The Sponsor attempted to explain these findings by the differences in the time interval between activation and injection.
- 4) The toxicology profile of the to-be-marketed formulation is worse than that of the earlier formulation(s) (please see p.90 of PT review)

Furthermore:

- 5) There was no concern about this interval in the pre-clinical or clinical studies. Consequently, some investigators, preclinical as well as clinical, may have used the drug early and the others late after activation.
- 6) The length of this time period, between the activation and injection, was never recorded.
- 7) The interval from activation to injection may be an important variable **affecting** preclinical and clinical safety of the drug.
- 8) The large fluctuations in some safety parameters among various trials, and particularly among different investigators within the trials might be attributable to an independent variable which remained, in effect, unaccounted for throughout the drug development.

**6. Clinical Background**

There are two intravenously administered ultrasound contrast agents that are approved for marketing by MBI.

#### Albunex

**Albunex** was the first approved sonographic contrast agent. It is a 5% solution of human albumin to which small amounts of stabilizers, sodium acetyl tryptophanate and sodium caprylate, are added. The microspheres are produced by sonication. The package insert also states: "The human albumin is held at 60 degrees Centigrade for 10 hours." **This is to show that a heat modification, stabilization, and/or denaturation occur.** The protein in microspheres makes up approximately 1% of the total protein in the liquid, and the remaining 99% is unchanged 5% human albumin.

Average size of microspheres is 3 um - 5 um, with maximum diameter 32 um.

The indication for Albunex states: "... is intended as an aid for ultrasound contrast enhancement of ventricular chambers, and improves endocardial border definition in patients with suboptimal echoes undergoing ventricular function and regional wall motion studies."

#### Optison

**Optison** is similar in composition to Albunex. **The similarity is such that the maximal diameter of albumin microspheres, reported in the package insert is the same, 32 um.** The mean diameter 2.0 um – 4.5 um is also very close to that of Albunex. The drug is also prepared by a temperature dependent process and shipped ready-made requiring only gentle mixing prior to use. **The only difference is the use of octafluoropropane gas which is incorporated into the formulation by a proprietary process.** The head space of the vial is also filled with octofluoropropane gas.

Optison indication states: "... for use in patients with suboptimal echocardiograms to opacify the left ventricle and to improve the delineation of the left ventricular borders" and is, therefore, almost identical to that of Albunex.

#### Similarities and differences between DEFINITY and other sonographic contrast agents

The other two agents Albunex and Optison have several common features by which they differ from DEFINITY:

- Composed of albumin microspheres
- Obtained by a heat-dependent manufacturing process
- Microspheres are of similar size, range 1 um – 32 um

Consequently, **there is very little common in terms of chemistry between Alburnex and DEFINITY**. For Optison, the only chemical similarity is the presence of a **fluoropropane in both Optison and DEFINITY**, but the clinical effect, stemming from the chemical composition only may not be profound as this is an inert gas.

The main **differences between DEFINITY and other approved sonographic contrast agents are profound** already from a chemical standpoint, but the most relevant are the physical properties:

- DEFINITY formulation, a mixture of lipids and water is inherently unstable because of the lipid content. On the other hand, for example, a heat-treated protein, such as in Alburnex and Optison, partially denatured or modified, as microspheres, will show the stable, or relatively stable properties of denatured protein. If the protein were not denatured or modified, it would simply desolve in an aqueous solution.
- Upon mixing the liquid with perfluoropropane gas in head space of the vial and subsequent transfer via syringe to atmospheric conditions, or upon mixing outside of the vial, it becomes even more unstable (in a more unstable environment – air).
- As it is unstable under normal conditions of handling in vitro, its physical stability in vivo cannot be guaranteed without an adequate empirical assessment.
- In addition, unstable and/ or unstabilized lipid-based liposomes in DEFINITY may easily re-arrange, coalesce, associate, split or otherwise modify with uncertain outcomes.
- As a result, a variety of effects on the safety profile in a number of body systems cannot be excluded.

In regard to indication, that for Optison is the most limited while that proposed for DEFINITY is most inclusive. The indication for Optison is aimed essentially at anatomical features, opacity of the left ventricle and delineation of left ventricular border. Alburnex tacitly adds a potential in cardiac functional studies, ventricular wall function and regional wall motion. DEFINITY attempts to extend the use also to the studies of renal and hepatic pathology.

The art of ultrasonography is limited in its efficacy in part due to operator dependence. The use of contrast may reduce the effect of the operator and ideally enhance the production of an effective image. A limiting safety issue is a potential damage of body tissues by the applied acoustic power which should be limited to avoid adverse thermal effects.

### **6.1 Relevant Human Experience**

### **6.2 Important Information from related INDs and NDAs**

### **6.3 Foreign Experience**

There is no foreign marketing of this ultrasound product.

## 6.4 Human Pharmacology, Pharmacokinetics, Pharmacodynamics

Please refer to the Biopharmaceutics Review.

## 6.5 Other Relevant Background Information

This drug has a long and complex regulatory history. At one point in 1997, a court decision enjoined the FDA from "continuing any approval and review procedures with respect to" this and several other products.

Ownership of an IND for development of this product was transferred from ImaRX Pharmaceutical Company to the present owner DuPont in January 1977, only several months earlier. Although all the pivotal trials were performed at the time the current owner, DuPont, already formally had had the rights to the drug product, the first two pivotal clinical trials started shortly after the transfer.

The drug formulation apparently was changed upon the transfer of ownership. Based on preclinical studies, the current drug formulation is more toxic than the original one (please refer to p.88, par. 2 and p.90, par. 4 and 5 of PT review).

Advice was provided by Agency but trials were ongoing and were not modified by the Sponsor.

## 7. Description of Clinical Dose Evaluation

Three indications pursued

This agent was originally developed for echographic cardiac exams, as apparent from the Summary of Clinical Studies table shown below.

### Change in formulation and route of administration

In the first four clinical trials, the original ImaRx formulation was used, but later it was substituted for DMP 115, a DuPont product, which is the to-be-marketed formulation. The product was mostly used as a bolus, with only a few exceptions. From completed trials, infusion of the contrast agent was used only in one trial of 64 patients. The safety database is not large enough to support an indication for the use of the drug during a continuous infusion. The safety data from the bolus injection of this product is not applicable to the drug infusion because the liposomes may change in infusate, as compared to the non-diluted formulation. **Data comparing the behaviour and fate of the liposomes given by infusion, as compared to bolus, and related safety should be submitted and reviewed prior to potential approval.**

**Summary of Clinical Studies (Vol.1, pp.178 - 188)**

Product	Trial Phase	Dose(uL/kg)	By	Freq.	Men	Women	Sites	Study
MRX <sup>^</sup>	900	1	5,10,20,50,100	Bolus	1x	30	0	C
MRX <sup>^</sup>	901	1	5,10,15,30	Bolus	5x	18	0	C
MRX	902	2	5, 10,15	Bolus	1x	29	27	C
MRX	903	2	50 only	Bolus	6x	14	3	C
DMP <sup>^</sup>	905	1	50 only	Bolus	1x	15	9	CPD
DMP	1	3	10,30,50	Bolus	2x	10	14	
DMP	4	3	5 or 10	Bolus	2x	69	18	8 C
DMP	5	3	5 or 10	Bolus	2x	70	54	9 C
DMP								4 C
DMP								4 C
DMP	17	3	10 only	Both	2x	45	19	C

<sup>^</sup> normal volunteers  
 \* ongoing trial  
 shaded - pivotal studies  
 C - cardiac

**Changes in dose from trial to trial and study to study**

It can also be seen from the reviewer's table that the dose as well as frequency of administration changed from trial to trial and from one pivotal study to another. Therefore, the **safety data are not directly comparable from one study to another.** However, it gives comprehensive information and helps with labeling decisions.

**Lack of dose response relationship, particularly in regard to safety**

The conventional wisdom that the smaller dose is always safer than the larger one may not hold, as the relationship between the drug dose and the drug liposome size distribution was not studied in this submission.

