

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-064

**Clinical Pharmacology and Biopharmaceutics
Review**

BACKGROUND

The following requests were made by the Reviewing Medical Division regarding Clinical Pharmacology issues in the Approvable Letter dated 10/8/99:

1. The pharmacokinetics data lack sufficient information to validate the results of the octafluoropropane elimination profile. Specifically, study 905 was performed to measure the elimination of the octafluoropropane gas. The data to validate the assay were not submitted. In order to resolve this deficiency, please provide the assay validation data. Alternatively, if these data are not available or not sufficient, the mass balance study should be repeated.
2. The elimination data lack sufficient information on the fate of the microsphere shell. Although the application states that the lipid components are endogenous substances, data to characterize the elimination were not submitted. In order to resolve this deficiency, please provide an analysis of the pharmacokinetic literature on these components as a liposome. If literature data are not available, then studies are needed to demonstrate whether and how the microsphere shell is metabolized and/or eliminated.

After considering the Applicant's Responses, a follow up request or Request for Additional Information on assay methodology was communicated to the Applicant on 3/6/2000, which the Applicant submitted a response on 3/31/2000. The following request was made:

1. Please submit the following information from the Final Report (Method validation) entitled "In-vivo kinetics of the Perfluoropropane Component of MRX-115 in the Dog", conducted by [redacted] dated 7/31/97. It appears that blood and expired air samples from dogs were analyzed on 10/24/96 or between 10/24 and 10/28/96 (according to the 'Quality Assurance Statement' page).
 - a. Please submit standard curve(s) (plots and numerical values), generated on 10/24/96 or from all of the subsequent days between 10/24/96 and 10/28/96, i.e., from days which samples were actually analyzed.
 - b. Please submit quality control (QC) sample information from days which samples were actually analyzed, i.e., above dates.
 - c. If applicable, please provide assay performance information, e.g., between-day variation, intra-day variation, accuracy, precision, etc., from days which dog blood and expired air samples were actually analyzed.
2. For Study DMP 115-905 normal subject pharmacokinetic study, please provide standard curve (a plot and numerical values), QC, and assay performance information from the actual sample analysis day, i.e., from the day which the collected human blood and expired air samples were actually analyzed.

If samples were analyzed on multiple days due to number of samples, please submit all standard curves and related assay information obtained on those days that blood and expired air samples were analyzed.

COMMENTS REGARDING ASSAY AND LIPID METABOLISM INFORMATION

1. Assay information

The gas chromatography assay used to measure the elimination of the gas in Study 905 was identical to that used in a dog study, Study MRI 4490-F (In-Vivo Kinetics of the PFP component of MRX-115 in the Dog). The assay method and validation supported both dog and human studies. Additionally the Applicant submitted a copy of the validation report.

Dog PK study

Submission dated 3/31/2000 contained standard curves and QCs from various days. Standard curve concentrations used 0.00015, 0.00074, 0.0012, 0.0031, 0.0062, 0.0185, 0.062, 0.111, 0.222, 0.556 $\mu\text{L/mL}$. Standard curves appear to have correlation coefficients >0.99 . The average recovery for QC samples were 100.4 ± 25.1 , 99.5 ± 13.7 , and $90.9 \pm 10.9\%$ for 0.00074, 0.0031, and 0.111 $\mu\text{L/mL}$ concentrations, respectively.

Study 115-905 human PK study

Submission dated 3/31/2000 contained standard curves and QCs from various days. Standard curve concentrations used 0.00015, 0.00038, 0.00074, 0.0012, 0.0031, 0.043, and 0.0124 $\mu\text{L/mL}$. Standard curves appear to have correlation coefficients >0.99 . The average recovery for QC samples were 148.3 ± 107.5 , 102.5 ± 16.7 , and $102.1 \pm 94\%$ for 0.00074, 0.0012, and 0.0124 $\mu\text{L/mL}$ concentrations, respectively. It is expected that at lowest concentration the SD is relatively large compared to other two QC concentrations.

2. Lipid metabolism information

Submission dated 2/7/2000 the Applicant responded that the elimination of the lipid components was presented in the rat study report, RDR 98-12 (Pharmacokinetics, Distribution, Metabolism and Excretion of ^{14}C -DMP 115 Following an Intravenous Dose to Conscious Sprague Dawley Rats).

The three lipids listed in the formulation, DPPA, DPPC, and DPPE, are naturally occurring in man as blood lipids. In plasma alone, the concentrations of phosphatidyl ethanolamine (DPPE), phosphatidyl choline (DPPC), and phosphatidic acid (DPPA) are 0.02, 1.46, and 1.16 mg/mL , respectively (Goodman *et al.*, 1964, Masoro *et al.*, 1968, Schrade *et al.*, 1960, Williams *et al.*).

In summary, the labeled component of the DMP 115 (DPPE [^{14}C]-MPEG 5000) showed no major metabolism through the time points prior to 1 hour post-injection in plasma. The HPLC in urine at 4 hours shows 90% of the radioactivity in the form of ^{14}C MPEG 5000. The total activity in the urine at 4 hours was only 8.5% of the injected dose. The ^{14}C -MPEG 5000 DPPE is metabolized to ^{14}C -MPEG5000 LPE and ^{14}C -MPEG 5000. Since DPPE is naturally occurring and the metabolism occurs on this part of the molecule, any fragments of the DPPE would undergo normal physiological biotransformations. The ^{14}C -MPEG 5000 fragments are predominantly excreted renally.

The amount of lipid in DMP 115 administered to a 70 kg person would be 0.0002 mg/mL DPPE, 0.0002 mg/mL DPPC, and 0.00003 mg/mL DPPA. This would represent approximately 0.86% DPPE, 0.016% DPPC and 0.002% DPPA of the naturally occurring levels in plasma. These levels would suggest that the lipids in DMP 115 would not contribute significantly to the naturally occurring levels already present in man.

Reviewer's Comment: The information submitted by the Applicant is acceptable.

**APPEARS THIS WAY
ON ORIGINAL**

RECOMMENDATION

The amendment #5 submitted by the Applicant on 2/7/2000 has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II (OCPB/DPE II; HFD-870).

The re-submission contains necessary information that was requested in the Approvable Letter on 10/8/99. Therefore, the re-submitted information is considered adequate. However, it is noted that there exists a lack of pharmacokinetic information from the intact microbubbles.

At present time, there are no comments that need to be conveyed to the Applicant regarding Definity, except that the Applicant should address the Labeling issues (Clinical Pharmacology and Biopharmaceutics Review dated 8/23/99) appropriately.

/S/

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

7/22/00

Concurrence:

/S/

John Hunt
Deputy Director
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

7/26/00

CC: HFD-160 NDA 21-064; DIV FILE; /CSO/TNguyen (1X); /OCPB/DLEE
HFD-870 /OCPB/JHUNT, SHuang(1X)
CDR Attn: Barbara Murphy

APPENDIX

Additional Information regarding Definity drug product

SYNOPSIS

DuPont had submitted an original New Drug Application (21-064) on 12/8/98, and was seeking approval of Definity as a contrast agent for use in echocardiography. The recommended dose for Definity is a single dose of 10 $\mu\text{l}/\text{kg}$ by slow I.V. bolus injection over 30-60 seconds, followed by a 10 ml saline flush. A second 10 $\mu\text{l}/\text{kg}$ dose may be administered to prolong optimal imaging. The drug product did not have any substantial changes in its formulation during the entire drug development process. Therefore, it was considered that there is one formulation for Definity.