**Optimal dose was not determined in advance**

The dose ranging trials were MRX - 900, - 901 and - 902 and studied, respectively, 30 and 18 normal volunteers, and 56 patients, a total of 104 subjects. The dose could not be reliably chosen for Phase 3 trials which continued to explore doses between 5ul/kg and 30 ul/kg.

**Changes in formulation and the drug dose not conducive to rigorous safety evaluation**

In addition, it is recognized that only a small number of patients was studied outside of the context of pivotal trials. Thus, the emphasis in the safety evaluation has to be on the pivotal trials with only a supportive information available from the other trials. The

use of more than one formulation throughout the development of this drug should not be overlooked, and likewise, the effect of the time period between the activation and injection, which may potentially influence drug stability and the safety profile.

**Potential sources of enormous variability in safety profile among different investigators**

Finally, the large number of investigative sites and investigators should be seen as a **potential source of large variability both in regard to safety as well as efficacy**. This includes the differences not only in the drug activation, on the Vialmix shaker, as one administered dose may contain 10x less total liposomes than the next (6/24/99 submission, p. 8, Table 2, last column). Agitation and re-agitation by hand shaking may increase the number of liposomes larger than 10um in diameter 4 fold and 7 fold respectively (6/24/99 submission, p.11, Table 11). Also, use of different transducers may differ as a particular type of transducer was not specified in protocol. In addition different techniques of imaging by various investigators may play role since ultrasonography is known to be operator dependent. Demographic and disease related factors among patients, variable treatment of patients among various hospitals (for example 17 medical centers for study #1, DMP 115-004 and -005) , concomitant medications, etc. could also contribute to the variability.

**8. Clinical Studies**

The reviewer examined the Sponsor's trials to find answers to hypotheses looking for evidence to support the applicant's claims.

**8.1 Sponsor's pivotal trials DMP 115 – 004 (Vol. 62 – Vol.68) and DMP 115 – 005 (Vol.69 - Vol.74).**

**8.1.1 Sponsor stated Objectives**

The primary objectives were:

- *The ability of DMP-115 to visually demonstrate left ventricular cavity enhancement*
- *The safety of two single IV doses of DMP in patients referred for echographic ventricular function studies*

The secondary objectives were:

- *The ability of DMP 115 to improve endocardial border delineation in patients referred for echocardiographic evaluation of ventricular function who demonstrated suboptimal unenhanced images*
- *Duration of ventricular enhancement*

- *The ability of contrast enhancement provided by DMP 115 to impact the following diagnostic attributes:*
  - *Diagnostic confidence*
  - *Quality of wall-motion detection*

*The number of myocardial views that displayed continued enhancement following DMP 115 administration when seven different myocardial views mimicking a complete ventricular function evaluation were obtained*

*The number of non-diagnostic echocardiographic images (four or more non-evaluable segments) that become diagnostic (one or zero segments non-evaluable) after DMP 115 administration*

### 8.1.2 Design

The first pivotal trials in patients were originally designed as randomized double blind, multicenter and placebo-controlled studies. However, **the intent (i.e doubleblind) did not materialize since the activated drug (milky, white emulsion) is visually easily distinguishable from saline (clear liquid), used as placebo, by color and consistency.** The investigator was instructed that “The product should appear as a milky white suspension following agitation.” in protocol (Vol.62, p.254, par.1). He/she was not instructed that the syringes should be masked, or covered.

### 8.1.3 Protocol

Saline was administered as placebo. Each drug dose was administered through an 18- to 20-gauge needle situated in a large forearm vein. Each bolus injection was followed immediately by a 10-ml saline flush at a rate 1ml/sec. Two injections 30 min apart were administered. Up to 10 centers were planned in trial DMP 115- 004, but only 8 of them finally participated. In trial DMP 115 - 005 the plan was for 10 centers and 9 actually participated. Patients received either placebo, 5 ul/kg DMP 115, or 10 ul/kg DMP 115. Each of the patient groups who received the drug was about twice as many as that receiving placebo. A total of 87 were eventually enrolled in DMP 115 – 004 and 124 patients in DMP 115 - 005. Sonographic 2-D fundamental images were obtained starting immediately post-injection to be blindly read. The primary criterion of effectiveness was the Ventricular Cavity Enhancement.

The two imaging sessions were held at least 30 min apart. *“The sonographer began post-injection imaging using the 4- or 2-chamber view that qualified the patient for the trial. Imaging in the qualifying view continued for 30 seconds before imaging in the second apical view began: images in the second apical view were again acquired for 30 seconds. The sonographer alternated between the 4- and 2-chamber apical views, obtaining approximately 30 seconds of images in each view for the remainder of the 5 minute imaging session.”* (Vol.62, p. 33, par.3, l.1)

Images from the first baseline and post-injection imaging session (5 ul/kg dose) were recorded on a dedicated super VHS videotape. Following at least a 30-min period, the patient again underwent a baseline and a post-injection (10 ul/kg dose) imaging session. The sonographer, instrument, transducer, and instrument settings used during the first imaging session were to be used for the second imaging session. The sonographer acquired images for at least 10 seconds per view (apical 4- and 2-chamber; parasternal long axis; subcostal 4-chamber and mid-ventricular apical and basal short axis).

Further details can be found in respective section of this submission (Vol.62, p.34, par.2, l.1).

#### Blinded read

Readers were blinded to patient information and trial medication (DMP 115 and placebo). The order in which images were presented was randomized. *The readers were presented with only single-beat cine loops for evaluation.*

*For each patient, four single-beat images (two per apical view) were selected for evaluation of endocardial border delineation:*

- *Apical 4-chamber view, Baseline*
- *Apical 2-chamber view, Baseline*
- *Apical 4-chamber view, Post-injection 1*
- *Apical 2-chamber view, Post-injection 1*

*Analogous views were also obtained for use in grading ventricular cavity enhancement. Those were read in a pairwise fashion from a split screen format. All 4-chamber views were presented first.*

*In addition, seven paired image sets were constructed:*

- *Apical 4-chamber view, Post-injection 2*
- *Apical 2-chamber view, Post-injection 2*

- *Parasternal long-axis view, Baseline and Post-injection 2*
- *Mid-ventricular short axis view, Baseline and Post-injection 2*
- *Apical short-axis view, Baseline and Post-injection 2*
- *Basal short axis view, Baseline and Post-injection 2*
- *Subcostal short-axis view, Baseline and Post-injection 2*

*Those were presented to readers in a split-screen format and were used to evaluate the number of myocardial views with contrast enhancement.*

### **8.1.3.1 Population**

#### *Inclusion Criteria*

- *"Were 18 years of age (or the age of legal consent) or older with at least two of six ventricular border segments non-evaluable in either an apical 4- or 2-chamber view"*
- *women were non-pregnant ...*
- *Provided verbal assurance of willingness to return for Visits 2, 3 and 4 for safety follow-up.*
- *Provided written signed informed consent ...*
- *Were able to communicate effectively with trial personnel."*

#### *Exclusion Criteria*

- *"Alcohol and drug addiction*
- *Inability to provide blood samples*
- *Serious mental illness*
- *Recent investigational use*
- *Clinically unstable, had a history of an acute disease that could influence the patient's ability to return for follow-up e.g., unstable angina, acute myocardial infarction, etc.*
- *Known right-to-left shunt"*

### **8.1.3.2 Efficacy Endpoints**

*In regard to the first endpoint, evaluated by blinded read, the endocardial border delineation, "each reader will evaluate each image to determine segment evaluability." The six left ventricular myocardial segments of each view will be graded as 0 = nonevaluable, 1 = evaluable and 9 = not applicable (segment not in image selected)".*

*For the second endpoint, left ventricular cavity opacification "Scoring ... will be performed using a pairwise presentation of the first imaging session baseline, and*

DMP, or placebo images .... The readers will grade ventricular cavity enhancement using the following scale: 0 = no contrast enhancement

- 1 = Weak Contrast Enhancement
- 2 = Adequate Contrast Enhancement
- 3 = Full Contrast Enhancement
- 9 = Excessive Contrast Enhancement"

The duration of contrast enhancement was evaluated separately.

The third endpoint was what the sponsor referred to as diagnostic and patient management attributes. "During the paired presentation of optimal images selected for ventricular cavity opacification, the readers will be asked to determine whether the addition of contrast agent: 1 = impaired, 2 = failed to impact, or 3 = improved

- a) their diagnostic confidence in interpreting the images
- b) their ability to detect wall motion abnormalities, and,
- c)

Also, each reader was asked whether the post-injection image:

- "Distorted or impaired the reader's ability to evaluate the information seen in the baseline image,
- Provided no new information over the baseline image
- Provided information not seen in the baseline image that could a) result in a change in patient management, b) identify new findings, or c) eliminate the need for an additional test."

Separately, number of views with contrast enhancement was to be evaluated with similar a scale. "Each of the 7 views acquired (apical 2-chamber, apical 4-chamber, parasternal long axis, subcostal 4-chamber, and 3 short axis views (mid, apical and basal) will be evaluated in a paired presentation". (Vol. 62, p.221, par.3, l.3)

There were two additional efficacy criteria. *The Salvage of Non-Diagnostic Examinations* was defined as

- At least four non-evaluable segments (score of 0) in a single apical view at baseline
- A total of zero or one non-evaluable segment (score of 0) in the same apical view after Injection 1

The ventricular videodensitometry was measured to quantitatively evaluate changes in the ventricular cavity. Independent values were recorded for regions of interest in the apex and mid-cavity for baseline images and post-treatment images at end-diastole, mid-systole and end-systole.

#### 8.1.3.2.1 Protocol deficiencies related to efficacy

The main weakness of all these **efficacy measures** resulting from the blinded read stems from an uncertainty whether or not these parameters are conducive to an objective assessment, or whether they are merely **subjective opinions of the readers** based on no objective, or quantitative criteria. As far as it can be determined, no details on such items are present in this submission. The instruction to the readers only referred to vague, all encompassing, terms such as contrast, enhancement, or information and nothing else. Although there was a training session for the readers, no other specifics on the substance of the difference between an enhanced and unenhanced image is presented throughout the submission. It is of significance that this very issue was discussed with the Sponsor during the pre-Phase 3 meeting on November 19, 1996.

The Sponsor also submitted evaluations similar to those described under the blinded read, but obtained by various investigators familiar with other patient information. This is referred in the submission as Institutional read. However, the information so obtained is usually confounded by bias from pre-existing knowledge of the patient and should be viewed with caution.

### 8.1.3.3 Statistical considerations

Please refer to the Statistical Review. The reader is also referred to the Statistical Review for planned blinding and randomization schemes. Otherwise, the efficacy issues will be commented upon throughout this review.

### 8.1.3.4 Safety considerations

Following safety parameters were obtained:

- AEs (*"Each patient was queried using non-leading questions (e.g. "How do you feel?")*)
- 12-lead ECG - pre-injection, within 60 min after the first injection, 24 hr, 48 hr and 72 hr post-injection
- vital signs - pre-injection, 7 min after the first and second injection, then 15 min, 20 min, 35 min after each injection and then approximately 24 hr, 48 hr and 72 hr post-injection (Vol. 62, p.55, par 2, l.1 and Protocol, Vol. 62, p. 217, par.2, l.1)
- serum chemistry, hematology and urinalysis - pre-injection, 60 - 90 min after the first injection, 24 hr, 48 hr and 72 hr post-injection
- history and physical - pre-injection, 60 min, 24 hr, 48 hr and 72 hr post-injection

As the drug is composed of liposomes, an effect on microcirculation should be expected until proven otherwise. Such an effect is usually most pronounced when the concentration of the particles is the highest, i.e. immediately post-injection. ECG monitoring was not done immediately after injection, about 30 min after the second drug injection (Vol. 62, p.55, par 2, l.1 and Protocol, Vol. 62, p. 217, par.2, l.1). This means that first ECG data was obtained only 1 hr after first exposure to the drug (1 hr, 24 hr, 48 hr, and 72 hr).

#### 8.1.4 Results

##### 8.1.4.1 Population enrolled

The patient population in the first trial (DMP 115 – 004) included 87 subjects in 8 centers, and in the second trial (DMP 115 – 005) 124 patients in 9 centers, all in the US. The only disease specific characteristic of note, common to all these patients, was that they had *“at least two of six ventricular border segments non-evaluable in either an apical 4- or 2-chamber view”* on the initial cardiac sonographic exam. Also of special significance, patients with an unstable disease were excluded.

##### 8.1.4.2 Efficacy endpoint outcomes

###### Endocardial Border Delineation

The first efficacy endpoint, endocardial border delineation, was evaluated on the following scale:

- a) 0 = evaluable
- b) 1 = nonevaluable, and
- c) 9 = not applicable.

The unit of observation was the ventricular border segment(s) (non-evaluable without the contrast) which was examined with DMP 115, as a contrast agent, to show a degree, if any, of increase in information from the echographic examination as compared to a placebo sonogram done with saline. As noted earlier, this was a rather subjective assessment, without any specifics of the grading categories given, albeit performed by blinded readers.

This reviewer agrees with the Statistical Reviewer, Mahboob Sobhan, Ph.D. in regard to the conclusion made from the results presented. Please refer to the Statistical Review and Evaluation, p.6, par.2. As Table 2.1.B.3 shows, the use of the contrast agent, DMP 115, contributed to a real (statistically significant), but partial (blinded readers split across the board) gain in the diagnostic information, as examined and described, only in one (DMP 115 – 004) of two trials in this study, regardless of DMP 115 dose.

**Table 2.1.B.3**  
Percent (%) of Patients showing Improvement in EBD in At Least One Segment by Study and Blinded Read.

Study #	Apical View	Placebo / 5 / 10 $\mu$ L/kg (p-value)		
		Reader 1	Reader 2	Reader 3
DMP 115-004	4-chamber	44/ 97/87 (p<.05)*	44/72/63 (NS)	35/79/82 (p<.05)*
	2-chamber	33/76/69 (p<.05)*	39/61/50 (NS)	40/55/54 (NS)
DMP 115-005	4-chamber	54/65/67 (NS)	64/76/82 (NS)	67/75/79 (NS)
	2-chamber	42/44/43 (NS)	52/54/63 (NS)	59/68/65 (NS)

\* Significant difference from placebo for both doses.

One blinded reader was successful in obtaining the information gain with contrast for both 2-chamber and 4-chamber views, while the second was not successful with any of them. The third showed improvement with the 4-chamber view, but not with 2-chamber view.

Although some improvement was seen numerically also in the second trial, none of the differences between the drug and placebo reached statistical significance. There was no difference due to the drug dose.

**Left Ventricular Border Opacification**

The grading scale for the ventricular cavity enhancement was:

- 0 = No Contrast Enhancement
- 1 = Weak Contrast Enhancement
- 2 = Adequate Contrast Enhancement
- 3 = Full Contrast Enhancement
- 9 = Excessive Contrast Enhancement

The ventricular cavity enhancement following the drug administration was established by pairwise comparisons of baseline and DMP or placebo images. There was no blinded read of **unpaired** baseline, DMP 115 and placebo images. Even more importantly, as there was no patient in whom all the three treatments were compared this evidence should be viewed as indirect. This evaluation is by definition only an indirect one. As the number of patients who received placebo (saline) was small, the general conclusions about the results can hardly be made.

Nevertheless, the sponsor claims a significant increase in the ventricular cavity enhancement due to the drug as shown in Vol. 42, Table 14. Given the caveats attached, as mentioned above, the clinical importance of these conclusions should not be overestimated. Particularly, when no certain clinical meaning can be associated, for example, with the difference between a patient with a weak contrast as opposed to that with a full contrast enhancement.

Additional limitations were mentioned already in the discussion found at: 8.1.3.2.1 Protocol Deficiencies Related to Efficacy, in the preceding pages of this review.