Definity is a non-pyrogenic suspension of phospholipid-encapsulated perfluoropropane (PFP) microbubbles. Initially the 2 ml vial will contain phospholipid blend (3 phospholipids), propylene glycol, glycerin, water for injection and a headspace containing PFP gas. Microbubbles, as a suspension, are prepared by shaking with the aid of an agitator, Vialmix™. PFP gas, as stated by the Applicant, is currently marketed as an intracocular injection for retinal reattachment procedures. Clinical trials with Definity were developed and performed under IND (drug code name DMP 115). To support the Human Pharmacokinetics and Bioavailability section of NDA 21-064, the original Application contained 3 studies (1 pharmacokinetic and 2 pharmacodynamic studies) and supporting information regarding metabolism of the shell, phospholipids:

Study DMP 115-900	A Phase I study to determine the safety and tolerance of single rising doses of MRX-115, an intravenous ultrasound contrast agent, in healthy adult male subjects
Study DMP 115-901	A Phase I study to determine the safety and tolerance of multiple doses of MRX-115, an intravenous contrast agent, in healthy adult male subjects
Study DMP 115-905	A Phase I, open-label evaluation of the pharmacokinetics of perfluoropropane following the intravenous administration of DMP 115 in normal subjects and subjects with chronic obstructive pulmonary disease

From Clinical Pharmacology and Biopharmaceutics perspective, Studies 900 (5, 10, 20, 50 and 100 $\mu\text{l}/\text{kg}$) and 901 (5, 10, 15, 30 $\mu\text{l}/\text{kg}$) did not provide significant information regarding dose-imaging quality information. At best, these two studies provided preliminary information for phase 2 dose PD ranging studies. These phase 2 PD studies were reviewed by the Reviewing Statistician. For Study 900, it was concluded that dose-related response was not observed in relation to opacification and recommended conducting further studies. For Study 901, due to the small number of subjects, it was not possible to identify any dose-related trends in any of the data collected in this study. Study 905 (50 $\mu\text{l}/\text{kg}$) results showed that pharmacokinetic parameters for PFP gas were similar in 12 healthy men and women and 12 COPD patients. No gender differences were noted.

It was also noted in the original OCPB Review that the initial NDA submission contained minimal pharmacokinetic information (i.e., limited expired PFP gas pharmacokinetics) and negligible pharmacokinetic/pharmacodynamic data. There was a discussion on obtaining PK information from "intact" microbubbles.

In all, the original OCPB Review stated that from a clinical pharmacology and biopharmaceutics perspective, Studies 900 and 901 provided no pharmacokinetic data and no useful pharmacodynamic data. However, Study 905 did provide some pharmacokinetic data for PFP gas in men and women. Nevertheless, due to the nature of the microbubble drug product, it is considered that there is minimal information to allow for an approvable status.

FORMULATION

Component	Concentration/ml
Lipid Blend DDPA DDPC MPEG5000 DPPE	0.75 mg Mole % ratio
Perfluoropropane Gas	5% in the Headspace
Propylene Glycol, USP	103.5 mg
Glycerin, USP	126.2 mg
Sodium Chloride, USP	6.8 mg
Water for Injection, USP	
Sodium Hydroxide, NF	Only as necessary to adjust pH
Hydrochloric Acid, NF	Only as necessary to adjust pH

Injectate Characteristics	Concentration/ml
Perfluoropropane Gas	
Number of Microbubbles	$- 1.2 \times 10^{10}$

INDICATIONS AND USAGE

The proposed indications and usage as indicated by the Applicant under INDICATION AND USAGE section in the original package insert:

DRAFT

DOSAGE AND ADMINISTRATION

The proposed dosage for Definity in the original Application was (from the package insert's Dosage and Administration section):

Bolus Administration:

Draft

Infusion

Draft

REVIEWER'S GENERAL REVIEW COMMENTS FROM THE ORIGINAL NDA OCPB REVIEW

1. PFP GAS MASS-BALANCE INFORMATION

The Applicant should provide PFP gas mass-balance information. Study 905 provides PEP gas collection data (Ae: 0-5 minutes continuous collection). From the majority of plots, at 5 minutes post injection, it appears that PEP gas is still being eliminated, i.e., cumulative slope is still at a rising phase. This Reviewer feels that the sampling scheme was not appropriate to capture the PFP lung expiration data. Hence, the mass balance of PFP is difficult to obtain from the data set.

2. ELIMINATION HALF LIFE OF PFP GAS AND PERSISTENCE OF IMAGE ENHANCEMENT RELATIONSHIP

Although the Applicant explored the dose-enhancement relationship, the results were not conclusive. No useful information can be extracted from 900 and 901 studies. Study 905 design is not optimal; thus, it is concluded that substantial supportive information was not submitted in Clinical Pharmacology section of this NDA.

3. PFP GAS PROTEIN BINDING INFORMATION

It appears that the NDA package lacks PFP gas protein binding and distribution information. However, this Reviewer expects, as with other inert gases, that PFP gas has a relatively low partition coefficient. Therefore, PFP protein binding is expected to be minimal due to the low partition coefficient of the gas in blood.

4. ASSAY INFORMATION

The Applicant did not submit any assay information.

5. GENDER ANALYSIS

In Study 905, the Applicant stated that gender differences were not noted. This Reviewer concurs with the Applicant's conclusion. However, due to the nature of the drug product and the small number of subjects utilized in the Study 905, the results regarding gender analysis must be considered with caution.

REVIEWER'S COMMENTS TO THE APPLICANT FROM THE ORIGINAL NDA OCPB REVIEW

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

1. PFP GAS PROTEIN BINDING INFORMATION

It appears that the NDA package lacks PFP gas protein binding and distribution information. The Applicant is encouraged to provide this information.

2. PHARMACOKINETICS OF INTACT MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of DMP 115, i.e., the "intact PFP- filled" microbubbles. Ideally "intact" microbubble pharmacokinetic information is needed with respect to the dosage proposed in the package insert. The Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method in order to definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. In addition, the Applicant is encouraged to explore in vitro methods to provide

information on the microbubbles in terms of microbubble "fragility and stability." One such in vitro method that can be explored is the microbubble "fragility" test: Addition of the microbubbles in blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. Furthermore, the Applicant may explore relevant animal models (e.g., microscopic examination of nail-bed capillary or cannulated cat mesenteric artery), if any. The Applicant is encouraged to correspond with the Pharm/Tox review team to explore the feasibility of using animal models to obtain microsphere fragility information. Once such information is obtained the data/information should be submitted to the agency for review.

3. PHARMACOKINETICS OF LIPID COMPONENTS, THE MICROSPHERE SHELL

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. The Applicant stated that substances are endogenous lipids and are well characterized. In addition, MPEG5000 was not evaluated; however, extensive PK studies have been previously performed in rats. Although it is reasonable not to perform any PK or metabolism studies on DPPC, DPPA and MPEG5000 in humans, it was raised during the pre-NDA Meeting that supportive information regarding lipids and MPEG5000 should be submitted. This information is still requested.

4. PFP GAS MASS-BALANCE INFORMATION

Please submit any 'mass balance information' on DMP 115 microbubbles.

5. PFP GAS ASSAY INFORMATION

The Applicant did not submit any assay information regarding PFP gas,
Please submit this information.

**APPEARS THIS WAY
ON ORIGINAL**

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-064

REVIEWER: David J. Lee, Ph.D.

DRUG: Definity™ (Perflutren)
(10 µl/kg; supplied as a 2 ml glass vial)

SUBMISSION DATE: 12/8/98
STAMPED DATE: 12/9/98
ROUGH DRAFT: 7/14/99, 7/20/99
FINAL: 8/23/99

SPONSOR: DuPont Pharmaceuticals Company, North Billerica, MA

TYPE OF SUBMISSION: Original NDA 1S

SYNOPSIS

DuPont Pharmaceuticals Company has submitted a New Drug Application (21-064) on 12/8/98. DuPont is seeking approval of Definity as a contrast agent for use in echocardiography (see Indications and Usage section). The recommended dose for Definity is a single dose of 10 µl/kg by slow I.V. bolus injection over 30-60 seconds, followed by a 10 ml saline flush. A second 10 µl/kg dose may be administered to prolong optimal imaging (Dosage Administration section).

Definity is a non-pyrogenic suspension of phospholipid-encapsulated perfluoropropane (PFP) microbubbles. Initially the 2 ml vial will contain phospholipid blend (3 phospholipids), propylene glycol, glycerin, water for injection and a headspace containing PFP gas. Microbubbles, as a suspension, are prepared by shaking with the aid of an agitator, Vialmix™. PFP gas, as stated by the Applicant, is currently marketed as an intraocular injection for retinal reattachment procedures.

Clinical trials with Definity were developed and performed under IND (drug code name DMP 115). To support the Human Pharmacokinetics and Bioavailability section of NDA 21-064, the Applicant has submitted 3 studies (1 pharmacokinetic and 2 pharmacodynamic studies) and supporting information regarding metabolism of the shell, phospholipids.