A slightly different position on this issue was taken by the reviewing statistician Dr. Sobhan (Please see p.5, par.3 of his review), but I agree with his overall conclusion that a favorable result stemming from the assessment of this endpoint is vague at best.

Videodensitometry

**Table 2.1.B.4**  
**Videodensitometric Measurements (at End-Diastole) of Left Ventricular Enhancement**  
**Change from baseline by Region and Apical Views: Study DMP 115-004 and DMP 115-005\***

Study #/Regions	N	Apical 4-chamber View		Apical 2-Chamber View	
		Baseline Mean(SD)	Change from Baseline Mean(SD)	Baseline Mean(SD)	Change from Baseline Mean(SD)
<b>Study DMP 115-004:</b>					
<b>Apex</b>					
Placebo	17	15.4(6.6)	3.8(8.5)	18.0(11.7)	2.4(12.4)
DMP 5 µL/kg	33	22.6(15.0)	15.8(16.4)*	20.7(14.4)	12.7(13.6)*
DMP 10 µL/kg	33	22.5(18.0)	19.1(17.3)*	22.7(18.0)	13.0(15.8)*
<b>Mid-Chamber</b>					
Placebo	17	13.2(6.2)	2.6(7.0)	15.3(7.3)	1.5(8.0)
DMP 5 µL/kg	33	18.7(13.1)	13.8(13.7)*	17.1(10.9)	12.8(14.3)*
DMP 10 µL/kg	33	19.1(15.7)	15.8(13.2)*	20.4(17.0)	11.8(13.1)*
<b>Study DMP 115-005:</b>					
<b>Apex</b>					
Placebo	24	26.0(21.5)	2.0(8.3)	27.8(19.8)	-0.7(10.1)
DMP 5 µL/kg	50	29.7(21.3)	14.4(14.5)*	28.3(20.5)	14.8(15.1)*
DMP 10 µL/kg	49	29.7(20.0)	23.5(21.5)*	27.5(19.2)	22.4(17.5)*
<b>Mid-Chamber</b>					
Placebo	24	20.5(17.3)	0.7(2.7)	21.0(16.5)	1.0(4.0)
DMP 5 µL/kg	50	24.0(18.0)	14.9(14.2)*	23.5(18.2)	15.3(17.3)*
DMP 10 µL/kg	49	23.8(15.8)	20.8(22.7)*	22.6(15.7)	20.1(18.6)*

\* Significantly different from placebo and from baseline (p<.05)

The statistical review also concluded that the use of DMP contrast increased videodensitometric values when measured in both apex and mid-chamber and both apical views at end-systole and end-diastole. Although this improvement may potentially be helpful for the interpretation of cardiac sonograph, an exact clinical meaning of such a gain is not clear and was not assessed directly in these trials.

In conclusion, the evaluation of the primary endpoint, ventricular cavity enhancement, was based on an indirect inference. Although the results suggest an improvement in clinical information due to DMP 115 contrast, the procedure used to obtain this information was not methodologically and statistically sound.

The Sponsor was **successful** in showing the diagnostic gain in the secondary endpoint, endocardial border delineation, due to the administration of DEFINITY, **in one of two trials**. In the trial considered acceptable, one of the blinded readers saw improvement in both 2- and 4-chambers views, one did not see a statistical improvement at all and the third saw the improvement only in 4-chamber view. **The results do not support the efficacy claim for EDB fully.**

#### 8.1.4.3 Safety outcomes

##### Adverse Drug Events

Discussion on ADEs in individual studies is deferred towards the end of this review where they will be considered globally as well as sub-analyzed.

##### Vital signs

Vital signs were collected at baseline and at various time intervals after the drug injection (2min, 10m, 15m ...24h, 48h, 72h) as depicted in the data table starting on page 24 for **the diastolic blood pressure**. The shaded area (investigator F, Site 6 DMP115 – 004; reviewer's table p. 3 and 4 in Appendix J; p. 3 and 4; Appendix J different capital letters for different investigators indicate DMP 115 – 004 and small caps DMP 115 – 005, respectively) suggests that the primary data may not be reliable and, thus, it is excluded from analysis. One patient is described to have a constant DBP of 80 mmHg for all the 13 time points measured. Two other subjects have only a single change in those 13 measurements and another two subjects only two changes out of 13 measurements. Emphasis here is on diastolic blood pressure as a large percentage of patients showed the change of clinical concern (greater than 20%).

A summary table (Table 1, next page) for the diastolic blood pressure is provided below. The main categories for the analysis consist of those patients with more than a 20% change in DBP which were a) over and below the baseline, and b) over or below

**Table 1**

**Twenty or More Percent Change in DBP in Patients  
(by investigator in DMP 115 - 004 and 115 - 005)**

Investigator	Over baseline		Below baseline		Over preceding value		Below preceding value	
	%	n/N	%	n/N	%	n/N	%	n/N
A	62.5%	5/8	8.75%	1/8	37.5%	3/8	25%	2/8
B	16.6%	1/6	16.6%	1/6	16.6%	1/6	0	0/6
C	42.9%	6/14	50%	7/14	57.1%	8/14	35.7%	5/14
D	43.7%	7/16	56.2%	9/16	68.7%	11/16	68.7%	11/16
E	25%	2/8	12.5%	1/8	37.5%	3/8	37.5%	3/8
F*	0%	0/0	0%	0/0	0%	0/0	0%	0/0
G	33.3%	1/3	33.3%	1/3	0%	0/3	33.3%	1/3
H	60%	3/5	60%	3/5	80%	4/5	40%	2/5
<b>Trial 004</b>	<b>higher scores (3)</b>	<b>51.7 %</b>	<b>15/29</b>	<b>54.3% 19/35</b>	<b>71.8%</b>	<b>23/35</b>	<b>55.2%</b>	<b>16/29</b>
	<b>lower scores (4)</b>	<b>32.2%</b>	<b>10/31</b>	<b>16% 4/25</b>	<b>28.0%</b>	<b>7/25</b>	<b>25.8%</b>	<b>8/31</b>
	<b>Total 004</b>	<b>41.6%</b>	<b>25/60</b>	<b>38.3% 23/60</b>	<b>50%</b>	<b>30/60</b>	<b>40%</b>	<b>24/60</b>
* primary data for DBP not genuine								
a	100%	6/6	0%	0/6	100%	6/6	33.3%	2/6
b	50%	1/2	0%	0/2	50%	1/2	0%	0/2
c	50%	6/12	33.3%	4/12	50%	6/12	50%	6/12
d	15%	3/20	30%	6/20	20%	4/20	25%	5/20
e	25%	6/24	12.5%	2/24	20.8%	5/24	12.5%	3/24
f	33.3%	1/3	33.3%	1/3	33.3%	1/3	66.6%	2/3
g	57.1%	4/7	28.6%	2/7	77.7%	7/9	71.4%	5/7
h	0%	0/11	27.3%	3/11	27.3%	3/11	9.1%	1/11
i	28.6%	2/7	14.3%	1/7	28.6%	2/7	14.3%	1/7
<b>Trial 005</b>	<b>higher scores (4)</b>	<b>63%</b>	<b>17/27</b>	<b>31.7% 13/41</b>	<b>69%</b>	<b>20/29</b>	<b>53.6%</b>	<b>15/28</b>
	<b>lower scores (5)</b>	<b>18.5%</b>	<b>12/65</b>	<b>11.8% 6/51</b>	<b>23.8%</b>	<b>15/63</b>	<b>18.8%</b>	<b>12/64</b>
	<b>Total 005</b>	<b>31.5%</b>	<b>29/92</b>	<b>20.6% 18/92</b>	<b>38.0%</b>	<b>5/92</b>	<b>27.2%</b>	<b>25/92</b>

the preceding measurement of DBP. The latter documents sudden large swings in DBP which may make the patient prone to stroke or a cardiac event. Source data for this table is the Database for Diastolic Blood Pressure, and particularly its rightmost half on pp.2 – 14, Appendix J. Partial summaries for individual patients (20% or more change in regard to baseline and preceding values) appear in the rightmost 4 columns extending from p. 9 through p.14, Appendix J. A special notation (U or ###) depicts the instances when the of primary data was not collected, or the respective value could not be calculated because of lack of primary data.

As Table 1 shows, a **large portion of patients exhibit changes which are of clinical concern (>20%)**. These are not only increases over the baseline and drops below the baseline, but also the sudden increases and drops from one measurement to another.

**There are large differences in this vital sign parameter recorded among individual investigators.** As the sub-analysis for DMP 115 – 004 reveals, the potentially clinically significant changes in DBP reached more than 50% in the population of patients encompassing more than half of all subjects in the trial (Trial 004, higher scores). A similar result can be seen also in over one third of all subjects in trial DMP 115 – 005.

The large differences among investigators are a **safety concern, but it was overlooked by the Sponsor**. In some subcategories, as apparent from the table, this variation is absolute ( 0% -100%). That suggests that the use of the drug is unsafe. In the absence of other explanations, particularly from the Sponsor, the working hypothesis should be that the individual investigators prepared the drug differently from one another, or the properties of the drug changed, affecting safety. Another possibility is the variation in patient population, but this must be considered less likely for the extremes in patient selection are unlikely because patients with “*a recent history of acute disease*” were excluded.

As noted in the preclinical section of this review, the Sponsor might not have recommended the optimal conditions for the use of this drug. The drug might have been used it when it is less than adequate, or when its safety has been compromised.

#### Electrocardiography

A 12- lead ECG was obtained at the baseline and at 1 hr, 24 hrs, 48 hrs and 72 hrs after the first exposure to the drug, as shown in Database for Electrocardiography Parameters, pp.47 – 51 of this review. Some essential ECG parameters were listed, or calculated by the Sponsor. **The drug was given by bolus injection in 2 doses approximately 30 min apart.**

The entire database of some of the quantitative parameters is provided for completeness. A shaded area (investigator d, Site 4 DMP 115 – 005, p. 9 and 10,

**Table 2**

**Thirty or More Unit Change in QTc in Patients  
(by investigator in DMP 115 - 004 and 115 - 005)**

Investigator	Over baseline	Over preceding value
A	12.5% 1/8	0% 0/8
B	0% 0/6	0% 0/6
C	14.3% 2/14	14.3% 2/14
D	18.7% 3/16	43.8% 7/16
E	37.5% 3/8	37.5% 3/8
F	12.5% 1/8	12.5% 1/8
G	0% 0/4	0% 0/4
H	20% 1/5	0% 0/5
<b>Trial 004</b>	<b>higher scores (4)</b>	<b>20.9% 9/43</b>
	<b>lower scores (4)</b>	<b>7.6% 2/26</b>
	<b>Total 004</b>	<b>15.9% 11/69</b>
		<b>28.3% 13/46</b>
		<b>0% 0/23</b>
		<b>18.8% 13/69</b>
a	33.3% 2/6	50% 3/6
b	66.6% 2/3	66.6% 2/3
c	26.6% 4/15	26.6% 4/15
d*		
e	8.3% 2/24	12.5% 3/24
f	20% 1/5	20% 1/5
g	0% 0/8	0% 0/8
h	9.1% 1/11	9.1% 1/11
i	0% 0/8	0% 0/8
<b>Trial 005</b>	<b>higher scores (4)</b>	<b>31.0% 9/29</b>
	<b>lower scores (4)</b>	<b>5.8% 3/51</b>
	<b>Total 005</b>	<b>16.2% 13/80</b>
		<b>17.5% 14/80</b>

\* primary data for QTc not genuine

reviewer's table, Appendix K); capital letters indicate DMP 115 - 004 and small caps DMP 115 - 005, respectively) shows the primary data for PR and QRS intervals not considered not accurate (compared to the rest of data, hardly any change with time can be observed) by this reviewer, and thus, excluded from analysis. Results of the analysis of QTc changes were calculated by this reviewer and entered into a several page table accompanied by graphs for easier interpretation (QTc changes in Patients, pp. 1 - 6, Appendix K of this review). The shaded lines designate the patients which received "placebo". The use of placebo is discussed in the Overview of Safety section, later in this review.

A summary table (reviewer's Table 2, preceding page) for the QTc prolongation is provided below. **The main categories for the analysis consist of those patients with more than a 30 msec increase.** The latter is considered indicative of a drug effect (potential increased risk for the life threatening arrhythmia Torsades de Pointes (TdP). A table and the graphic presentation, shown on pages 1 - 6, Appendix K of this review, depict not only the increases, but also decreases in QTc as compared to the baseline as well as the preceding value. Obviously, a large swing in QTc, even a decrease, will likely be accompanied by a prolongation at one time or another, in order for the QTc interval to stabilize about the baseline.

As the summary table (previous page) as well as the tables graphs reveal (Appendix K, starting on page 1), **a large proportion of patients exhibit the changes which are of clinical concern (>30 msec).** These are not only increases over the baseline and drops below the baseline, but also the sudden increases and drops from one measurement to another.

Another safety concern is **the large differences in QTc prolongation among the patient groups treated by different investigators.** As the sub-analysis for DMP 115 - 005 reveals (Table 2, previous page, lower half, Trial DMP 115 - 005), the potentially alarming QTc prolongations reached more than 30% in the population of patients being cared for by half of the investigators (Trial DMP 115 - 005, higher scores, investigators a, b, c and f). A somewhat lower, but still a significant result can be seen also in more than one half subjects in trial DMP 115 - 004.

The large among investigators (sites) differences (variation) is of safety concern, but it was entirely overlooked by the Sponsor. In some subcategories, as apparent from the table, this variation is large (0% - 66%). This suggests, once again, as before for the DBP, that the use of the drug in these trials is unsafe. In the absence of other explanations, particularly from the Sponsor, the working hypothesis should be that the individual investigators prepared the drug differently from one another, or the properties of the drug changed, affecting safety. The Sponsor should address the reasons for these differences.

## 8.2 Sponsor's pivotal trials DMP 115 – 006 (Vol. 76 – Vol. 83) and DMP 115 – 007 (Vol.84 - Vol.91).

### 8.2.1. Sponsor stated Objectives

The primary objectives were:

- *obtained prior to and following the administration of DMP 115 to . measures determined by Magnetic Resonance Imaging (MRI).*

The secondary objectives were:

- *To compare the accuracy of ecgocardiographic end-diastolic volume and end-systolic volume (ESV) measurements obtained prior to and following the administration of DMP 115 to ventricular measures determined by MRI*
- *To examine the safety of two single IV doses of DMP in patients referred for echographic ventricular function studies*
- *To examine the percentage of patients who show an improvement in the number of ventricular segments correctly identified following DMP 115 administration as determined by MRI during wall motion evaluation*
- *To determine the percentage of patients with non-diagnostic echocardiographic images who become diagnostic after DMP administration*
- *To assess the accuracy of echographic second harmonic imaging wall motion, EF, EDV, and ESV measurements following the administration of DMP 115 of ventricular measure determined by MRI (Vol.76, p.199, par 2)*

### 8.2.2 Design

The pivotal trials in patients were originally designed as randomized double blind, multicenter and placebo-controlled studies. However, **the intent (i.e doubleblind) did not materialize since the activated drug (milky, white emulsion) is visually easily distinguishable from saline (clear liquid), used as placebo, by color and consistency.** The investigator was instructed that "The product should appear as a milky white suspension following agitation." in protocol (Vol.69, p.260, par.1). He/she was not instructed that the syringes should be masked, or covered.