Study DMP 115-900	A Phase I study to determine the safety and tolerance of single rising doses of MRX-115, an intravenous ultrasound contrast agent, in healthy adult male subjects
Study DMP 115-901	A Phase I study to determine the safety and tolerance of multiple doses of MRX-115, an intravenous contrast agent, in healthy adult male subjects
Study DMP 115-905	A Phase I, open-label evaluation of the pharmacokinetics of perfluoropropane following the intravenous administration of DMP 115 in normal subjects and subjects with chronic obstructive pulmonary disease

It appears that this drug product did not have any substantial changes in its formulation during the entire drug development process. The Reviewing Chemist stated that he is not aware of any substantial changes (if substantial changes, then, it warrants additional data to support the differences) in the formulation during its development process. Therefore, the Reviewer considers that there is one formulation for Definity.

Regarding the status of the NDA review, it appears that Reviewing Medical Officer recommends not approvable. Review Chemist indicated that the current NDA submission is approvable. In addition, according to the Pharm/Tox and Microbiology Reviewers, both reviews are still pending.

From Clinical Pharmacology and Biopharmaceutics perspective, Studies 900 (5, 10, 20, 50 and 100 $\mu\text{l}/\text{kg}$) and 901 (5, 10, 15, 30 $\mu\text{l}/\text{kg}$) do not provide significant information regarding dose-imaging quality information. At best, these two studies provided preliminary information for phase 2 dose PD ranging studies. These phase 2 PD studies are reviewed by the Reviewing Statistician.

Briefly, for Study 900, it was concluded that dose-related response was not observed in relation to opacification and recommended conducting further studies. For Study 901, due to the small number of subjects, it was not possible to identify any dose-related trends in any of the data collected in this study.

Study 905 (50 $\mu\text{l}/\text{kg}$) results showed that pharmacokinetic parameters for PFP gas were similar in 12 healthy men and women and 12 COPD patients. No gender differences were noted.

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. The Applicant stated that substances are endogenous lipids and are well characterized. In addition, MPEG5000 was not evaluated; however, extensive PK studies have been previously performed in rats. However, although it is reasonable not to perform any PK or metabolism studies on DPPC, DPPA, and MPEG5000 in humans, this Reviewer clearly stated during the pre-NDA Meeting that the Applicant needed to submit any supportive information regarding the lipids and MPEG5000. Therefore, as far as MPEG5000 is concerned, this Reviewer directs one to the Pharm/Tox Review for input on its PK and metabolism in rats.

Note: DPPA:
DPPC:
MPEG5000 DPPE:

The current NDA submission contains minimal pharmacokinetic information (i.e., limited expired PFP gas pharmacokinetics) and negligible pharmacokinetic/pharmacodynamic data. Ideally "intact" microbubble pharmacokinetic information should have been provided with respect to the dosage proposed in the package insert. In order to obtain this information in the future, the Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method so it could definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. Once such information is obtained the data/information should be submitted to the agency for review.

In conclusion, from a clinical pharmacology and biopharmaceutics perspective, Studies 900 and 901 provided no pharmacokinetic data and no useful pharmacodynamic data. However, Study 905 did provide some pharmacokinetic data for PFP gas in men and women. Nevertheless, due to the nature of the microbubble drug product, it is considered that there is minimal information to allow for an approvable status.

BACKGROUND

Current methods for determining cardiac function are invasive and expensive, i.e., intracoronary injection for procedures such as coronary angiography or contrast ventriculography.

A simplified procedure, such as gas-filled microbubbles by ultrasound method was pursued. Gas-filled microbubbles are proposed as a contrast-enhancing medium in clinical ultrasound to visualize left ventricular wall motion, left ventricular border definition,

As indicated above (SYNOPSIS Section) the following studies were submitted in the NDA:

1. Study DMP 115-900: dose-ranging PD study;
2. Study DMP 115-901: dose-ranging PD study;
3. Study DMP 115-905 PK/PD study.

The related drugs to this application are:

PMA	Albunex, albumin (human) 5%, sonicated (Molecular Biosystems, Inc.)
NDA 20-899	Optison Intravenous Injection, albumin human 1% with perfluoropropane microbubbles (Molecular Biosystems, Inc.)

PERFLUOROPROPANE (PFP) GAS CHEMISTRY

PFP Molecular Formula: C_3F_8 ; $CF_3-CF_2-CF_3$

Stability: Inert. Degradation dose not occurs. Pharmaceutical stability is considered infinite.

Physicochemical properties: Carbon-fluorine bonds are stable. Colorless gas. The Applicant provided the following physicochemical properties:

Boiling point (1 atm): -36.7° C
 Freezing point (1 atm): -183° C
 Liquid density at 20° C: 1.350 g/ml
 Molecular weight: 188.02 g/mol

FORMULATION

Component	Concentration/ml
Lipid Blend DDPA DDPC MPEG5000 DPPE	0.75 mg Mole % ratio
Perfluoropropane Gas	% in the Headspace
Propylene Glycol, USP	103.5 mg
Glycerin, USP	126.2 mg
Sodium Chloride, USP	6.8 mg
Water for Injection, USP	
Sodium Hydroxide, NF	Only as necessary to adjust pH
Hydrochloric Acid, NF	Only as necessary to adjust pH

Injectate Characteristics	Concentration/ml
Perfluoropropane Gas	0.15 ± 0.10
Number of Microbubbles	$- 1.2 \times 10^{10}$

INDICATIONS AND USAGE

The proposed indications and usage as indicated by the Applicant under INDICATION AND USAGE section in the package insert:

DRAFT

DOSAGE AND ADMINISTRATION

The proposed dosage for Definity is as follows (from the package insert's Dosage and Administration section):

Bolus Administration

Infusion

REVIEWER'S GENERAL COMMENTS

1. PFP GAS MASS-BALANCE INFORMATION

The Applicant should provide PFP gas mass-balance information. Study 905 provides PEP gas collection data (Ae: 0-5 minutes continuous collection). From the majority of plots, at 5 minutes post injection, it appears that PEP gas is still being eliminated, i.e., cumulative slope is still at a rising phase. This Reviewer feels that the sampling scheme was not appropriate to capture the PFP lung expiration data. Hence, the mass balance of PFP is difficult to obtain from the data set.

2. ELIMINATION HALF LIFE OF PFP GAS AND PERSISTENCE OF IMAGE ENHANCEMENT RELATIONSHIP

Although the Applicant explored the dose-enhancement relationship, the results were not conclusive. No useful information can be extracted from 900 and 901 studies. Study 905 design is not optimal; thus, it is concluded that substantial supportive information was not submitted in Clinical Pharmacology section of this NDA.

3. PFP GAS PROTEIN BINDING INFORMATION

It appears that the NDA package lacks PFP gas protein binding and distribution information. However, this Reviewer expects, as with other inert gases, that PFP gas has a relatively low partition coefficient.

Therefore, PFP protein binding is expected to be minimal due to the low partition coefficient of the gas in blood.

4. ASSAY INFORMATION

The Applicant did not submit any assay information,

5. GENDER ANALYSIS

In Study 905, the Applicant stated that gender differences were not noted. This Reviewer concurs with the Applicant's conclusion. However, due to the nature of the drug product and the small number of subjects utilized in the Study 905, the results regarding gender analysis must be considered with caution.

NDA REVIEW ISSUES:

1. PHARMACOKINETICS OF THE INTACT MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of DMP 115, i.e., the "intact PFP- filled" microbubbles. However, the Applicant did assess the pharmacokinetics of PFP gas, some of which may or may not have been encapsulated.

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

Toxicity due to the intact microbubbles

There are pharmacology and toxicology safety concerns due to the intact microbubbles. At this time the reader is referred to the Pharm/Tox Review and encouraged to follow the Pharm./Tox concerns outlined within the text.

2. PHARMACOKINETICS OF PFP GAS IN EXPIRED AIR AND IN BLOOD

PFP gas pharmacokinetic information has been obtained from Study 905. A non-compartmental model

approach was taken to calculate the pharmacokinetic parameters. See individual study reviews attached (Appendix).

PFP exhibited a very fast half-life for distribution into expired air for all subjects. This can be attributed to a high, first-pass extraction ratio of the pulmonary capillary bed for gases.