The criteria for effectiveness described in the original protocol to be evaluated by both blinded readers and institutional readers were:

- Echographic image evaluation

- Segmental Wall Motion Evaluation
- 
- Magnetic Resonance Image Evaluation

### 8.2.3 Protocol

Each drug dose was administered through an 18 - to 20-gauge needle situated in a large forearm vein. Each bolus injection was followed immediately by a 10-ml saline flush. Two injections at least 30 min, but not 2 hrs apart were administered. Up to 4 centers were planned in trial DMP 115 - 006 with 67 patients. In trial DMP 115 - 007 the plan was for 4 centers and 59 patients participated. Patients received 10 ul/kg DMP 115 once by a bolus and later on also as a slow IV push in a modified randomized design. A total of 67 were eventually enrolled in DMP 115 – 006 and 59 patients in DMP 115 - 007. Sonographic 2-D fundamental imaging was to be used for calculation of ventricular size and an assessment of wall motion starting immediately post-injection to be blindly read. **The primary criterion of effectiveness was**

**changed to the measurement of left ventricular cavity size by a new methodology proposed by the**

As far as it can be determined, this methodology was never validated for the suggested use.

#### Echographic imaging

The two imaging sessions were held at least 30 min, but not more than 2 hrs apart. Protocol called for at least a 20 minute work out period (Vol. 76, p.202). One injection in these 2 trials was to be administered over approximately 30 – 60 seconds and the other during 2 minutes. *“ Before image acquisition, the ultrasound unit was set for optimal image evaluation as determined during the unenhanced imaging procedure and was not further adjusted. ... Fundamental 2-D gray-scale images were obtained before and after the first DMP 115 injection beginning with the acquisition of pre-contrast baseline images. .... The second image acquisition began with second harmonic baseline imaging. ... Fundamental baseline images were obtained using the same settings as those used during the first injection of contrast agent for optimal baseline fundamental images.”* (Vol.76, p.36 - 38)

Images from post-injection imaging sessions were recorded on SVHS videotapes.

Blinded read

*“The readers ... identified the single-beat cine loops in each view to identify end-diastolic and end-systolic frames. The frames with single largest and smallest endocardial dimensions were selected. The endocardial borders were manually traced by each sonographer, and EDV and ESV were calculated using the method within the* (Vol.76, p.42, par.2)

Blinding was accomplished by blinding the readers to patient information and image type (unenhanced or enhanced (Vol. 76, p.40, par.3). The order in which images were presented was randomized.

Although the original protocol described a much more elaborate blinded read, in reality, only two blinded read assessments were made. The first was a new nonvalidated method of measuring the left ventricular cavity size by a method of using estimates of EDV and ESV. It used a paired format in presentation of images.

The other was an evaluation of wall motion abnormalities from a split screen showing coupled 4- and 2-chamber views with up to six split screen sets of images evaluated per patient. Here, the images were presented in an unpaired format.

### 8.2.3.1 Population

#### *Inclusion Criteria*

- *“Were 18 years of age (or the age of legal consent) or older with at least two of six contiguous myocardial segments of the ventricular border deemed non-evaluable in either the apical 4- or 2-chamber view (within 90 days of trial)*
- *If women were non-pregnant ...*
- *Have undergone a routine echographic assessment within 90 days of trial*
- *Provided written signed informed consent ...*
- *Were able to communicate effectively with trial personnel.”*

#### Exclusion Criteria

- *“Alcohol and drug addiction*
- *Inability to provide blood samples*
- *Serious mental illness*
- *Recent investigational use*
- *Clinically unstable, had a history of an acute disease that could influence the patient’s ability to return for follow-up e.g., unstable*

- *angina, acute myocardial infarction, marked ventricular ectopy, atrial fibrillation with variable ventricular response*
- *Had existing NYHA Class IV CHF or severe COPD*
- *Required cardiovascular medications during the imaging session*
- *Presented with a contradiction relative to MRI imaging"*

### 8.2.3.2 Efficacy Endpoints

As per modified protocol, in regard to the first endpoint, evaluated by the blinded read, the segmental wall motion, *"Each reader will evaluate each image to determine segmental wall motion. The six left ventricular myocardial segments of each view will be graded as 0 = non-evaluable, 1 = normal/hyperkinetic, 2 = hypokinetic, 3 = dyskinetic/akinetic."*

For the second endpoint, left ventricular cavity size, *"Following EDV, ESV calculation the patient's ventricular function assessment based on will be categorized by the sponsor into the following:*

- 0 = Normal*
- 1 = Mildly impaired*
- 2 = Moderately impaired*
- 3 = Severely impaired"*

The Sponsor performed MRI-based comparator studies for the wall motion calculations. The supportive literature based studies submitted by the Sponsor are retrospective and lack the adequate data to validate MRI as an adequate standard of truth for LVEF (Vol.77). MRI-based wall motion evaluation determination are not FDA approved diagnostic procedures at this time. As stated by the secondary clinical review "The Agency has not accepted MRI as a standard of truth for cardiac studies because there are no data from prospective studies that have been submitted to the Agency to support MRI as a valid standard." (Please refer to Clinical Team Leader's Review, p. 13, par. 1)

Although the Sponsor originally planned to consider the as the primary efficacy measure, the Sponsor withdrew the claim for as an indication after the study completion (Vol.1, p.233, par.2, 1.5 and p.236, par. 2, 1.5). This is mentioned here for completeness.

The secondary effectiveness measures for these trials as described in the clinical study report were:

- *Difference from MRI*
- *Difference in relative error from MRI in end diastolic volume*
- *Difference in relative error from MRI in end diastolic volume*

- *Contiguous endocardial border length*
- *Segmental wall motion percentage*
- *Wall-motion percentage by region*
- *Improvement in Endocardial Border Delineation*
- *Salvage of non-diagnostic echographic examinations*

The duration of contrast enhancement was evaluated separately in studies DMP 115 - 006 + 007.

### 8.2.3.2.1 Protocol Deficiencies Related to Efficacy

The main weakness, again, of all these **efficacy measures** resulting from the blinded read stems from an uncertainty whether or not these parameters are conducive to an objective assessment, or whether they are **subjective opinions** of the readers based on no objective, or quantitative criteria. As far as it can be determined, no details on such items are presented in this submission. The instruction to the readers only referred to vague, all encompassing, terms such as contrast, enhancement, or information and nothing else. Although there was a training session for the readers, no other specifics on the substance of the difference between an enhanced and unenhanced image is presented throughout the submission. This issue was discussed with the Sponsor during the pre-Phase 3 meeting on November 19, 1996.

The Sponsor also submitted evaluations similar to those described under the blinded read, but obtained by various investigators familiar with other patient information. This is referred in the submission as Institutional read. However, the information so obtained is usually confounded by investigator's bias.

As already mentioned, the Sponsor does not want to make a claim for and, therefore, all the secondary effectiveness criteria related to the are deemed moot. It can be used only as supportive evidence. Likewise, the cardiac volumes obtained by MRI, a non-validated standard of truth, can be considered no more than supportive evidence.

**The concept of a comparison of the and wall motion evaluations with the respective MRI obtained parameters cannot be considered to have a firm scientific and regulatory footing.**

There were two additional efficacy criteria. *The Salvage of Non-Diagnostic Examinations was defined as*

- *At least four non-evaluable segments (score of 0) in a single apical view at baseline*

- *A total of zero or one non-evaluable segment (score of 0) in the same apical view after Injection 1*

*The ventricular videodensitometry was measured to quantitatively evaluate changes in the ventricular cavity. Independent values were recorded for regions of interest in the apex and mid-cavity for baseline images and post-treatment images at end-diastole, mid-systole and end-systole.*

### **8.2.3.3 Statistical considerations**

Please refer to the Statistical Review. The reader is also referred to the Statistical Review for planned blinding and randomization schemes. Otherwise, the efficacy issues will be commented upon throughout this review.

### **8.2.3.4 Safety considerations**

Following safety parameters were obtained: 1) AEs (*Each patient was queried using non-leading questions (e.g. "How do you feel ?")*); 2) 12-lead ECG – pre-injection and within 60 - 90 min after the first injection; 3) vital signs - pre-injection, 3 min, 5 min, 10 min after each injection and 30 min, 60 min and 24 hr after the second injection (Vol. 76, p.59, par 2, l.3 and Protocol, Vol. 76, p. 219, par.2, l.3); 4) serum chemistry, hematology and urinalysis – pre-injection, 60 – 90 min after the first injection and 24 hr post-injection; 5) history and physical – pre-injection and at 24 hr post-injection.

As the drug is composed of liposomes, an effect on microcirculation should be expected until proven otherwise. Such an effect is usually most pronounced when the concentration of the particles is the highest, i.e. immediately post- injection. ECG monitoring was not done immediately after the injection, but only about 30 min after the second drug injection (Vol. 76, p.59, par 2, l.3 and Protocol, Vol. 76, p. 219, par.2, l.3). This means that the first ECG data was obtained 1 hr after the first exposure to the drug (1 hr, 24 hr). The required information about the main metabolites was not obtained in advance nor was the data to assess immunogenicity.

## **8.2.4 Results of Trial DMP 115 – 006 and Trial 115 - 007**

### **8.2.4.1 Population enrolled**

The patient population in this study included 136 subjects in 8 centers in the US. The only common specific characteristic of note, common to all these cardiac patients, was that they had *"at least two of six ventricular border segments non-evaluable in either an apical 4- or 2-chamber view"* on the initial cardiac sonographic exam. Also of special significance, patients with unstable disease were excluded.

### 8.2.4.2 Efficacy endpoint outcomes

#### Primary Efficacy Measure

As per the original protocol, the Sponsor intended to perform these two trials to support the indication for *...* and the wall motion as among the secondary measures. None of those goals truly materialized as addressed elsewhere in this review.

#### Secondary Efficacy Measure

The Sponsor described two instances asserting that the results of these two trials support, separately, the claims made.

**However, as seen in the Clinical Study Report (Vol.76, p.53, par 2) “the endocardial border delineation” within the context of this trial meant something else than described elsewhere throughout the submission. According to the Sponsor what is understood here is the following: “An improvement in endocardial border delineation occurred when a segment that was scored as non-evaluable (0) at baseline was scored as evaluable (normal or hyperkinetic [1], hypokinetic [2], akinetic [3], or dyskinetic [4]) in the corresponding apical view after injection with DMP 115.”**

#### Wall motion

Therefore, the substance of this evaluation is wall motion rather than anything to do with the endocardial border.

The Sponsor reports: *“For the unpaired Blinded Reads, the median values for difference in segmental wall motion match versus MRI comparator in the Trials DMP 115 - 006 and - 007 were 29.0% and 7.9%, respectively. For the paired Blinded reads, the median values for difference in segmental wall motion match versus MRI comparator for these two trials were 40.1% and 16.9%.”* (Vol.1, p.225, par 2)

Consequently, the factual agreement between the new procedure and proposed comparator (MRI) from the two trials improved by 18.4% for the unpaired Blinded read and by 28.5% for the paired Blinded read. This result is marginal considering the reference standard, MRI. The respective “absolute” (Vol.77, p.232) agreement between MRI and contrast angiography in regard to evaluation of hypokinesia only was about 60% in the sole article available in the literature (Vol. 77, p.232, Fig.8). MRI was reported to have a 60% agreement with contrast cineangiography and, in 28.5%

instances improves with the DEFINITY results.

**Endocardial Border Length**

One of the secondary efficacy parameters evaluated was the Endocardial Border Length.

It turned out that the Endocardial Border Length was the **only** parameter which showed a clear benefit of DMP 115 in this study. According to the Statistical Review and Evaluation by Dr. Sobhan, the improvement due to DEFINITY was seen by both trained

<b>Table 2.2.B.3</b>						
<b>Mean (SD) Endocardial Border Length (EBL) by both Apical 2- and 4-chamber Views at End-Systole and End-Diastole, Study DMP 115-006 and DMP 115-007.</b>						
<b>View</b>	<b>Endocardial Border Length –Blinded Read</b>					
	<b>Reader 1</b>		<b>Reader 2</b>		<b>Reader 3</b>	
	<b>Study-006</b>	<b>Study-007</b>	<b>Study-006</b>	<b>Study-007</b>	<b>Study-006</b>	<b>Study-007</b>
	<b>Mean(SD)</b>	<b>Mean(SD)</b>	<b>Mean(SD)</b>	<b>Mean(SD)</b>	<b>Mean(SD)</b>	<b>Mean(SD)</b>
<b>Apical 4-chamber</b>						
End-Diastole	8.1(3.3)	4.0(2.7)	4.5(2.6)	9.2(5.9)	7.0(2.3)	8.8(4.8)
Baseline	13.5(5.2)*	7.1(5.5)*	6.8(3.3)*	11.5(7.5)*	7.2(3.1)	7.8(4.2)
Post-DMP						
End-Systole	7.6(3.2)	3.8(2.6)	4.5(2.7)	7.3(5.6)	6.9(2.6)	9.1(5.0)
Baseline	11.5(4.4)*	5.9(5.3)*	5.3(3.1)	8.7(6.3)*	6.5(2.8)	7.8(4.2)
Post-DMP						
<b>Apical 2-chamber</b>						
End-Diastole	8.0(3.4)	4.3(2.6)	4.7(2.8)	7.8(5.3)	6.9(1.6)	7.9(3.8)
Baseline	12.8(5.2)*	5.7(4.7)*	5.8(2.6)*	8.2(6.5)	6.2(1.9)	7.4(4.1)
Post-DMP						
End-Systole	7.1(3.3)	4.1(2.4)	4.3(2.6)	6.5(5.1)	6.0(1.5)	7.3(3.3)
Baseline	10.6(5.0)*	5.5(4.4)*	4.4(2.3)	6.9(6.3)	5.6(1.4)	7.2(4.1)
Post-DMP						

\* Significant change from baseline (paired t-test, p<0.05)

blinded readers, but not by the untrained blinded reader. I agree with his assesment in this respect. However, **the broader context and/or clinical meaning of this finding is yet to be defined.** From the regulatory standpoint, as of now, this result is an isolated finding without its clinical correlate.

#### 8.2.4.3 Safety outcomes

The safety of this trial was evaluated as a part of global assessment. Please refer to section 10. Overview of Safety, p.67, of this review.

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#### **8.3.4.3 Safety outcomes**

Please refer to section 10. Overview of Safety, p.67, of this review, for a global assessment.

### **9. Overview of Efficacy.**

#### **9.1 Population**

#### **9.2 Efficacy Findings and Significance**

**A clearcut, clinically meaningful benefit of DEFINITY as a sonographic contrast agent for cardiology was demonstrated only in one (DMP 115 – 004) of two trials designed to demonstrate it.** The other trial failed to confirm that result. The evaluation scale used in this study (evaluable, or nonevaluable, or not applicable) was somewhat ambiguous.

Although the second pivotal study (DMP 115 – 006 and – 007) documented some improvement in the wall motion evaluation by a comparison with MRI, the modality used as a comparator, the MRI, is not specifically approved for the evaluation of wall motion. Nonetheless, it is sometimes used for that purpose as a part of medical practice. However, the literature article search and review, submitted in support of this approach, yielded only a single remotely related item.

## 10. Overview of Safety

Some safety evaluation during the development of this drug was performed, but whether the approach taken is satisfactory is open to questions.

### Placebo-controlled trials

As an overall approach to safety evaluation, the Sponsor chose the route of placebo controlled trials. The overall safety of the drug cannot be evaluated with any confidence in part because of the choice of placebo, selection of subjects and number of subjects. Placebo choice in Phase 1 (glycerol, propylene glycol and saline) was different than in Phase 3 (saline).

### Placebo used in trials with normal volunteers

In all the safety trials (DMP - 900 and DMP - 901) with **normal volunteers** (10 subjects in DMP - 900 and 6 subjects in DMP - 901), the Sponsor selected as placebo a mixture of glycerol, propylene glycol and saline (Vol.53, p.22, par.2; Vol. 55, p.26, par.3). The organic components may be present in the drug formulation. However, no evidence is presented that this formulation has been prepared similarly (activation) as the drug. In addition, there is no reference that the use of this placebo has been properly validated for the patient population.

### Drug differences due to manufacturing

Furthermore, the drug formulation used in these trials was different from that used in pivotal trials and intended to be marketed. The earlier formulation which was used in these early clinical experiments, MRX 115, had, in pre-clinical experiments, a much more favorable toxicological profile than that used later and intended for marketing, DMP 115. Therefore, comparison of to-be-marketed product with placebo is not valid.