PFP blood pharmacokinetic information has been obtained from Study 905. A non-compartmental model approach was taken to calculate the pharmacokinetic parameters. See individual study reviews attached (Appendix).

Table 1. Blood PEP Pharmacokinetic Parameters - All Normal Subjects versus COPD Subjects

Pharmacokinetic Parameter	All Subjects Normal Mean (SD)	All Subjects COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	8	11		
Tmax (min) ^a	1.17 (0.67-2.17)	1.67 (1.17-3.17)		0.055
Cmax (μL/mL) x 10 ³	3.60 (2.46)	2.95 (1.80)	91 (54-154)	0.768
t _{1/2} (min)	1.28 (0.40)	1.95 (1.86)	97 (57-166)	0.932
AUCinf (μL/mLxmin) x 10 ³	8.12 (2.22)	7.62 (3.59)	90 (65-124)	0.571
CL (L/hr)	2481 (980)	2777 (1096)	102 (71-144)	0.940
V _{ss} (L)	112 (52)	181 (133)	112 (70-179)	0.692
CL _{lung} (L/hr) ^c	1478 (898)	1268 (665)	60 (33-107)	0.141

a tmax is presented as median (range); b Adjusted for sex differences;
c For the comparison of CL_{lung}, 7 normal subjects and 10 COPD subjects were used.
GLS = Geometric least squares mean

Table 2. Blood PFP Pharmacokinetic Parameters - Normal Males Versus COPD Males

Pharmacokinetic Parameter	Male Normal Mean (SD)	Male COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	3	8		
tmax (min) ^a	1.67 (1.17-2.17)	2.17 (1.17-3.17)		0.329
Cmax (μL/mL) x 10 ³	2.43 (0.71)	2.70 (2.09)	88 (36-213)	0.794
t _{1/2} (min)	1.34 (0.54)	2.35 (2.05)	138 (57-334)	0.518
AUCinf (μL/mLxmin) x 10 ³	6.71 (1.37)	8.09 (4.03)	111 (67-185)	0.715
CL (L/hr)	3151 (801)	2815 (874)	87 (59-129)	0.532
V _{ss} (L)	152 (40)	218 (138)	123 (60-251)	0.607
CL _{lung} (L/hr) ^b	1716 (1395)	1476 (562)	-	0.969

a tmax is presented as median (range).
b Ratio and 90% CI was not presented as there were only 2 males included in the normal group.
GLS=Geometric least squares mean

Table 3. Blood PEP Pharmacokinetic Parameters - Normal Females versus COPD Females

Pharmacokinetic Parameter	Female Normal Mean (SD)	Female COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-Test p-value
Number of Subjects	5	3		
tmax (min) ^a	1.17 (0.67-1.67)	1.17 (1.17-1.67)		0.337
Cmax (μL/mL) x 10 ³	4.30 (2.94)	3.60 (0.25)	96 (51-179)	0.901
t _{1/2} (min)	1.24 (0.36)	0.86 (0.43)	65 (37-112)	0.177

AUCinf ($\mu\text{L}/\text{mL}\times\text{min}$) $\times 10^3$	8.97 (2.30)	6.38 (2.11)	70 (46-107)	0.153
CL (L/hr)	2079 (907)	2675 (1819)	122 (58-254)	0.626
Vss (L)	88 (45) 80 (29)	99 (46-213)	-	0.989
CL _{lung} (L/hr) ^b	1383 (827)	437 (224)	-	0.084

a t_{max} is presented as median (range).

^b Ratio and 90% CI was not presented as there were only 2 females included in the normal group.

GLS=Geometric least squares mean

Table 4. PFP Ratios of Pharmacokinetic Parameters - All Males Versus All Females

	All Male Mean (SD)	All Female Mean (SD)	Female/Male ^a GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	11	8		
t _{max} (min) ^a	2.17 (1.7-3.17)	1.17 (1.17-1.67)*		0.005
C _{max} ($\mu\text{L}/\text{mL}$) $\times 10^3$	2.63 (1.78)	4.04 (2.26)	167 (99-281)	0.105
t _{1/2} (min)	2.08 (1.80)	1.10 (0.41)	63 (37-107)	0.147
AUCinf ($\mu\text{L}/\text{mL}\times\text{min}$) $\times 10^3$	7.71 (3.49)	8.0 (2.47)	103 (74-143)	0.879
CL (L/hr)	2907 (829)	2302 (1229)	74 (52-106)	0.159
Vss (L)	200 (121)	85 (37)*	47 (29-75)	0.012
CL _{lung} (L/hr) ^b	1524 (687)	1113 (823)*	49 (27-87)	0.044

*p < 0.05; a Adjusted for group differences; b t_{max} is presented as median (range)

c for the comparison of CL_{lung} 10 male subjects and 7 female subjects were used; GLS = Geometric least squares mean

Table 5. Blood PFP Pharmacokinetic Parameters - Normal Males Versus Normal Females

	Normal Male Mean (SD)	Normal Female Mean (SD)	Female/Male GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	3	5		
t _{max} (min) ^a	1.67 (1.17-2.17)	1.17 (0.67-1.67)		0.124
C _{max} ($\mu\text{L}/\text{mL}$) $\times 10^3$	2.43 (0.71)	4.30 (2.94)	159 (81-312)	0.228
t _{1/2} (min)	1.34 (0.54)	1.24 (0.36)	94 (62-145)	0.802
AUCinf ($\mu\text{L}/\text{mL}\times\text{min}$) $\times 10^3$	6.71 (1.37)	8.97 (2.30)	132 (94-186)	0.168
CL (L/hr)	3151 (801)	2079 (907)	62 (35-110)	0.154
Vss (L)	152 (40)	88 (45)	52 (25-110)	0.140
CL _{lung} (L/hr) ^b	1716 (1395)	1383 (827)		0.787

a t_{max} is presented as median (range); ^b ratio and 90% CI was not presented as there were only 2 males in the normal group.

GLS=Geometric least squares mean

Table 6. Blood PFP Pharmacokinetic Parameters COPD Males Versus COPD Females

	COPD Male Mean (SD)	COPD Female Mean (SD)	Female/Male GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	8	3		
t _{max} (min) ^a	2.17 (1.17-3.17)	1.17 (1.17-1.67)		0.055
C _{max} ($\mu\text{L}/\text{mL}$) $\times 10^3$	2.70 (2.09)	3.60 (0.25)	174 (73-414)	0.272
t _{1/2} (min)	2.35 (2.05)	0.86 (0.43)	44 (18-111)	0.138

AUCinf ($\mu\text{L}/\text{mL} \times \text{min} \times 10^3$)	8.09 (4.03)	6.38 (2.11)	83 (48-143)	0.548
CL (L/hr)	2815 (874)	2675 (1819)	87 (52-145)	0.622
V _{ss} (L)	218 (138)	80 (29)	42 (20-87)	0.057
CL _{lung} (L/hr) ^b	1476 (562)	437 (224)*		0.007

*p \leq 0.05; a tmax is presented as median (range); b ratio and 90% CI was not presented as there were only 2 females in the COPD group.
GLS=Geometric least squares mean

Table 7. % Dose excreted : Ae (0-5 minutes continuous collection)

Normal Subjects

	Male	Female	All Subjects
N	5	6	11
Mean	36.44	60.82	49.74
SD	24.39	18.05	23.72
Min			
Max			

COPD Subjects

	Male	Female	All Subjects
N	9	3	12
Mean	52.21	32.94	48.71
SD	12.92	22.17	15.60
Min			
Max			

4. PHARMACOKINETICS OF LIPID COMPONENTS, THE MICROSPHERE SHELL

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. The Applicant stated that substances are endogenous lipids and are well characterized. In addition, MPEG5000 was not evaluated; however, extensive PK studies have been previously performed in rats. However, although it is reasonable not to perform any pharmacokinetic or metabolism studies on DPPC, DPPA and MPEG5000 in humans, this Reviewer clearly stated during the pre-NDA Meeting that the Applicant needs to submit any supportive information regarding lipids and MPEG5000. Therefore, the Applicant still needs to submit the requested information. As far as MPEG5000 is concern, this Reviewer directs one to the Pharm/Tox Review for input on its PK and metabolism in rats.