### Comparison between normal volunteers and patients

Likewise, it is **not valid** to compare the studies done in normals to those performed on patients. In the **Safety Update** on April 7, 1999 (p. 000255, Table 35), the Sponsor concluded that **68.8%** of the **normal volunteers** had a 20% to 40% change in diastolic blood pressure while on placebo (glycerol, propylene glycol and saline), implying that there is no effect of the drug, since the normals receiving the drug showed a similar change. In addition, 18.8% and 25% of these "normals" had a 40% to 60% change in pulse rate and respiratory rate, respectively, due to this "placebo". All these 16 subjects were males of an average age 27.7 years (Vol.53, p.44 and Vol.55, p.65).

### Small number of patients treated with placebo

Secondly, the Sponsor performed the placebo-controlled safety trials (DMP 115 - 004 and DMP 115 - 005) **in patients** using saline as the placebo. The placebos, therefore, are not comparable between the trials with normals and patients. In addition, the trials selected for safety evaluation deal with severely ill, but not in acute distress, cardiac patients in whom a great variety of abnormalities in numerous safety parameters are to be expected. To compensate for this variability a proper design requires an adequate number of subjects, to insure that patients with various disease conditions (such as coronary artery disease, valvulopathy, congestive heart failure, dilated cardiomyopathy, conduction abnormalities, etc.), or usual combinations of disease conditions (hypertension, diabetes, etc.) or risk factors (smoking, lack of exercise, etc.) are accounted for. As only a total of 42 patients received placebo (saline), it is doubtful that all common cardiac conditions or their combinations were adequately represented, or proportionately represented in regard to the entire patient population. Therefore, there **is not a reasonable probability that the results obtained with placebo can reasonably serve as a control** to the results in patients (556) receiving the drug, as the Sponsor seems to imply.

**Safety evaluation in patients hampered with non-homogeneity of patient population**

Thirdly, the selection of patient population for the placebo-controlled trials is also odd. As the cardiovascular system should be expected to be affected by the drug consisting of particles around 10 um in size, the patients with cardiovascular disease are an improper population to assess as they bring with them such a large variability component, particularly in regard to ECG and vital signs determination, that even a large drug effect would necessarily pale in comparison. Thus, the drug effect in regard to safety may be confounded with and overwhelmed by the large disease component.

**Improper timing of key safety measurements**

As the drug is composed of liposomes, an **effect on microcirculation** should be expected until proven otherwise. Such an effect is usually most pronounced when **the concentration of the particles is the highest**, i.e., immediately post-injection. It is, therefore, unfortunate that the Sponsor selected to initiate the safety most pertinent monitoring (ECG), except for AE monitoring, in a reliable manner only about 30 min after the (second) drug injection (Vol. 62, p.55, par 2, l.1 and Protocol, Vol. 62, p. 217, par.2, l.1). This means that **useful ECG data was obtained only 1 hr after the first exposure to the drug** (1 hr, 24 hr, 48 hr and 72 hr). It is also doubtful that ADEs can be adequately monitored while the patient undergoes echographic imaging. **This is only to emphasize that adequate safety data within the first 3 min and up to 1 hr do not exist.**

**Lipid-based drug**

Finally, it is not only the liposomes per se that are of concern in regard to impaired hemodynamics when a lipid-based drug, including fat droplets are introduced into the pulmonary, or other vasculature. The latter is more, or less an acute problem. A more distant effect is the **endothelial injury caused by fatty acids released from impacted fat droplets** by lipoprotein lipase, with ensuing increased microvascular permeability and fluid leakage into interstitial spaces. This and related abnormalities of carbohydrate and lipid metabolism, increased capillary fragility and abnormal neurohumoral response to stress are usually associated with a condition described as fat embolism syndrome.

#### Potential danger of fat embolism

The mortality from fat embolism is less than 10% and it is usually described in association with fresh long bone fractures where it occurs in 2 to 25% cases (Cecil, Textbook of Internal Medicine, 1988 and 1996). There are no laboratory tests that are diagnostic of fat embolism. The diagnosis is based on the presence of at least one of the following features within the first 72 hours after traumatic fracture: 1) otherwise unexplained dyspnea, tachypnea, arterial hypoxemia and diffuse alveolar infiltrates; 2) unexplained confusion or other signs of cerebral dysfunction; 3) petechiae over the upper half of the body, including the axillae, conjunctivae, and oral mucosa (Cecil, Textbook of Internal Medicine, 1988 and 1996). All of these singly or in combination were observed and reported during the safety evaluation of this drug as AEs, the petechiae potentially confused with rash, along with hemodynamic-cardiovascular effects. The deaths occurring within days of the drug injection also fit this category.

The trials were not designed to control for the variability in safety parameters

Consequently, as the Sponsor mishandled the design and execution of the placebo-controlled trials, **it cannot be reliably determined whether any effect seen could be due any other factor, except for the drug.** In other words, only the drug exposure was a parameter common to all the patients. Therefore, for the sake of this safety review, any abnormality of the safety parameters throughout the entire NDA submission should not be formally attributable to anything else but the drug, although it could have been potentially be due to a disease. Pooling the patient populations with an array of disease conditions for safety evaluation may even confound those drug effects, which could have been apparent without such a pooling. Therefore, the placebo-control safety evaluation is not applicable. Furthermore, **as the presumed active drug component all but disappears by 30 min post-injection, the failure to evaluate the complete safety concurrently (i.e. ECG and vital signs) may also disqualify the drug on safety grounds,** at least until the time that a convincing safety database is available.

Placebo used in patients was clearly recognizable by color and consistency

It may be recalled from the chemistry section of this review that the drug formulation upon activation will become a milky white emulsion. Saline, on the other hand, a clear transparent will not change its color upon shaking, "activation", on a shaker except, perhaps, for a few bubbles which will quickly disappear. Therefore, **the investigator, or a designated technician always knew exactly (and in advance of imaging and safety assessment) which patient had received the drug and which received placebo.** Adjustment of evaluation procedures, even inadvertently, could not be excluded. Thus, the safety and efficacy assessment throughout all the pivotal studies could be biased.

#### Drug substance

As every evaluation of drug safety and efficacy should include a clear understanding what the drug in question is, or what it should be, it is felt that this should be an early point of the overall safety discussion.

#### Liposome or a lipid-encapsulated microbubble?

In the introductory sentence, introducing the main section on Drug Substance (Vol.6, p.206, par.2, l.1), the Sponsor described the drug as follows: ***"The substance is a lipid-encapsulated Perfluoropropane (SG897) microbubble, in the size range of 1 to 10 um in diameter."***

It is noted only a page down in the same section of this NDA submission (Vol.6, p.207, par.3, l.2), that: ***"..Perfluoropropane in activated DMP115 vials is consistent with that of PFP in the gas phase. These experiments showed that PFP in activated DMP 115 is a gas dispersed in liquid, and not a solute."*** It would appear, therefore, that the presumably active component of the drug is not a gas in the ***"lipid encapsulated ..... microbubble"*** as stated in the introductory sentence, and reiterated numerous times throughout this NDA submission, but rather it is a gas dispersed in saline, something like CO<sub>2</sub> in soda, or O<sub>2</sub> which fish can breath in fresh water, a liposome, in short.

#### Lipid needed to prevent a rapid dissipation of perfluoropropane from the drug blend

In addition, the Sponsor continues (Vol.6, p.208, par. 1, l.1): ***"The role of the phospholipids in stabilizing the gas in the DMP 115 formulation was demonstrated by comparing the NMR signal obtained with and without lipids present in the formulation. For the formulation that did not contain the lipid blend only a faint signal could be detected using the typical acquisition conditions. However, the sample that contained the lipid blend in the formulation had a strong signal"***. Therefore, it appears that lipids facilitate the presence of a larger amount of the PFP gas in a unit volume of solution.

#### Use of NMR to determine particulate nature of the drug