Note: DPPA:
DPPC:
MPEG5000 DPPE:

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COMMENTS TO THE APPLICANT

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

1. PFP GAS PROTEIN BINDING INFORMATION

It appears that the NDA package lacks PFP gas protein binding and distribution information. The Applicant is encouraged to provide this information.

2. PHARMACOKINETICS OF INTACT MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of DMP 115, i.e., the "intact PFP-filled" microbubbles. Ideally "intact" microbubble pharmacokinetic information is needed with respect to the dosage proposed in the package insert. The Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method in order to definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. In addition, the Applicant is encouraged to explore in vitro methods to provide information on the microbubbles in terms of microbubble "fragility and stability."

One such in vitro method that can be explored is the microbubble "fragility" test: Addition of the microbubbles in blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. Furthermore, the Applicant may explore relevant animal models (e.g., microscopic examination of nailbed capillary or cannulated cat mesenteric artery), if any. The Applicant is encouraged to correspond with the Pharm/Tox review team to explore the feasibility of using animal models to obtain microsphere fragility information. Once such information is obtained the data/information should be submitted to the agency for review.

3. PHARMACOKINETICS OF LIPID COMPONENTS, THE MICROSPHERE SHELL

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. The Applicant stated that substances are endogenous lipids and are well characterized. In addition, MPEG5000 was not evaluated; however, extensive PK studies have been previously performed in rats. Although it is reasonable not to perform any PK or metabolism studies on DPPC, DPPA and MPEG5000 in humans, it was raised during the pre-NDA Meeting that supportive information regarding lipids and MPEG5000 should be submitted. This information is still requested.

4. PFP GAS MASS-BALANCE INFORMATION

Please submit any 'mass balance information' on DMP 115 microbubbles.

5. PFP GAS ASSAY INFORMATION

The Applicant did not submit any assay information regarding PFP gas,
Please submit this information.

RECOMMENDATION

The Human Pharmacokinetics and Bioavailability section of NDA 21-064, for Definity that was submitted by the Applicant on 12/8/98 has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II (OCPB/DPE II; HFD-870).

The current NDA submission, The Human Pharmacokinetics and Bioavailability Section, contains minimal pharmacokinetic information and negligible PK/PD data. Ideally "intact" microbubble pharmacokinetic information should have been provided with respect to the dosage proposed in the package insert. In order to obtain this information in the future, the Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method so it could definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. Once such information is obtained the data/information should be submitted to the agency for review.

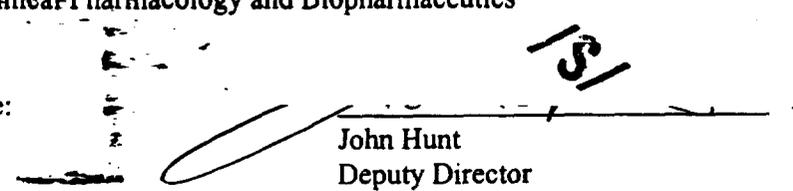
Regarding the approval status of the application, this submission is considered approvable. The items covered under 'Comments to the Applicant' section and the Labeling Comments (to be covered under a separate review) should be conveyed to the Applicant as appropriate.



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

2/31/99

Concurrence:



John Hunt
Deputy Director
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

3/3/99

Clinical Pharmacology/Biopharmaceutics Briefing: Attendees: John Hunt, Peter Honig, Nakissa Sadrieh,
8/30/99

APPENDIX I

Summary of Individual Studies

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Study results:

I. 900 Study (Attachment: Synopsis): The recommended dose for Definity is a single dose of 10 µl/kg

Title: *A Phase I Study to Determine the Safety and Tolerance of Single Rising Doses of MRX-115, an Intravenous Ultrasound Contrast Agent, in Healthy Adult Male Subjects*

Study 900 was a randomized, single-center, single-blind, placebo-controlled, single ascending dose trial in 30 healthy men (18-45 years of age) to assess degree of contrast opacification due to the following doses: 5, 10, 20, 50 and 100 µl/kg. Three to 4 subjects were administered at each dosing level. Efficacy was assessed by:

1. An evaluation of the following two echocardiography endpoints:
 - A. Visual scoring for contrast opacification in the left ventricular cavity and for the septal, lateral, medial, and posterior walls of the left ventricle (myocardial walls), and
 - B. Changes from baseline in videodensitometry measurements for the left ventricular cavity and each of the myocardial walls.
2. Contrast opacification was scored independently by two blinded readers, and changes from baseline in the videodensitometry measurements were provided by one blinded reader.

Safety: The Applicant indicated that there was no clinically significant changes form baseline for any subject for any hematology parameter, serum chemistry parameter, or immunology test. There were no clinically significant ECG findings, nor were there any clinically significant findings during physical and neurological examinations. No deaths or serious AEs were reported during the trial. Seven subjects experienced a total of 10 AEs; the majority was mild or moderate in severity and all resolved. Two subjects in the 5 µl/kg group reported 4 AEs (two of headaches and one each of tachycardia and vasodilation). One subject in the 10 µl/kg group reported one AE of headache. One subject in the 50 µl/kg group reported an AE of throat infection. No AEs were reported in the 20 or 100 µl/kg groups.

Efficacy: The Applicant indicated that in general, the mean visual scores for contrast opacification were higher in the drug groups ($p < 0.05$) compared with placebo.

Region	Dose and readers
Left Ventricle	At 5 µl/kg – 1 reader, and at 10, 20, 50 and 100 µl/kg – 2 readers
Septum	At 5 µl/kg – 1 reader, and at 20, 50 and 100 µl/kg – 2 readers
Lateral Wall	At 50 µl/kg – 1 reader, and at 100 µl/kg – 2 readers
Posterior Wall	At 20 µl/kg – 2 readers, and at 50 µl/kg – 1 reader, and at 100 µg/kg – 2 readers
Medial Wall	At 5 and 50 µl/kg – 1 reader, and at 100 µl/kg – 2 readers

Again, in general, the mean changes from baseline in videodensitometry were higher in the drug groups ($p < 0.05$) compared with placebo.

Region	Dose and readers
Left Ventricle	At 5, 10, 20, 50 and 100 µl/kg

Septum	At 20, 50 and 100 $\mu\text{l/kg}$
Lateral Wall	At 10, 20 and 50 $\mu\text{l/kg}$
Posterior Wall	At 10 and 20 $\mu\text{g/kg}$
Medial Wall	At 50 $\mu\text{l/kg}$

In summary:

1. Left ventricular cavity enhancement was demonstrated at all dose levels tested. In addition, myocardial tissue (myocardial wall) opacification was also observed by Reader 1 at all dose levels;
2. Reader 2 only observed opacification at the higher ($\geq 20 \mu\text{l/kg}$) dose levels.

Conclusion: A dose-related response was not observed in relation to opacification, indicating an ability to observe maximum enhancement at all dose levels.

Reviewer's Comment: *It should be noted that each dosing group had 3 – 4 subjects. Variability among the two-blinded readers was observed. There may be inherent variability associated with the current imaging modality as well. This Reviewer recommends that Reviewing Statistician may review this study for validity of the Applicant's claim. However, this reviewer concurs with the Applicant's conclusion that there was no dose-related response in relation to opacification.*

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II. 901 Study (Attachment: Synopsis): The recommended dose for Definity is a single dose of 10 µl/kg

Title: *A Phase I Study to Determine the Safety and Tolerance of multiple Rising Doses of MRX-115, an Intravenous Ultrasound Contrast Agent, in Healthy Adult Male Subjects*

Synopsis: Study 901 was a randomized, single-center, single-blind, placebo-controlled, multiple administration, ascending dose trial in 18 healthy men (18-45 years of age) to assess degree of contrast opacification due to the following doses: 5, 10, 15, 30 µl/kg. In addition, 4 healthy men were rechallenged with intradermal skin testing at 50 µl, followed by 10 µl/kg and 90 µl/kg. Two to 4 subjects were administered at each dosing level. Efficacy was assessed by:

1. An evaluation of the following two echocardiography endpoints:
 - A. Visual scoring of left ventricular cavity enhancement;
 - B. Visual scoring for myocardial enhancement of each wall (antero-septal, anterior, antero-lateral, infero-lateral, inferior, and infero-septal);
 - C. Visual scoring of endocardial border delineation by wall for each patient, as well s global improvement (overall);
 - D. Changes from baseline in ventricular cavity end-diastolic videodensitometric measurements;
 - E. Changes from baseline in myocardial wall videodensitometric measurements.
2. The first 4 assessments were scored independently by two blinded readers, and one blinded reader provided the changes from baseline in the myocardial wall videodensitometry measurements.

Safety: The Applicant indicated that there were no deaths or serious AEs were reported during the trial. Two subjects experienced a total of two AEs during the main study; one subject had a frontal headache (15 µl/kg) and another subject had an abdominal urinalysis result (5 µl/kg).

Efficacy: The Applicant stated the following:

1. In general, the % of patients who demonstrated optimal enhancement of the left ventricle were higher than those of placebo;
2. Evaluation of myocardial wall enhancement was limited, due to the dosing strategy and the use of fundamental imaging;
3. Variability among the two blinded readers was observed for endocardial border delineation score;
4. For both ventricles, the magnitude of increase in signal intensity measured by videodensitometry following the first injection was larger for the dose groups compare to placebo;
5. Based on the results, doses 5 to 15 µl/kg are recommended for further examination of cavity opacification.

In summary:

1. AE events are considered not serious;
2. Due to the small number of subjects, it was not possible to identify any dose-related trends in any of the data collected in this study.

Conclusion: It was not possible to identify any dose-related trends in any of the data collected in this study.

Reviewer's Comment: *This reviewer concurs with the Applicant's conclusion that it was not possible to identify any dose-related trends in any of the data collected in this study due to the small number of subjects utilized.*

III. 905 Study (Attachment: Synopsis): The recommended dose for Definity is a single dose of 10 µl/kg

Title: *A Phase I, Open-Label Evaluation of the Pharmacokinetics of Perfluoropropane Following the Intravenous Administration of DMP 115 in Normal Subjects and Subjects with Chronic Obstructive Pulmonary Disease*

Synopsis: Study 905 was a Phase I, open-label, safety and pharmacokinetics study in 12 healthy men and women and 12 COPD patients. All subjects received a single I.V. bolus, 50 µl/kg dose. Whole blood and expired air were sampled at baseline and at frequent intervals up to 15 minutes following administration. PFP gas concentrations in expired air and blood were determined using a validated gas chromatographic method. Optional Doppler ultrasound measurements were performed immediately pre-dose, and at frequent intervals up to 20 minutes post-dose, for generation of a relative blood microbubble Doppler intensity-time curve for comparison to blood concentrations of PFP.

Dose selection: 50 µl/kg was selected since the recovery of PFP at a clinically relevant dose was desired. The Applicant acknowledged the challenge of PFP quantitation inherent to this study; a dog study showed PFP recovery at doses less than 100 µl/kg was at or below the level of quantitation.

PK and metabolism of the lipid components:

DPPC and DPPA were not evaluated in this study since both substances are endogenous lipids and are well characterized. In addition, MPEG5000 was not evaluated; however, extensive PK studies have been previously performed in rats.

Reviewer's Comment: *Although the Applicant's proposal is reasonable not to perform any PK or metabolism studies on DPPC and DPPA, this Reviewer clearly stated during the pre-NDA Meeting that the Applicant needs to submit any supportive information regarding DPPC and DPPA. As far as MPEG5000 is concern, the reader should refer to Pharm/Tox Review for input on its PK and metabolism in rats.*

Subjects: Healthy 6 men and 6 women (18 years of age or older); approximately equal numbers of men and women COPD patients (e.g., emphysema or bronchitis). Resting forced expiratory volume over 1 second (FEV1) was to be <70% of predicted and baseline SaO₂ was to be ≥ 90%.

Note:

DPPA:

DPPC:

MPEG5000 ~~DPPC~~

Blood sampling: At baseline, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 10 and 15 minutes post injection, relative to the start of the injection (not the end of the injection); each sample was immediately transferred to an individual glass vial.

Expired air sampling: At baseline, all expired air during the first 30-sec, the 2nd 30 seconds, and the post injection intervals 1-2, 2-3, 3-4, 4-5, 10-11, 15-16 minutes post injection. Three 8-ml air samples were extracted from each bag and injected into individual glass vials.

Reviewer's Comment: *According to Appendix E (Volume 38), cumulative elimination of PFP in expired air was plotted between 0 – 5 minutes post administration. From the majority of plots, at 5 minutes post injection, it appears that PEP gas is still being eliminated, i.e., cumulative slope is still at a rising phase. This Reviewer feels that the sampling scheme was not appropriate to capture the PFP lung expiration data. Hence, the mass balance of PFP is difficult to obtain from the data set, if possible.*

Doppler Ultrasound: Recordings of auditory signals from a Doppler ultrasound probe, placed over the radial artery, were obtained within 2 hours prior to injection and continued for 15 minutes post injection or until the signal returned to baseline, whichever was longer.

The data were saved in analog format at the sampling. The data were later played back at the same speed of recording, which the signals from the audio output were input into Dynamic Data Acquisition Board (the board's capability of sampling at 51.2 kHz). was used to sample the signal continuously at 5 Hz. The transferred Doppler data files were imported into Excel and the Doppler amplitude envelope was prepared by determining the maximum response for every 6 second segment of data. The resulting Doppler vs. time data was used for estimation of maximum signal intensity and time to maximum signal intensity (t_{max}) for each subject. Additionally, data were averaged over 30 second intervals (5 data points per 30 second interval) and the resultant average Doppler signal intensity vs. time data were used to determine an approximate time interval for a 20 dB decrease in signal intensity, representing a signal decrease of 99%, i.e., each 10 dB decrease was equal to a 90% signal decrease.

PK parameters: Blood – t_{max}, C_{max}, AUC, AUC_{last}, AUC_{inf}, t_{1/2}, CL, V_{dss}
Expired air – cumulative excretion of PFP in expired air, CL_{lung}

PK/PD analysis:

Non-compartmental methods using was used to estimate the PK parameters. Statistical analyses were carried out using 5 and/or SAS version Following log_e-transformation of the data, parameters were analyzed using ANOVA with terms for study group (normal or COPD) and gender in the model. Geometric least squares mean ratios and 90% CI were calculated. In addition, median t_{max} will be compared with non-parametric Wilcoxon's Rank Sum Test. The Hodges-Lehman estimator associated with the Wilcoxon's Rank Sum Statistics for the median difference and the corresponding distribution free CI were calculated.

In addition to obtaining parameters, an attempt was to be made to develop a model for the simultaneous fitting of the blood and expired air concentration data. The data from the Doppler probe, if obtained, were to be used to generate a Doppler-signal-intensity time curve for comparison to blood concentration of PFP.

% Dose excreted via the lungs - cumulative amount PFP in air / Dose_{PFP}

DosePFP - Estimated to be 3.85 $\mu\text{l}/\text{kg}$, based on the conclusion of a study, DPDG/GCS/13, performed to evaluate the conc. of PFP gas per ml of drug at 15 min. post-shaking. The study concluded that the mean amount of PFP gas/ml was 77 $\mu\text{l}/\text{ml}$ (Appendix G.1). Since the dose administered in this study was 50 $\mu\text{l}/\text{kg}$, the actual PFP dose administered corresponded to 3.85 $\mu\text{l} * \text{kg}$.

Reviewer's Comment: A consult was placed to Chemistry Reviewer in order to obtain concentration information from DPDG/GCS/13 document. Per package insert, it should be noted that PFP gas concentration is 150 $\mu\text{l}/\text{ml}$.

Reviewer's Comment: There is a possible concern relating to V_{dss} calculation. The equation proposed by the Applicant is : $V_{dss} = \text{DosePFP} * \text{AUMC}_{inf}/\text{AUC}_{inf}$. This equation presumes that 1) the system must respond linearly, i.e., follow first-order kinetics, 2) elimination of drug from the body must be directly from the plasma or central compartment. PFP elimination may be due to multiple routes.

Pharmacodynamic Analysis:

Originally, the statistical analysis plan specified that PK/PD modeling of the blood PFP concentration and Doppler data would be performed and that a suitable PD model (linear, log-linear, or E_{max}) would be fit to the Doppler data.

Efficacy Assessment: The diagnostic efficacy was not evaluated in this study. However, the ability of the drug to enhance Doppler ultrasound was utilized as a non-invasive method to assess the time course of ultrasound signal enhancement, post drug administration.

Amendments to the original protocol:

1. Changed the rate of administration of the drug to deliver over a 30 sec., rather than 10 sec.; changed the rate of administration of the 10 ml saline flush to deliver 10 ml saline over 30 sec.;
2. Expanded the inclusion criterion for COPD subjects to an acceptable window for the FEV1 from the previous cutoff of >35% to <70%, to the new window of $\geq 30\%$ to <70%.
3. Added the following exclusion criterion for COPD subjects: if they had received systemic steroids, or used inhalers greater than for times per day, or had undergone a recent change in their study medication, they would be excluded from study participation.
4. Changed the instructions for preparation of study drug to read, 'the vial should be allowed to stand for 15 minutes prior to withdrawing the dose into a syringe to allow for non-encapsulated gas to return to the headspace'.

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Results:

1. Study Subjects:

Table 1. Subject Study Summary

Subject Number	Study Group	DMP 15 Injection Time (sec)	Pharmacokinetic Analysis	Statistical Analysis	Pharmacodynamic Analysis
1	Normal	10	Na	N	Y
2	Normal	10	Na	N	Y
3	Normal	10	Na	N	Y
4	Normal	10	Yb	Y	Y
5	Normal	10	Y	Y	Y
6	Normal	10	Y	Y	Y
7	Normal	10	Y	Y	Nc
8	COPD	10	Yb	Y	Y
9	Normal	10	Y	Y	Y
10	Normal	10	Y	Y	Y
11	Normal	30	Y	Y	Y
12	Normal	30	Y	Y	Y
13	COPD	30	Y	Y	Y
14	COPD	30	Y	Y	Y
15	Normal	30	Na	N	Y
16	COPD	30	Y	Y	Y
17	COPD	30	Y	Y	Y
18	COPD	30	Y	Y	Y
19	COPD	30	Y	Y	Y
20	COPD	30	Y	Y	Y
21	COPD	30	Y	Y	Y
22	COPD	30	Y	Y	Y
23	COPD	30	Y	Y	Y
24	COPD	30	Na	N	Y

N-No, Y-Yes; a Had only Two (or less) detectable PFP blood concentrations; b Unable to determine CL_{low} ; c Incomplete Doppler data

2. Comparison of Normal versus COPD Subjects

All Subjects

The concentration-time curves of **blood PFP** for both normal subjects and COPD subjects showed a delayed but relatively a rapid rise to C_{max} for most study subjects (Table 2). After reaching C_{max} , PFP concentrations declined in a log-linear fashion for both normal and COPD subjects. Average concentration-time profiles for all subjects were similar between both groups (normal versus COPD). It should be noted that due to the low PFP concentrations obtained in this study, PFP concentrations were below detection limits in many blood samples; subsequently, the terminal slope of the individual PFP blood concentration-time curves in most subjects used only 3 points to estimate λ_z .

No statistically significant differences were observed in the comparison of normal versus COPD subjects,

There was a slight increase for COPD subjects in median t_{max} (difference of 0.50 min, perhaps due to

differences in injection times, 10 vs. 30 sec.); however, this was not different statistically for COPD subjects compared to normal subjects. CL_{lung} was 40% lower, on average, in COPD subjects compared to normal subjects. However, this difference was not statistically significant.

Table 2. Blood PEP Pharmacokinetic Parameters - All Normal Subjects versus COPD Subjects

Pharmacokinetic Parameter	All Subjects Normal Mean (SD)	All Subjects COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	8	11		
Tmax (min) ^a	1.17 (0.67-2.17)	1.67 (1.17-3.17)		0.055
Cmax ($\mu\text{L}/\text{mL}$) x 10 ³	3.60 (2.46)	2.95 (1.80)	91 (54-154)	0.768
t _{1/2} (min)	1.28 (0.40)	1.95 (1.86)	97 (57-166)	0.932
AUC _{inf} ($\mu\text{L}/\text{mL}\times\text{min}$) x 10 ³	8.12 (2.22)	7.62 (3.59)	90 (65-124)	0.571
CL (L/hr)	2481 (980)	2777 (1096)	102 (71-144)	0.940
V _{ss} (L)	112 (52)	181 (133)	112 (70-179)	0.692
CL _{lung} (L/hr) ^c	1478 (898)	1268 (665)	60 (33-107)	0.141

^a tmax is presented as median (range); ^b Adjusted for sex differences;

^c For the comparison of CL_{lung}, 7 normal subjects and 10 COPD subjects were used.

GLS = Geometric least squares mean

Males

On average, male COPD PEP concentrations were consistently higher than normal male subjects. However, it should be noted that there was a large variability associated with these concentration-time curves. No statistically significant differences were observed. A summary of the selected blood PEP pharmacokinetic parameters along with the percent ratio and 90% CI for COPD males compared to normal males is shown in Table 3.

Table 3. Blood PEP Pharmacokinetic Parameters - Normal Males Versus COPD Males

Pharmacokinetic Parameter	Male Normal Mean (SD)	Male COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	3	8		
tmax (min) ^a	1.67 (1.17-2.17)	2.17 (1.17-3.17)		0.329
Cmax ($\mu\text{L}/\text{mL}$) x 10 ³	2.43 (0.71)	2.70 (2.09)	88 (36-213)	0.794
t _{1/2} (min)	1.34 (0.54)	2.35 (2.05)	138 (57-334)	0.518
AUC _{inf} ($\mu\text{L}/\text{mL}\times\text{min}$) x 10 ³	6.71 (3.37)	8.09 (4.03)	111 (67-185)	0.715
CL (L/hr)	3451 (801)	2815 (874)	87 (59-129)	0.532
V _{ss} (L)	152 (40)	218 (138)	123 (60-251)	0.607
CL _{lung} (L/hr) ^b	1716 (1395)	1476 (562)	-	0.969

^a tmax is presented as median (range).

^b Ratio and 90% CI was not presented as there were only 2 males included in the normal group.

GLS=Geometric least squares mean

Females

Female COPD PFP concentrations were slightly higher than normal female subjects on average. As was the case in the previous comparisons (male subjects), a large variability was observed for the mean concentration-time profiles. Lung clearance of PFP appeared to be reduced by 66% in females with COPD compared to normal females, but proved not to be statistically significant (p-value < 0.084). However, it should be noted that in this comparison there were a limited number of females in the COPD group (n=2 for COPD CL_{lung} calculations). Subsequently, comparison of PFP CL_{lung} should be done with caution. A summary of the selected blood PFP pharmacokinetic parameters along with the percent ratio and 90% CI for COPD females compared to normal females is shown in Table 4.

Table 4. Blood PFP Pharmacokinetic Parameters - Normal Females versus COPD Females

Pharmacokinetic Parameter	Female Normal Mean (SD)	Female COPD Mean (SD)	COPD/Normal GLS Ratio % (90% CI)	F-Test p-value
Number of Subjects	5	3		
t _{max} (min) ^a	1.17 (0.67-1.67)	1.17 (1.17-1.67)		0.337
C _{max} (μL/mL) x 10 ³	4.30 (2.94)	3.60 (0.25)	96 (51-179)	0.901
t _{1/2} (min)	1.24 (0.36)	0.86 (0.43)	65 (37-112)	0.177
AUC _{inf} (μL/mLxmin) x 10 ³	8.97 (2.30)	6.38 (2.11)	70 (46-107)	0.153
CL (L/hr)	2079 (907)	2675 (1819)	122 (58-254)	0.626
V _{ss} (L)	88 (45) 80 (29)	99 (46-213)	-	0.989
CL _{lung} (L/hr) ^b	1383 (827)	437 (224)	-	0.084

^a t_{max} is presented as median (range).

^b Ratio and 90% CI was not presented as there were only 2 females included in the normal group.

GLS=Geometric least squares mean

3. Comparison of Male versus Female Subjects

All Subjects

Mean concentrations of females were consistently higher compared to males. For all subjects, the mean total blood clearance of PFP was approximately 26% lower on average in females than in males; the difference was not statistically significant. This slight decrease in systemic blood clearance resulted in a 67% higher C_{max} with only a 3% higher AUC in females on average; these differences were also not significant.

T_{max} was shown to be significantly reduced in females compared to males (p-value 0.01). V_{ss} was also shown to be decreased in females compared to males (p-value = 0.01). On average, in all subjects (male and female) CL_{lung} was shown to be significantly lower (51%) in females compared to males (p-value = 0.05).

A summary of the percent ratio and 90% CI for PFP pharmacokinetic parameters in all subjects (females compared to males) is shown in Table 5.

Table 5. PFP Ratios of Pharmacokinetic Parameters - All Males Versus All Females

	All Male Mean (SD)	All Female Mean (SD)	Female/Male ^a GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	11	8		
t _{max} (min) ^a	2.17 (1.7-3.17)	1.17 (1.17-1.67)*		0.005
C _{max} (μL/mL) x 10 ³	2.63 (1.78)	4.04 (2.26)	167 (99-281)	0.105
t _{1/2} (mm)	2.08 (1.80)	1.10 (0.41)	63 (37-107)	0.147
AUC _{inf} (μL/mLxmin) x 10 ³	7.71 (3.49)	8.0 (2.47)	103 (74-143)	0.879
CL (L/hr)	2907 (829)	2302 (1229)	74 (52-106)	0.159
V _{ss} (L)	200 (121)	85 (37)*	47 (29-75)	0.012
CL _{lung} (L/hr) ^b	1524 (687)	1113 (823)*	49 (27-87)	0.048

*p < 0.05; a Adjusted for group differences; b t_{max} is presented as median (range)

c for the comparison of CL_{lung} 10 male subjects and 7 female subjects were used; GLS = Geometric least squares mean

Normal Subjects

Mean concentrations for males were consistently lower than females throughout PFP blood sampling; there was a large standard error associated with most time points.

While no statistically significant differences were observed for all pharmacokinetic parameters (C_{max}, AUC_{inf}, t_{1/2}, CL, V_{ss} or CL_{lung}), total blood PEP clearance was approximately 38% lower on average in females than in males. Similarly, PFP C_{max} and AUC_{inf} were increased (59% and 32%, respectively) in females compared to males. In contrast, a shorter median (difference) t_{max} (-0.50 mm) was noted for females compared to males. Lung clearance was shown to be similar in males and females, 1716 versus 1383 L/hr, respectively. A summary of the selected blood PFP pharmacokinetic parameters along with the percent ratios and 90% CI for females compared to males is shown in Table 6.

Table 6. Blood PFP Pharmacokinetic Parameters - Normal Males Versus Normal Females

	Normal Male Mean (SD)	Normal Female Mean (SD)	Female/Male GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	3	5		
t _{max} (min) ^a	1.67 (1.17-2.17)	1.17 (0.67-1.67)		0.124
C _{max} (μL/mL) x 10 ³	2.43 (0.71)	4.30 (2.94)	159 (81-312)	0.228
t _{1/2} (mm)	1.34 (0.54)	1.24 (0.36)	94 (62-145)	0.802
AUC _{inf} (μL/mLxmin) x 10 ³	6.71 (1.37)	8.97 (2.30)	132 (94-186)	0.168
CL (L/hr)	3151 (801)	2079 (907)	62 (35-110)	0.154
V _{ss} (L)	152 (40)	88 (45)	52 (25-110)	0.140
CL _{lung} (L/hr) ^b	1716 (1395)	1383 (827)		0.787

a t_{max} is presented as median (range); b ratio and 90% CI was not presented as there were only 2 males in the normal group.

GLS=Geometric least squares mean

COPD Subjects

Similar to the above comparison, mean PEP concentrations for male subjects were lower than their female counterparts. No statistically significant differences were observed. Total blood PFP clearance was shown to be no different between males and females with COPD (2815 versus 2675 L/hr). As in the previous comparison (normal subjects), Cmax was shown to be increased by 74% on average in females compared to males. However, again, this difference was not statistically significant (p-value = 0.27).

A noteworthy shorter median (difference) tmax (-0,50 mm) was seen in females compared to males (p-value = 0.06). Also, there was a trend for Vss (p-value = 0.06) to be decreased in females compared to males with COPD. However, females had a significantly lower CL_{lung} than their COPD male counterparts. It should be noted that in this comparison there were a limited number of females in the COPD group (n=2 for COPD CL_{lung} calculations). Subsequently, comparison of PFP CL_{lung} should be done with caution. A summary of the selected blood PEP pharmacokinetic parameters along with the percent ratio and 90% CI for females compared to males is shown in Table 7.

Table 7. Blood PFP Pharmacokinetic Parameters COPD Males Versus COPD Females

	COPD Male Mean (SD)	COPD Female Mean (SD)	Female/Male GLS Ratio % (90% CI)	F-test p-value
Number of Subjects	8	3		
tmax (min) ^a	2.17 (1.17-3.17)	1.17 (1.17-1.67)		0.056
Cmax (μL/mL) x 10 ³	2.70 (2.09)	3.60 (0.25)	174 (73-414)	0.272
t½ (min)	2.35 (2.05)	0.86 (0.43)	44 (18-111)	0.138
AUCinf (μL/mLxmin) x 10 ³	8.09 (4.03)	6.38 (2.11)	83 (48-143)	0.548
CL (L/hr)	2815 (874)	2675 (1819)	87 (52-145)	0.622
Vss (L)	218 (138)	80 (29)	42 (20-87)	0.057
CL _{lung} (L/hr) ^b	1476 (562)	437 (224)*		0.007

*p<0.05; a tmax is presented as median (range); b ratio and 90% CI was not presented as there were only 2 females in the COPD group.
GLS=Geometric least squares mean

4. Statistic analysis concerns:

The following parameters are noteworthy with p-values:

- a. female/male in COPD Subjects CL_{lung} - 0.007
- b. female/male in All subjects tmax - 0.005; Vss - 0.012; CL_{lung} - 0.048
- c. COPD/Normal females CL_{lung} - 0.084
- d. COPD/Normal all subjects tmax - 0.055

Reviewer's Comment: *Although above parameters appear to be statistically different when compared, some of the differences are due to variability associated with limited number of subjects in that particular group, e.g., number of females in the COPD group (n=2 for COPD CL_{lung} calculations). Therefore, a caution should be exercised in interpreting above results. The data may be inconclusive in detecting gender differences due to small number of subjects. However, overall, it appears that there are no gender differences.*

5. % Dose excreted: Ae (0-5 minutes continuous collection)

Normal Subjects

	Male	Female	All Subjects
N	5	6	11
Mean	36.44	60.82	49.74
SD	24.39	18.05	23.72
Min			
Max			

COPD Subjects

	Male	Female	All Subjects
N	9	3	12
Mean	52.21	32.94	48.71
SD	12.92	22.17	15.60
Min			
Max			

Reviewer's Comment: From the majority of plots, at 5 minutes post injection, it appears that PEP gas is still being eliminated, i.e., cumulative slope is still at a rising phase. This Reviewer feels that the sampling scheme was not appropriate to capture the PFP lung expiration data. Hence, the mass balance of PFP is difficult to obtain from the data set, if possible. This is based on the conclusion of a study, DPDG/GCS/13. The study concluded that the mean amount of PFP gas/ml was 77 $\mu\text{l/ml}$ (Appendix G.1). Since the dose administered in this study was 50 $\mu\text{l/kg}$, the actual PFP dose administered corresponded to 3.85 $\mu\text{l} * \text{kg}$. However, per package insert, it should be noted that PFP gas concentration is 150 $\mu\text{l/ml}$.

6. Mean Change from baseline Doppler Signal-Time Profile

The Applicant reported Doppler Signal intensity. On average the signal intensity increased to 30dB with microbubbles at approximately 1 – 2 minutes post injection. The profile indicated that intensity returns to the baseline gradually, reaching the baseline at approximately 10 minutes (evaluation of the time for a 99% decrease in Doppler intensity).

Conclusion: Pharmacokinetic parameters are presented for normal and COPD subjects. No gender differences were noted.

Reviewer's Comment: This reviewer concurs with the Applicant's conclusion that gender differences are not noted in the study.

APPEARS THIS WAY
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APPEARS THIS WAY
ON ORIGINAL

Abuse Liability Review:

N/A

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL