

The following observations were made during the gestation period: mortality and clinical observations, body weight and food consumption. At the time of cesarean section, the pregnant females were weighed and examined for gross alterations. The uterus and ovaries were examined for implantation and corpora lutea, early and late resorptions were determined. Fetuses were sexed, weighed and examined for external abnormalities. The fetuses were also examined for soft tissue abnormalities and skeletal abnormalities according to established procedures. Variations and malformations were reported. All data were statistically analyzed.

Results: Three high-dose females were found dead on days 16, 17 and 19 of gestation. After dosing, these animals had tremors, muscle rigidity, ataxia, erythema, convulsions, hypoactivity, prostration and/or dyspnea. These symptoms were also seen immediately after dosing in 6 other high-dose females and one mid-dose female that survived till necropsy.

Mean body weights and body weight changes were similar among groups, with minor non-significant incidental changes that were not treatment-related due to their lack of consistency and dose-responsiveness.

Mean food consumption values were generally similar among groups. Mean values for the mid and high-dose groups were significantly higher at several time points throughout gestation when compared with the placebo, but not when compared with the saline control.

Other than a pale liver in a placebo female and pale lungs in 2 high-dose females that were found dead, there were no significant observations upon gross necropsy.

Mean gravid uterine weight, corrected terminal weight, and net body weight change from day 0 were similar among groups.

Pregnancy rates were 95, 100, 95, 95 and 100% for groups 1-5. No dead fetuses were found in any of the groups. The mean number of corpora lutea, implantation sites, resorptions and live fetuses were similar among groups. One female in the mid-dose group had no viable fetuses. Preimplantation loss values were generally higher among groups although these appeared slightly higher for groups 3 and 4.

No fetal external variations were noted.

Soft tissue malformations consisted of malformations of the heart and/or greater vessel in one placebo and mid-dose group fetus. Additionally, one placebo group fetus had the above malformations plus renal agenesis.

Fetal skeletal malformations consisted of one fetus in each of the groups (except the saline control group) with vertebral anomaly with/without associated rib anomaly, one fetus in groups 3 and 4 with fused thoracic centra, one fetus in group 1 and 2 with major fusion of the sternabrae and one fetus in group 5 with micromelia. Another group 5 fetus also had a vertebral anomaly with/without associated rib anomaly and a major fusion of sternabrae and fused ribs. No statistical trends were found in these observations, however, fetal and litter incidences of total skeletal malformations in group 5 were generally higher than those of group 1 and 2.

Conclusions: Doses of 0.5, 2.5 and 7.5 ml/kg were used from days 7-20 of gestation in New Zealand White rabbits. Two control groups including a 0.9% saline and a placebo group dosed with 7.5 ml/kg were used. On day 29 of gestation, all surviving rabbits were subjected to cesarean section and necropsy. Three rabbits died in the 7.5 ml/kg dose group within one hour

post dose on days 16, 17 and 19. Clinical signs included muscle rigidity, prostration, convulsions, dyspnea and/or tremors. Similar observations were observed in surviving animals in the 7.5 ml/kg and in one animal in the 2.5 ml/kg dose group. No effects on body weight changes and food consumption were noted. No changes in uterine weight and other cesarean section parameters were reported. No visceral and skeletal variations or malformations were reported. It is concluded that DMP 115 was not embryotoxic or teratogenic in rabbits at doses up to 7.5 ml/kg; therefore the fetal NOAEL is reported to be 7.5 ml/kg. The maternal NOAEL is reported to be 2.5 ml/kg.

Summary of developmental toxicology studies:

Five developmental toxicology studies were carried out. These included 1) a segment I study in rats, 2) a dose-range finding segment II study in rats, 3) a definitive segment II study in rats, 4) a dose-range finding segment II study in rabbits and 5) a definitive segment II study in rabbits.

1) In the segment I study, male rats were dosed for about 10 weeks, starting from 28 days prior to mating and through the mating period. Females were dosed for about 14 days, starting 7 days prior to mating and through gestation day 7. The animals were dosed with 0.1, 1.0 or 5.0 ml/kg of DMP 115 or 0.9% saline. On gestation day 13, all females were sacrificed and uterine evaluation was performed. Males were sacrificed after 10 weeks and reproductive organs were weighed. Semen evaluation was performed on the first 10 surviving males/group. One high dose male and one high dose female died on days 54 and 17, respectively. The cause of death was not determined. There were no effects noted on clinical observations, body weights and semen evaluation of any of the animals. No changes in pregnancy rates were noted amongst the groups. Pre and post-implantation losses were similar amongst the groups. The **NOEL was concluded to be 1.0 ml/kg** for both male and female rats. The **NOEL for reproductive effects was reported to be 5.0 ml/kg**.

2) In the dose-ranging segment II study in rats, doses of 1, 3 and 5 ml/kg were used by IV administration to pregnant rats between days 6-17 of gestation. Control groups received 0.9% saline. On day 20, the animals selected for cesarean section were anesthetized and necropsied. Mortalities in the maternal groups occurred at 3 ml/kg (2 animals) and 5 ml/kg (3 animals). The clinical signs were pale appearance, hypoactivity, prostration, labored and/or rapid breathing and in some cases convulsions. Surviving animals in the same groups showed similar signs but with decreased severity. Body weight change was slightly lower for the high dose (5 ml/kg) group. The mean postimplantation loss values were higher in the 3 and 5 ml/kg dose group. There were no fetal deaths or external abnormalities. The levels for the expanded study were set at 0.5, 1 and 2 ml/kg.

3) In the definitive segment II study in rats, doses of 0.5, 1.0 and 2.0 ml/kg were used between days 6 and 17 of gestation. Control animals received 0.9% saline or the placebo (inert ingredients of DMP 115). On day 20 of gestation, the animals selected for cesarean section were anesthetized and necropsied. No changes in clinical observations, body weight changes, gross pathological findings, differences in uterine weights or mean fetal body weights were noted. Five fetal malformations were reported, one in the saline control group and 2 in each the 0.5 and 2 ml/kg dose groups. These findings were considered to be incidental. Therefore, it is concluded that at doses up to 2 ml/kg, there are no reproductive, developmental or teratogenic effects in the rat.

4) In the dose-ranging segment II study in rabbits, doses of 0.5, 2.5, 7.5 and 10 ml/kg were used by IV administration between days 7-20 of gestation. No controls were used. On day 21 of gestation, surviving females were subjected to a cesarean section and necropsied. Four

animals died within one hour post dose; one in the 7.5 ml/kg group on day 10 and 3 in the 10.0 ml/kg dose group on days 7, 9 and 10 respectively. Clinical signs included pale appearance, convulsions, hyperactivity, prostration, and/or labored breathing. A trend towards a lower food consumption was noted in the 2.5-10 ml/kg treatment groups. Mean body weight change decreased in a dose-dependent fashion. No developmental effects were noted upon cesarean section. The dose level of 10 ml/kg/day of MRX 115 was associated with maternal toxicity, as evidenced by 3 maternal deaths. At the 7.5 ml/kg/day dose, the only sign of toxicity was one maternal death. Based on the results of this study, the doses chosen for the expanded developmental toxicity study in rabbits were 0.2, 2.5 and 7.5 ml/kg.

5) In the definitive segment II study in rabbits, doses of 0.5, 2.5 and 7.5 ml/kg were used from days 7-20 of gestation in New Zealand White rabbits. Two control groups including a 0.9% saline and a placebo group dosed with 7.5 ml/kg were used. On day 29 of gestation, all surviving rabbits were subjected to cesarean section and necropsy. Three rabbits died in the 7.5 ml/kg dose group within one hour post dose on days 16, 17 and 19. Clinical signs included muscle rigidity, prostration, convulsions, dyspnea and/or tremors. Similar observations were observed in surviving animals in the 7.5 ml/kg and in one animal in the 2.5 ml/kg dose group. No effects on body weight changes and food consumption were noted. No changes in uterine weight and other cesarean section parameters were reported. No visceral and skeletal variations or malformations were reported. It is concluded that DMP 115 was not embryotoxic or teratogenic in rabbits at doses up to 7.5 ml/kg, therefore the fetal NOEL is reported to be 7.5 ml/kg. The maternal NOEL is reported to be 2.5 ml/kg.

Based on the results of the developmental toxicity studies in rats and rabbits, it is concluded that in the segment I study, the NOEL in males and females is 1 ml/kg and the NOEL for reproductive effects is 5 ml/kg. In the rat segment II study, the maternal and fetal NOEL is 2 ml/kg. In the rabbit segment II study, the maternal NOEL is 2.5 ml/kg and the fetal NOEL is 7.5 ml/kg.

SPECIAL TOXICOLOGY STUDIES:

Study Title: Intravascular irritation study of MRX-115 in rabbits.

Study No: 97-09-52

Vol. 1-31, page: 261-273

Conducting laboratory and location:

Date of study initiation: 11/26/96

GLP compliance: GLP

QA- Reports: None

Design: The study examined the ability of MRX-115 to produce intravascular irritation using an intravenous retention method (3 minutes retention in the retro-auricular vein prior to flow restoration). MRX-115 or bromosulfophthalein sodium (BSP, positive control) was administered via the left retro-auricular vein to 3 females/group for 7-8 consecutive days. MRX-115 was injected within 2 hours of preparation. The right retro-auricular vein of all rabbits was injected with saline as a negative control. Rabbits were euthanized on day 8 or 9 for histological examination of the injection site.

Results: Both saline control and MRX-115 injected veins showed reddening around the site of needle insertion. There were no histological findings attributable to MRX-115. BSP positive control group showed dark red areas with poorly defined margins around the injected site in all animals from day 3 of administration. Histologic examination showed thrombus formation in 2 of 3 animals as well as hemorrhage, edema, inflammatory cells infiltration and fibroblast

proliferation around the vessel at the site of injection. In the remaining animal, endothelial cells were desquamated and extensive inflammation observed around the injection site.

Conclusion: The sponsor concluded that repeated intravascular administration of MRX-115 did not lead to significant irritation.

Reviewer's comments: agree.

Study Title: Intramuscular irritation study of MRX-115 in rabbits.

Study No: 97-09-53

Vol. 1-31, page: 274-290

Conducting laboratory and location:

Date of study initiation: 1/8/97

GLP compliance GLP

Design: The study examined the potential of MRX-115 to cause local intramuscular irritation in New Zealand white rabbits. A single 1 ml dose of MRX-115 or aqueous acetic acid (0.425% or 1.7%, positive control) was injected into the left musculus vastus lateralis (six female per were group). A single 1 mL dose of the negative control (saline) was injected into the right musculus vastus lateralis. MRX 115 was injected within 2 hours of preparation. Three animals per group were euthenized 2 or 14 days after dose administration, the muscles at the site of injection removed for gross and histopathological observation.

Results: Two days post MRX 115 administration, there were local muscular irritation (red/white discoloration, focal degeneration and necrosis of muscle fibers, hemorrhage and infiltration of inflammatory cells. The changes produced were greater than that produced by saline but were of similar magnitude to that produced by 0.425% acetic acid. Acetic acid (1.7%) produced more pronounced degeneration. Fourteen days after dose administration, few injection site abnormalities were observed in animals given saline, 0.425% acetic acid or MRX-115 whereas more pronounced irritation was observed in animals given 1.7% acetic acid.

Conclusion: The local muscular irritation produced by MRX-115 injection was equivalent to that produced by 0.425% acetic acid. It resolved by about 14 days post injection

Reviewer's comments: agree

Study Title: Eye Irritation test of MRX-115 injection (Aerosomes) in rabbits

Study No: T97-11-35

Vol: 1.31, page: 411-423

Conducting laboratory and location:

Date of study initiation: 03/6/97

GLP compliance GLP

QA- Reports Yes () No (x):

Lot No: 744-71-0004

Summary:

The study examined the potential of MRX-115 to cause ocular irritation in New Zealand white rabbits. The left eye of each of six females was exposed once to 100µL of MRX-115. MRX 115 was administered within 8 hours of activation. In three of the six rabbits, the MRX-115-exposed ocular surface, eyelids, and surrounding area were washed with saline after 10 seconds of

exposure. The right eye of each animal was exposed to 100 µL of saline and used as control. The cornea, iris, and conjunctiva were observed from 1 hour until 8 days after exposure. In either non-washed or washed eyes, no MRX-115-related abnormalities were observed in the cornea, iris, or conjunctiva. MRX-115 was not an ocular irritant in the study.

Reviewer's comments: agree.

Study Title: In vitro hemolysis test of MRX-115 injection in human blood.

Study No: T98-04-22

Vol. 1.31, pages: 425-432

Conducting laboratory and location:

Date of study initiation: 02/06/97

GLP compliance: GLP

QA- Reports Yes () No (x):

Lot No: 744-71-0004

Summary:

In vitro hemolysis tests of MRX-115 injection were performed using blood specimen from five healthy male volunteers. 1ml of the test solution was mixed with 0.1 ml of each blood specimen, and the mixture was then incubated at 37 °C for 30 minutes and centrifuged. Supernatants from the mixture were grossly examined and no evidence of hemolysis was observed.

Reviewer's comments: The sponsor used visual inspection as evidence of hemolysis. The preferred method of analysis is spectrophotometrical analysis with a positive control as part of the experimental design. However, it is noted that study R98-17 showed that DMP 115 lack edhemolytic potential when administered intravenously in dogs.

RDR-98-05: Assessment of intra-arterial administration of DMP115 in the anesthetized rat
Vol. 1.10 pp. 114-118. DuPont Pharmaceutical report dated 03/09/98 Lot # 4505Z GLP: No

Design: The study examined the potential of DMP115 to cause cerebral vasculature damage following intra-arterial administration into the carotid artery. The carotid artery was occluded caudally, and 100 µl/kg DMP 115 was administered over 20 seconds. 5 minutes later, the rats were euthanized and the brain removed for histopathological analysis.

Results and conclusions: The pathologist reported that microscopic examination of the brain did not reveal any abnormality. The sponsor concluded that DMP115, at a dose, that was ten times the projected human dose (based on body weight), did not adversely affect cerebral vasculature in the rats.

Reviewer's comments: This reviewer disagrees with the sponsor's conclusion. The rats were euthanized for histopathological analysis 5 minutes after administering DMP115. The time is probably too short to allow for elicitation of histopathological changes but may be sufficient for the manifestation of subtle metabolic consequences of the insult. Diffusion or perfusion studies together with enzyme analysis as indicators of neuronal damage should have been conducted to elucidate such changes. Additionally, the dose is not 10X the human dose, since the maximum human dose is 20µl/kg which translates to 0.8 times the human dose based on body surface area.

T98-10-29: Dose-response of DMP-115 and perfluoropropane in conscious rats following an intravenous dose. The study was conducted at DuPont Pharmaceuticals Company, N. Billerica, MA dated 08/3/98 Vol. 1.31 pps: 402-410. GLP: No

Design: This study examined the role of the PFP content of DMP-115 formulation in the toxicological profile differences between ImaRx Pharmaceutical Corporation and DuPont Pharmaceuticals Corporation sponsored studies. The toxicological studies were characterized by differences in dose of DMP-115 that produced clinical signs and death in rats (ImaRx 3-5 mL/kg Vs DuPont 1 mL/kg). The study evaluated the role played by formulation activation process, formulation handling, and how long after activation it was given to rats on PFP content and toxicity profile. In the ImaRx studies, DMP 115 was activated using _____ mixer and was used within 4 h of activation. In some studies, the activated formulation was transferred to an open vessel prior to injection. For DuPont studies, DMP 115 was activated on a mixer and administered within 30 min after activation, directly from the original sealed vial. It was reasoned that the method of formulation preparation during ImaRx studies might have allowed more of the PFP gas to diffuse out of the activated DMP 115 formulation compared with DuPont method resulting in lower toxicity.

Group	Dose ^a (mL/kg)	Condition ^b	# Males	# Females
1 Saline	3.0		2	2
2 DMP 115	1.0 (X8 MHDbsa)	Not activated	3	3
		Activated +5 min	6	6
		Activated + 15 min	6	6
3 DMP 115	3.0 (X24 MHDbsa)	Not activated	3	3
		Activated + 15 min	6	6

^aIntravenous injection in \approx 10 secs

^bDMP 115 was either not activated or activated with the specialized mixer as noted. Sealed, activated vials were allowed to stand 5 or 15 minutes after agitation and prior to injection.

In the second part of the study, PFP-containing headspace gas from vials containing DMP 115 (headspace gas contains \approx 89% PFP) was injected intravenously. This was to determine the similarity between the nature of the clinical signs and the number of deaths occurring in rats given a bolus injection of either air or PFP-containing headspace gas and DMP 115.

Group	Dose ^a (ml/kg)	# male rats
1 Air	1.0	2
2 DMP 115 vial headspace gas ^b	0.1	6
3 DMP 115 vial headspace gas ^b	0.2	5
4 DMP 115 vial headspace gas ^b	0.3	5
5 DMP 115 vial headspace gas ^b	1.0	6

^aIntravenous injection \approx 10 sec

^bInjection of head space gas from sealed vials of DMP 115 that were mixer-activated and allowed to stand for 5 minutes following activation.

In a separate experiment that was not part of the study, PFP concentrations in DMP 115 was determined at various times after mixer activation.

Results: No death occurred in rats given 1 ml/kg DPM 115 either 5 or 15 minutes after mixer activation. There were no clinical sign observed in any of the rats given 1 ml/ kg DMP 115, 15 minutes after mixer activation. In rats administered 1 ml/kg DMP 115, 5 minutes after mixer

activation, transient ataxia occurred in 2/12 rats. One animal died within 2 minutes after 3 ml /kg of DMP 115 fifteen-minutes after activation. Ataxia was observed prior to death. Transient rapid respiration 2/6, ataxia 2/6 and labored respiration 1/6 were observed in this group of animals.

Mortality in rats given DMP 115 vial head gas or air:

Group	Dose (ml/kg)	# of deaths
1 Air	1.0	0 of 2
2 DMP 115 vial headspace gas	0.1	0 of 6
3 DMP 115 vial headspace gas	0.2	3 of 5
4 DMP 115 vial headspace gas	0.3	4 of 5
5 DMP 115 vial headspace gas	1.0	4 of 6

Clinical signs observed prior to death included rapid breathing, dyspnea, ataxia, decreased motor activity and loss of righting reflex. The two rats administered bolus air injection did not show any clinical symptoms.

Average PFP concentrations in DMP 115 at various times after mixer activation

0 minute = 95±7
 5 minutes = 112±14^a
 15 minutes = 69±11^a
 30 minutes = 79±16^a
 60 minutes = 44±8^a

PFP concentrations were determined in six vials per time point. Amount of PFP present was determined using a headspace gas chromatographic method using an γ and a β detector. Values are expressed as the individual PFP concentration/ml of DMP 115 as well as the mean \pm % RSD percent relative standard deviation. ^aStatistically significant difference from 0 minute value using one way analysis of variance and Newman-Keuls test; P<0.05

Conclusions: The sponsor concluded that the study design and results did not allow any definitive conclusion regarding the role of PFP in the toxicity observed with DMP 115. The conclusion was based on the fact that there was a poor correlation between individual PFP concentration and time after activation, and that direct intravenous injection of PFP-containing head space gas was not the optimal model to evaluate the potential toxicity of PFP gas in DMP 115.

Reviewer's comments: While I agree with the sponsor that the value of this study is limited, nevertheless, one can reasonably conclude the following:
 Direct intravenous injection of PFP containing headspace resulted in clinical symptoms and death similar to those observed in the toxicological studies. The results were equivocal as to the role played by the time interval between activation and injection (5 or 15 minutes) in the toxicity of the product.

APPEARS THIS WAY
ON ORIGINAL

SPECIAL TOXICOLOGY SUMMARY

Study 97-09-52 showed that MRX 115 did not produce intravascular irritation when examined by repeated injection into the rabbit retroauricular vein using the intravenous retention method. However, it has the potential to cause muscular irritation equivalent to those elicited by 0.425% acetic acid as demonstrated by the results of study 97-09-03 showing that administration into the musculus vastus lateralis of rabbits led to focal degeneration, necrosis of muscle fibers hemorrhage and infiltration of inflammatory cells groups two days following administration. The signs of irritation resolved by 14 days post injection. MRX 115 did not cause eyes irritation in rabbits. The design of the experiment performed to assess the potential of DMP-115 to produce hemolysis in humans was inadequate since hemolysis was visually quantified. Study T97-11-35 showed that DMP 115 did not cause eyes irritation in rabbits.

T98-10-29 examined the role of the PFP content of DMP-116 formulation in the toxicological profile differences between ImaRx Pharmaceutical Corporation and DuPont Pharmaceuticals Corporation sponsored studies. The toxicological studies were characterized by differences in dose of DMP-115 that produced clinical signs and death in rats (ImaRx 3-5 mL/kg Vs DuPont 1 mL/kg). The study evaluated the role played by formulation activation process, formulation handling, and how long after activation it was given to rats on PFP content and toxicity profile. The second part of the study determined the similarity between the nature of the clinical signs and the number of deaths occurring in rats given a bolus injection of either air or PFP-containing headspace gas and DMP 115. No deaths occurred in rats given 1 ml/kg DMP 115 either 5 or 15 minutes after mixer-activation. There was no clinical sign observed in any of the rats given 1 ml/kg DMP 115, 15 minutes after mixer activation. In rats administered 1 ml/kg DMP 115, 5 minutes after mixer activation, transient ataxia occurred in 2/12 rats. One animal died within 2 minutes after 3 ml/kg of DMP 115 fifteen minutes after activation. Ataxia was observed prior to death. Transient rapid respiration 2/6, ataxia 2/6 and labored respiration 1/6 were observed in this group of animals.

Mortality in rats given DMP 115 vial head gas or air:

Group	Dose ^a (ml/kg)	# of deaths
1 Air	1.0	0 of 2
2 DMP 115 vial headspace gas ^b	0.1	0 of 6
3 DMP 115 vial headspace gas ^b	0.2	3 of 5
4 DMP 115 vial headspace gas ^b	0.3	4 of 5
5 DMP 115 vial headspace gas ^b	1.0	4 of 6

Clinical signs observed prior to death included rapid breathing, dyspnea, ataxia, decreased motor activity and loss of righting reflex. The two rats administered bolus air injection did not show any clinical symptoms. The sponsor concluded that the study design and results did not allow any definitive conclusion regarding the role of PFP in the toxicity observed with DMP 115. The conclusion was based on the fact that there was a poor correlation between individual PFP concentration and time, and that direct intravenous injection of PFP-containing head space gas was not the optimal model to evaluate the potential toxicity of PFP gas in DMP 115.

GENETIC TOXICOLOGY:

Study Title: Salmonella-Escherichia Coli/ Mammalian-Microsome reverse mutation assay preincubation method with a confirmatory assay

Study No: 98-07-02

Volume.1.31 and Pages: 38-83

Conducting Laboratory:

Date of Study Initiation/completion: 7/24/98-8/18/98

GLP Compliance: Yes

QA- Reports Yes (X) No ():

Drug Lot Number: PP97A-024

Test System: Salmonella strains TA98, TA100, TA1535, TA1537 and E.coli strain WP2uvrA
Test conditions: with and without Aroclor-induced rat liver microsomal enzymes.

Controls:

Tester strain	S9 Mix	Positive Control	Conc per plate
TA98	+	Benzo(a)pyrene	2.5µg
TA98	-	2-nitrofluorene	1.0µg
TA100	+	2-aminoanthracene	2.5µg
TA100	-	Sodium azide	2.0µg
TA1535	+	2-aminoanthracene	2.5µg
TA1535	-	Sodium azide	2.0µg
TA1537	+	2-aminoanthracene	2.5µg
TA1537	-	ICR-191	2.0µg
WP2uvrA	+	2-aminoanthracene	25.0µg
WP2uvrA	-	4-nitroquinoline-N-oxide	1.0µg

Vehicle controls were plated for all tester strains both in the presence and absence of S9 mix.

Dose Range: 60-1000µL per plate.

Study Design: dose ranging study, mutagenicity assay, confirmatory assay

Conformance with current ICH guidelines:

Tester strains: adequate

Top dose: Because of volume constrain, cytotoxicity was not observed at the highest concentration that could be tested.

Solubility: Precipitate was not observed at any dose.

Results: Negative

Conclusion MRX-115 at concentrations up to 1000µL/plate did not produce cytotoxicity in the tester strains and did not increase the frequency of revertant colonies with or without metabolic activation.

Reviewer's comments: agree

Study Title: DMP-115: Chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic confirmatory trial

Study No: T98-07-03

Volume.1.31 and Page: 115-160

Conducting Laboratory:

Date of Study Initiation/completion: 7/24/98-9/17/98

GLP Compliance: Yes

QA- Reports Yes (X) No ():

Drug Lot Number: PP97A-024

Test System: Culture Chinese hamster ovary cells

Test conditions: With or without Aroclor-induced rat liver S-9

Controls: non-activated positive contro; mitomycin C

Activated positive control; cyclophosphamide

Dose ranges:

Initial assay: 12.5-70 μ L/ml DMP115

Confirmatory assay: 12.5-70 μ L/ml DMP115

Conformance with current ICH guideline: Top dose was not toxic due to the fact that the osmolality of the drug precludes the testing of higher concentration.

Results: MRX-115 up to 70 μ L/ml did not significantly increase the incidence of chromosomal aberrations in CHO cells.

Study Title: DMP 115: In vivo rat micronucleus assay

Study No: T 98-07-04

Volume.1.31 and Page: 181-214

Conducting Laboratory: \

Date of Study Initiation/completion: 7/10/98-8/4/98

GLP Compliance: Yes

QA- Reports Yes (X) No ():

Drug Lot Number: PP97A-024

Controls: positive; cyclophosphamide, negative; saline

Study design:

Treatment DMP-115 ml/kg	Route	Volume	Male 24(Hr)	Female (24Hr)	Male (48Hr)	Female (48Hr)
0.25	IV	0.25	6	6	-	-
0.5	IV	0.50	6	6	-	-
1.0	IV	1.0	6	6	6	6
Saline	IV	1.0	6	6	6	6
Cyclophosphamide 60mg/kg	PO	10.0	6	6	-	-

Results & Conclusions: DMP 115 produced lethality in one female at the high-dose level. When compared to the vehicle control, DMP 115 did not produce a statistically significant decrease in the PCE:NCE ratio (i.e., bone marrow cytotoxicity) or a statistically significant increase in the percent micronucleated bone marrow polychromatic erythrocytes.

Reviewer's comments: Agree.

Study Title: Mutagenicity test on MRX-115 in the L5178Y TK⁺ mouse lymphoma forward mutation assay with a confirmatory assay

Study No: Study No: T95-07-21

Volume.1.31 and Page: 215-260

Conducting Laboratory:

Date of Study Initiation/completion: 12/21/94-2/1/95

GLP Compliance: Yes

QA- Reports Yes (X) No ():

Drug Lot Number: 744-71-001

Dr. Dundore reviewed the study.

Mouse lymphoma forward L5178Y heterozygous at the thymidine kinase locus (TK⁺) were exposed to MRX 115 at concentrations 10-70 µg/ml for a period of 4 hours with and without metabolic activation by a rat liver S9 fraction. After an expression of 2 days, cells were cloned for 10-14 day the number of colonies displaying the TK⁺ phenotypes were counted. Methy methanesulfonate 10 nm/ml and 15 nm/ml was used as positive control for non activation mutation studies, 20-methylcholanthrene at 2.0 and 4.0 µg/ml was used as positive control for metabolic activation. The results showed that MRX 115 did not produce cytotoxicity or increase the frequency of mutant colonies expressing the the TK⁺ phenotype.

GENETIC TOXICOLOGY SUMMARY

The sponsor conducted four genetic toxicology studies:

Study No: 98-07-02: Salmonella-Escherichia Coli/Mammalian reverse mutation assay

Study 98-07-04: In vivo rat micronucleus assay

Study 95-07-21: L5178Y TK⁺ mouse lymphoma forward mutation assay

Study 95-07-03: Chromosomal aberrations in Chinese hamster ovary (CHO) cells

DMP 115 was shown to be negative in all the tests.

**APPEARS THIS WAY
ON ORIGINAL**

OVERALL SUMMARY AND EVALUATION:

The goal of intravenous contrast agents is to enhance the diagnostic quality of ultrasound systems that have low signal to noise ratio. As such, echo contrast agents are intended to be passive indicators that enhance the scattered ultrasound signal without altering hemodynamic or other physiological parameters (Schwarz, Bezante, Chen, Phillips and Schlieff, J. Am Soc Echocardiogr 1996; 9 795-804). For echocardiography, the goal is to have a contrast agent that can be safely administered to produce prolonged, reproducible, ventricular opacification and myocardial perfusion without ventricular shadowing.

According to the sponsor, Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed as a contrast-enhancing agent for clinical ultrasound. The sponsor stated that the submitted data support the use of Definity for contrast-enhanced echocardiographic imaging of cardiac structure

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator. The sponsor specified a shaking frequency of around 4550 oscillations per minute. This amalgamator is equipped with an alarm that goes off when the frequency deviates by -10% or by +5%. The recommended dose as described in the product insert is as 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. The sponsor also suggested that Definity™ be administered via an i.v infusion of 1.3 mL added to 50 mL of preservative-free saline initiated at 4.0 mL/minute and titrated as necessary to achieve optimal image.

Efficacy Evaluation:

The sponsor submitted the results of several studies examining the ability of Definity™ to produce contrast enhancement in vitro, and echocardiography enhancement in a number of animal species in vivo. **Overall, this reviewer concurs that DuPont has provided sufficient pre-clinical experimental evidence to support the efficacy claim for Definity™ use in echocardiography.**

Efficacy studies were performed in dogs (studies RDRs 98-19, 98-20, 98-21, 98-22, 98-23, pigs (American Heart Journal, Vol. 1996, 132 938-945), and monkeys either as the primary objective or secondary to other investigations. In general, the ability of DMP 115 to elicit left ventricular opacification and image myocardial tissue perfusion, or to identify myocardial perfusion defects were examined. The results showed that Definity™ produced dose-related increases in the duration of left ventricular opacification, and in the video intensity duration of myocardial perfusion of the coronary arteries. In these species, the highest dose examined was generally limited by concomitant increase in ventricular shadowing reducing the resultant peak image intensity. The sponsor concluded that DMP 115 bubbles between 2-10 µm are the primary contributors for imaging using the fundamental mode while the 1-2 µm bubbles are the primary contributors for imaging in the second harmonic mode. Study RDR-98-26, an in vitro examination of the relationship between bubble concentration and ultrasound signal intensity demonstrated that changes in bubble concentration were directly correlated with signal intensity for both fundamental, and second harmonic modes of image acquisition. The video intensity of the second harmonic signal was significantly greater than the intensity of the fundamental signal. The sponsor posited that the enhanced signal resulted from changing the ultrasound

gain to take advantage of the additional dynamic range for the contrast signal in the second harmonic mode of image acquisition.

Administration of DMP-115 either as a bolus over 10 sec, or as an infusion over two minutes, or infused in saline at a rate of 9 μ l/kg for a 30 minute period was effective in providing echocardiography and measuring gated second harmonic myocardial perfusion in open-chested dogs. Both the bolus and the 2 minutes infusion method produced dose-dependent increases in image and duration of action. Ventricular shadowing occurred at a lower dose with bolus injection compared with infusion over 2 minutes. DMP-115 infused over 30 minutes resulted in steady state left ventricular opacification without ventricular shadowing but peak intensity was about half that of bolus injection. The data support the utility of administering DMP 115 as a bolus or as an infusion.

In addition, I agree with the sponsor's following key conclusions, as they affect the pre-clinical efficacy portion of the submission:

- Changes in the manufacturing process for the lipid component of the drug substance from an followed by does not affect the ability of DMP 115 to produce opacification of the left ventricle and perfusion of the coronary arteries. Moreover, comparison of six formulation lots manufactured at two different sites in terms of acoustic attenuation, rate of decay of attenuation and response of the attenuation to increasing ambient temperature and pressure in saline at 37°C demonstrated no significant difference.
- DMP-115 remains efficacious in producing echocardiographic demonstration of left ventricles and of myocardial perfusion following activation through mixing up to four days post-preparation. The responses produced at later stages following preparation are comparable to the response obtained immediately post preparation despite the fact that number of particles per ml remaining was less after four days. However, since the toxicological, immunological and other indices of safety were not examined, the sponsor's conclusion that the 4-days post activation preparation does not pose a risk to patients is not supported by the data. Interestingly, the number of particles decreased after four days, yet video intensity remained the same, suggestive that a lower dose of DMP 115 might be sufficient to obtain clinical efficacy.
- The duration of ultrasound signal generated was significantly greater when DMP 115 was diluted in canine blood compared to when dilution was in saline, both at room temperature/atmospheric pressure and at 37°C, with or without pressurization. For both media, increasing the pressure decreased microbubble life.
- DMP 115 did not induce hemolysis in the presence, or absence of clinically relevant levels of ultrasound power in an in vivo dog model.

Taken together, these results provide sufficient proof of concept, and demonstration of pre-clinical efficacy for the myocardial echocardiography indication.

Safety Evaluation

The submission addressed the safety issues of Definity™ from many perspectives.

Cardiovascular safety studies were performed in dogs, rabbits, pigs, and monkeys, with the dog being the most utilized species. The dog studies RDR 98-20, 98-21, 98-22, 98-23 were generally characterized by lack of effect on measured hemodynamic parameters including mean arterial pressure, pulmonary arterial pressure, heart rate and indices of myocardial contractility. However for most of these studies, since the primary end point was to demonstrate echocardiography efficacy, the doses employed were generally between multiples of 0.08-0.81 of the human dose based on body surface area. Ideally, a cardiovascular safety evaluation should employ high multiples of the human dose in an effort to characterize the likely targets of physiological insults. Hence how dogs will respond physiologically to equivalent amount of DMP 115 (Final formulation) to be administered to humans is not known at the present time. However, it is noted that for the toxicological studies, dogs administered higher multiples of the human dose showed responses suggestive of impairment of the cardiovascular system. DMP-115 did not alter the hemodynamic profile in anesthetized rabbits dosed at between 0.073 and 5.6 MHD.

The cardiovascular safety profile of DMP-115 was examined in anesthetized pigs at doses of 0.5, 1, 2, 5 and 10 $\mu\text{l}/\text{kg}$ (X 0.025-0.5 MHD). The highest dose evaluated produced a transient increase in mean pulmonary pressure that returned to baseline within 8 minutes after dosing. The authors ascribed the elevation in pulmonary arterial pressure to thromboxane-mediated pulmonary hypertension caused by pulmonary intravascular response to injected particles. The influence of known inhibitors of thromboxane metabolism was not investigated. Moreover, there was also a decrease in arterial oxygen saturation at the time of elevated pulmonary pressure. Although the sponsor claimed that the changes in oxygen saturation did not occur in other species, the time points at which measurements were taken in the studies were such that an adverse or indeed any effect on blood gases would have been difficult to detect. Be as it may, the key safety issue raised by the demonstrated effect of DMP 115 on arterial pulmonary pressure and oxygen tension, is the likely impact of this product on patients with compromised pulmonary functions. The sponsor believes that the pulmonary response is species dependent as there was no increase in pulmonary arterial pressure in rhesus monkeys at up to 100 $\mu\text{l}/\text{kg}$ (X1.6 MHDbsa) or in dogs at up to 25 $\mu\text{l}/\text{kg}$ (X0.675MHDbsa). However, these are low multiples of the human dose and should not be the basis of generalized conclusions.

Therefore, this reviewer is of the opinion that DuPont should have examined the cardio-pulmonary effect of DMP 115 at higher dose multiples and in an animal model with compromised pulmonary function.

The cardiovascular effect of DMP-115 was evaluated in both Rhesus and Cynomolgus monkeys (study # SC 950020) using ImaRx formulation. The results indicated that at doses between 0.5-100 $\mu\text{l}/\text{kg}$ (0.08 and 1.62 MHD), DMP-115 did not affect heart rate, pulmonary arterial pressure, systemic arterial pressure or pO_2 . For the Cynomolgus monkey studies, the animals were surgically implanted with radiotelemetric blood pressure and EKG transmitters. Following recovery, baseline cardiovascular data were obtained for two days prior to treatment. Doses of between 0.05-1 ml/kg, (0.008- 16.2 MHD) were administered as bolus injections. Cardiovascular parameters were unremarkable in 3 of the 4 animals. A marked and physiologically significant fall in blood pressure (-38%, 40 mm Hg), and heart rate (-46%, 95 beats per minute) was noted in one of the animals at 1 ml/kg (x16.2MHDbsa). The decreases rebounded within 10 minutes and returned to near baseline in 45 minutes. Continuous

electrocardiographs were obtained from 24 hours before to 7 days after DMP-115 administration. The consultant veterinary cardiologist report noted what was described as a possible ventricular premature depolarization originating from the specialized connective tissue of the left ventricle in one monkey, and prolonged ST segments with alterations in the configurations of T waves, which is consistent with electrolyte imbalance in another monkey. Although the results are suggestive of myocardial injury, he concluded that the findings did not reveal any abnormalities attributable to treatment.

However, it is pertinent to consider here additional electrocardiograph results obtained as part of toxicological studies in monkeys (T98-7-1) conducted using the final to be marketed formulation. All animals (n=6) intravenously administered DMP115 at 3 ml/kg (X48.6MHDbsa) showed abnormal electrocardiograms. Each monkey demonstrated evidence of ST-T segment depression within one minute of the start of infusion. The development of ventricular extrasystoles, ventricular tachycardia, first degree and complete atrioventricular block and the transient development of right bundle branch block followed this. The ST-T depression began to return toward baseline between 8 –10 minutes of the beginning of drug infusion but persisted longest in one female monkey. These signs are indicative of myocardial ischemia. All animals received supplemental oxygen for 3 to 6 minutes.

While it is noted that study RDR 98-7-1 was conducted at high multiple of human dose, nevertheless key information regarding the effect of DMP –115 on cardiovascular parameters like effect on blood pressure, pulmonary circulation and peripheral vascular resistance is lacking. Moreover, most of the symptoms developed by animals in the toxicology studies indicated overt signs of impairment of cardiovascular control mechanisms. Therefore, this reviewer believes that DuPont should be requested to perform a comprehensive cardiovascular study in at least one non-rodent species.

Pharmacokinetic Evaluation

The PFP component of DMP 115 is rapidly eliminated from the lungs following a single compartmental model in dogs. PFP was cleared rapidly from systemic circulation with the t_{max} occurring 10-45 seconds post-treatment. The mean area under the curve was 3.24 μ .sec/ml with a mean elimination half-life of 61 seconds and mean blood clearance of 19 ml/kg/sec. The pharmacokinetics, distribution and elimination of 14 C-DMP-115 were characterized in rats. A two compartmental model best described the results. 71.5% of total radioactivity was recovered in urine by 72 hours, 90% of which was in the form of 14 C-DPEG-5000. The feces contained 10.5%, liver 3.7%, skin 1.9%, muscle 1.4% and cage wash 2%. The remaining 5% distributed throughout other organs at less than 1% per organ. The tissues with the highest concentrations of activity were the liver (17.8%) and plasma (17.2%) at 4 hr post-injection. No major metabolite of the labeled component of DMP-115 (DPPE [14 C]-MPEG 5000) was produced through 15 min post-injection. At 1 hour, 60% of the activity was in the form of 14 C-MPEG 5000 LPE. At 4 hr, plasma levels were non detectable by HPLC. Fecal activity was below detection limit.

The observation that the PFP component is rapidly eliminated from the lungs raises interesting questions regarding how PK relates to efficacy; for example timing of imaging. The data presented suggest that following injection, echocardiographic imaging in dogs can be obtained for more than 15 minutes. This time period is in excess of time required for elimination of PFP from the body. A worst case scenario might then assume that PFP is not critical for imaging or that only PFP that has not been

incorporated into the microbubbles is being measured in expired air, and that the encapsulated PFP in the lipid blend is still in the body. Moreover, the questions remain what happen in the interim between administration and elimination of PFP? Do components stay intravascularly or is there tissue distribution? Is the rapid elimination of PFP inconsistent with the sponsor's concept of microbubble formation? In view of the importance of the lungs to elimination of PFP, the need for an understanding of how PFP would be handled in an animal model of compromised pulmonary function or in humans with pulmonary disorders cannot be overemphasized:

Immunotoxicology Evaluation:

The results of studies evaluating the potential of MRX 115 to elicit an anaphylactic reaction in guinea pigs were equivocal, however the results suggest that MRX 115 has the potential to cause active systemic anaphylaxis reaction characterized by retching, pawing at the nose/mouth, and head shaking. Evidence for the elicitation of passive cutaneous antigenicity reaction was at best weak. No effects were noted on plasma histamine, tryptase and Complement (SC5b-9) levels in Cynomolgus monkeys administered DMP 115 as a single intravenous administration although it is unclear at the present time whether responses would have been triggered in monkeys following repeated administration. The sponsor raised the possibility that the clinical signs that were observed in dogs following the administration of DMP 115 might be pseudo-allergic in nature rather than an immune-mediated response. According to the sponsor, dogs are reported to exhibit pseudo-allergic (anaphylactoid) response following intravenous administration of lipids. This response does not require prior sensitization and is characterized by one or more of the following: mast cell degranulation, histamine release, and respiratory and cardiovascular effects.

These results point to species differences in the immunotoxicology response to administered DMP115. Therefore, this reviewer opines that additional pre-clinical studies examining the question of immunotoxicity will be of limited value. According to the medical reviewer, "For young normal volunteers, immunogenic effect was observed as a systematic substantive (almost doubling in some instances) elevation in serum IgG in all subjected tested whether they received drug or placebo (glycerol, propylene glycol and saline)".

To this end it is recommended that the question of the immunotoxicity of DMP 115 be pursued further in humans. I therefore recommend that human studies be performed to evaluate the influence of DMP 115 on complement, immune complexes, lymphokines and other hematological indicators of immunogenicity.

Reproductive & Developmental Toxicology Evaluation:

Five developmental toxicology studies were carried out. These include 1) a segment 1 study in rats, 2) a dose-range finding segment 2 study in rats, 3) a definitive segment 2 study in rats, 4) a dose-range finding segment 2 study in rabbits and 5) a definitive segment 2 study in rabbits. None of the findings were suggestive of any adverse effects on reproductive and developmental capabilities. However, it is noted that the reproductive and developmental toxicology studies were not conducted with the final to be marketed formulation. DuPont has committed to repeating these studies beginning in the second quarter of 1999 with final reports in the second quarter of 2000 (21-064, BP of 2/1/99). DuPont went on to say that "Information that is currently presented in our NDA application supports a Category C classification and that the

results of the ~~scheduled~~ toxicity studies will be taken into consideration with regard to labeling claims. **I agree with DuPont's request.**

Genetic Toxicology Evaluation:

The sponsor conducted four genetic toxicology studies:

Study No: 98-07-02: Salmonella-Escherichia Coli/Mammalian reverse mutation assay

Study 98-07-04: In vivo rat micronucleus assay

Study 95-07-21: L5178Y TK⁺ mouse lymphoma forward mutation assay

Study 95-07-03: Chromosomal aberrations in Chinese hamster ovary (CHO) cells

DMP 115 was shown to be negative in all the tests.

The studies are adequate, and I agree with sponsor's conclusion.

Special Toxicology Evaluation:

Study 97-09-52 showed that MRX 115 did not produce intravascular irritation when examined by repeated injection into the rabbit retroauricular vein using the intravenous retention method. However, it has the potential to cause muscular irritation equivalent to that elicited by 0.425% acetic acid. The results of study 97-09-03 showed that administration of DMP 115 into the musculus vastus lateralis of rabbits led to focal degeneration, necrosis of muscle fibers hemorrhage and infiltration of inflammatory cells, two days following administration. The signs of irritation resolved by 14 days-post injection. MRX -115 did not cause eye irritation in rabbits. The design of the experiment performed to assess the potential of DMP-115 to produce hemolysis in humans was inadequate since hemolysis was visually quantified. However, other results submitted suggest that DMP 115 has no hemolytic potential.

While it is noted that no perivascular irritation study was conducted, I agree with the sponsor's conclusions regarding overall lack of significant irritancy.

Toxicology Evaluation:

It is very important to note that there are differences in the way the sponsor calculated the multiple of the human dose and the way that this reviewer did the same calculation.

The sponsor's calculation is based on body weight while I used body surface area. It is now the policy of the FDA's Pharmacology/Toxicology Coordinating Committee to use body surface area instead of body weight to calculate conversion factors between species. Moreover for several studies, the sponsor assumed the human dose to be 10 μ L/kg. However since the package insert suggested that "The recommended dose is as a single dose of 10 μ L/kg by slow i.v bolus injection over 30-60 seconds, followed by a 10 mL saline flush. A second, 10 μ L/kg dose may be administered to prolong optimal imaging" I assumed the worst case scenario that 20 μ L/kg would be administered. Therefore my calculated MHDs will be less than the sponsor's calculated values.

Both ImaRx Pharmaceutical Corporation and DuPont Pharmaceuticals Company conducted toxicity studies. However, the studies differ in 1), the timing of injection after mixer activation (within four hours for ImaRx studies, and within 60 minutes for the DuPont studies). 2), the dose that produced lethality; with the ImaRx studies generally showing a higher safety margin. The DuPont studies were conducted using the to be marketed formulation and characterized by increased severity in clinical and pathological symptoms. The thrust of the toxicological

evaluation will focus on DuPont studies since it is the to be marketed formulation. The clinical implication of the differences between ImaRx and DuPont studies will be highlighted, especially in the light of the reasons deduced by DuPont, to be responsible for the observed differences.

For acute toxicity studies in rats, a minimum lethal dose of 1 ml/kg (X8 MHDbsa) was established. Immediately prior to death, clinical signs including abnormal respiration, ataxia, decreased motor activity and increased heart rate were observed. ImaRx formulation was used for dog acute single dose toxicity study. The dogs were given 0.01, 1 and 2 ml/kg (X2.7, X27 or X 54 MHD). Clinical signs were observed with doses as low as 0.01 ml/kg (X2.7 MHDbsa) on day 1 only. The signs included, pale gums, urinary or fecal incontinence, salivation, hypoactivity, dyspnea, polypnea, cold to touch, tremors, and/or abnormal respiratory sounds. Urinary and fecal incontinence were also noted in the group of animals that received the degassed liposomes. In view of the fact that the ImaRx formulation showed lowered toxicity in the rat studies, one might assume that the MHD calculated will be lower for DuPont's formulation.

In study T98-7-1, submitted midway through the review process, Cynomolgus monkeys were administered a single dose of DMP 115 at 3 ml/kg (48 MHDbsa). There were no deaths. Clinical signs exhibited by animals consisted of decreased muscle tone, unresponsiveness, abnormal respiration, (increased, decreased or stopped), salivation, pale gums, vocalization, partly closed eyes, yawning, chewing behavior, dilated pupils, urination, defecation, and or tremor. All animals received supplemental oxygen for 3 to 6 minutes. Five of the animals recovered by 30 minutes, the remaining animal exhibited hunched posture and yawning at 60 minutes post dose. All six animals administered DMP 115 at 3 ml/kg showed abnormal electrocardiograms with electrocardiographic evidence of ST-T segment depression within one minute of the start of infusion. The development of ventricular extrasystoles, ventricular tachycardia, first degree and complete atrioventricular block and the transient development of right bundle branch block followed this. The ST-T depression began to return toward baseline between 8 -10 minutes of the beginning of drug infusion but persisted longest in one female monkey.

Five repeat-dose toxicity studies were performed in rats. For both ImaRx and DuPont studies, administration of MRX-115 resulted in some lethality. Death occurred immediately after dosing. Prior to death, prostration, dyspnea, abnormal respiration, convulsion and loss of consciousness were observed. No histopathological findings were evident in studies T95-7-29, T95-7-22, and T97-9-15. In study T97-11-15, in addition to the clinical signs elicited, histologically, there were lung lesions characterized by minimal to moderate perivascular and peribronchiolar eosinophilic infiltrates. The changes correlated with hematologic eosinophil increases, alveolar macrophage accumulation, bronchiolar goblet cell hypertrophy and hyperplasia, hemorrhage and/or interstitial pneumonia. In addition, there was an increased incidence of lymphoid hyperplasia within enlarged bronchial, mediastinal lymph nodes as well as increased extramedullary hematopoiesis. Study T98-3-46 was a combined acute and subchronic toxicity study that utilized both the ImaRx and DuPont formulations. The study established similar NOEL for the two preparations and there were no significant differences in profile between them. Study 98-3-46 reported a NOEL of 0.1ml/kg (0.8 MHDbsa). When one combines the results of T97-11-5 and T97-9-15, 17/31 male rats, and 13/30 female rats died at 1ml/kg (X8 MHDbsa)

Three repeat-dose toxicology studies were performed in dogs all the using the ImaRx formulation (T95-7-28, T95-6-34, and TT95-8-42). None of the studies established a NOEL. Clinical signs began on day 1 or days 3 and 4. The signs from all the studies included, pale

gums, polyuria, salivation, abnormal respiratory sound, increase in histamine level, changes in coagulation system.

Two repeat-dose toxicology studies (28 days) were performed in cynomolgus monkeys (T-95-7-24, T98-5-2). NOELs were 1 ml/kg and 0.3 ml/kg (16.2 and 4.86 XMHDbsa respectively). For the T98-5-2 study, 1/6 monkeys died and 4/6 exhibited an acute response after receiving 3.0 ml/kg on day 1. Clinical signs included abnormal respiration, decreased heart rate, pale gums, dilated pupils, salivation, urination, and vocalization. Similar signs including loss of consciousness were observed beginning days 15 and 27 for four of twelve animals that received 1 ml/kg. There were no major histopathological findings.

Key Issues Emerging From The Toxicology Evaluation:

At least 2 key issues emerged from the toxicological studies:

- The low MHD level at which toxicity or death occurred in these studies and the scientific and theoretical considerations for the adverse reactions observed.
- The impact of the time interval between activation and injection on manifestation of toxicity.

A low multiple of the human dose (MHD) at which NOEL occurred characterized both the acute and repeat dose toxicity studies. The NOEL was not identified for the dog studies. A similar spectrum of clinical signs in rats, dogs and cynomolgus monkeys administered intravenous doses of DMP 115 was observed. The signs occurred during or soon after dosing. Clinical signs exhibited by animals included some, or all of the following; decreased muscle tone, unresponsiveness, abnormal respiration, (increased, decreased, stopped), salivation, pale gums, vocalization, partly closed eyes, yawning, chewing behavior, dilated pupils, urination, defecation, and or tremor. In addition, monkeys that were administered DMP 115 at 3 ml/kg showed abnormal electrocardiograms with electrocardiographic evidence of ST-T segment depression within one minute of the start of infusion. The rapidity of the changes is suggestive of a rapidly occurring alteration in the cardiopulmonary status of the animals involved. It is reasonable to suspect that this may have been caused in part by entrapment of the microbubbles in vital organs such as the lungs.

The sponsor investigated the role played by PFP gas in the acute toxicity of the product (T98-10-29). The underlying hypotheses are 1) The PFP content of the administered DMP 115 formulation might in part be responsible for the clinical signs and death attributed to DMP 115 in rats. 2) The longer the time interval between activation and injection, the higher the probability of allowing more of the PFP gas to diffuse out of activated DMP 115 formulation resulting in lowered toxicity (explaining the role that time interval between activation and injection plays in the elicitation of toxicity). PFP-containing headspace was injected intravenously. There were several deaths. Clinical signs observed prior to death included rapid breathing, dyspnea, ataxia, decreased motor activity, and loss of writhing reflex. Symptoms that were remarkably similar to those elicited in rats by DMP 115. There was poor correlation between the individual PFP concentrations, and time between activation and injection. The sponsor used this lack of correlation, and the fact that direct intravenous injection of PFP-containing head space was not the optimal model to evaluate the potential toxicity of PFP gas contained in DMP 115 to conclude that the results regarding the role of PFP in toxicity is equivocal. I agree with the sponsor that the outcome of the study might represent a worst case scenario of the role of PFP. Nevertheless, these results are worrisome to this reviewer since they imply that following

activation, not all the PFP contained in the head space become encapsulated in lipid to form the microbubbles.

In view of the fact that the formulation contains a lipid blend, let us now briefly examine the possible role that fat embolism plays in the manifestation of toxicity. According to the Cecil Textbook of Internal Medicine 1998, the diagnosis of fat embolism is based on the presence of at least one of the following within the first 72 hours after traumatic fracture: otherwise unexplained dyspnea, tachypnea, arterial hypoxemia, and diffuse alveolar infiltrate on the chest radiography. Some of the cardio-pulmonary symptoms manifested in the toxicological studies certainly fit the description.

Whatever the origin of the clinical symptoms, the final decision should be based on clinical risk/benefit analysis as seen by the medical reviewer.

It is the considered opinion of this reviewer that the time interval between activation and injection may contribute to the manifestation of DMP 115 toxicity. This probably became apparent to DuPont following the results of Study T95-7-15 that showed excessive mortality in rats as compared with previous results obtained by ImaRx. It is noted that a study by DuPont (T98-3-46) that compared both formulations (ImaRx and DuPont final formulations) indicated no significant difference in the dose that caused toxicity in rats. Nevertheless, DuPont went on to say:

" While the basis for the increased toxicity (clinical signs) and difference in toxicity profile (lung lesions) of DMP 115 in the DuPont rat studies compared to the ImaRx rat studies is not known. It may be related to differences in how the formulation was activated, how the formulation was handled after activation, and /or how long after activation it was given to rats in these studies. In the ImaRx studies, DMP 115 was activated and injected within 4 hours after activation. In addition, during some of the ImaRX studies, the DMP 115 was transferred to a secondary container prior to injection. In contrast, during the 1-month studies in rats and monkeys conducted by DuPont, DMP 115 was activated and administered within 60 minutes after activation, directly from the original sealed vial. These differences in activation and handling may have affected the physical characteristics of the formulation resulting in a lower no-effect dose of DMP 115 for clinical signs in rats and monkeys and elicitation of lung lesions in rats. The results of the pre-clinical toxicity studies sponsored by DuPont are considered to be relevant for determining the potential toxicity of the DMP 115 because the studies were conducted under conditions that are representative of clinical use" (Vol. 1.9 pps 77-78)

The question then becomes; how was the condition of clinical use determined, and whether the results of the pre-clinical studies played any role in driving such determination.

It is reasonable to conclude that efficacy was not compromised by the manner in which ImaRx conducted their studies since the sponsor gave no indication that the echocardiography results obtained from studies conducted by ImaRx were inferior to those obtained from studies conducted by DuPont. If ImaRx activation procedure led to a reduction in the clinical signs of toxicity as well as the toxicity profile without compromising efficacy, one would have expected DuPont to adopt the same strategy in the conduct of their investigation and to allow the strategy to drive the determination of the manner of conduct of the clinical trials and eventually how the product is to be used clinically. It seems to this reviewer that this was not the case.

To this end, this reviewer recommends that we ask the sponsor for the scientific basis of the determination of the timing of DMP 115 in clinical use. Furthermore, to ask whether such determination should not be re-examined in light of the pre-clinical results.

Overall, although this reviewer has outstanding issues to be communicated to the sponsor, it is my considered opinion that the application is approvable pending successful resolution of these issues.

Conclusions: The application is **approvable** pending successful resolution of issues to be communicated to sponsor.

Labeling Review:

Draft Labeling

DRAFT LABELING

RECOMMENDATIONS: From pre-clinical pharmacology and toxicology perspective, this application is approvable pending successful resolution of the comments to be forwarded to the sponsor.

External comments to sponsor:

1. Pre-clinical toxicity study results conducted by DuPont were characterized by lower no-effect dose of DMP 115 required for elicitation of clinical signs and patho-physiology compared with the ImaRx studies. In view of your claim that *"the results of the pre-clinical toxicity studies sponsored by DuPont are considered to be relevant for determining the potential toxicity of the DMP 115 because the studies were conducted under conditions that are representative of clinical use"* (Vol 1.9 pps 77-78):

Please explain 1) how the condition of clinical use was determined; 2) whether the results of the pre-clinical studies played any role in driving such determination, and 3) whether a re-examination of the conditions of clinical use is not warranted in light of the pre-clinical results.

2. We recommend that a cardiovascular safety study be conducted in conscious Cynomolgus monkeys in view of the myocardial toxicity (abnormalities in the ECG profile) described in study T 98-7-1. We suggest that the study include sufficient numbers of animals of both sexes. We suggest that you monitor parameters including but not limited to MAP, HR, ~~LV dp/dt~~, pulmonary arterial pressure, organ blood flow continuous EKG including a chest lead monitoring, pulmonary and blood gasses assessments. These experiments need not be terminal but should capture the pharmacological and safety dose profile of DMP 115 including NOELs for the parameters evaluated.
3. We recommend that the question of the Immunotoxicity of DMP 115 be pursued further in humans. Such a study should evaluate the influence of DMP 115 on complements, immune complexes, lymphokines and other hematological indicators of immunogenicity
4. We recommend that a study be conducted to assess the effects of DMP 115 on microcirculation. Any number of established model systems could be used (cat mesentery,

ImaRx formulation	0.1, 1, 2 ml/kg			sound, emesis, hypoactivity. No major histopathological findings
Dog (T95-6-34) ImaRx study ImaRx formulation	7-day repeat-dose 0.1 ml/kg	None	-	Clinical signs that became prominent beginning day 3. Cyanosis of oral or conjunctiva mucosa, depression, changes in the coagulation system.
Dog (T95-8-42) ImaRx study ImaRx formulation	7-day repeat-dose 0.01, 0.03, 0.1 ml/kg	None	-	Clinical signs beginning post-dose day 4 became apparent. Dyspnea, ataxia, red discoloration of the perioral area, increase in histamine level.
Cynomolgus monkeys (T95-7-24) ImaRx formulation	28-day repeat-dose 0.5, 1, 10 ml/kg	1 ml/kg	16.2	1/3 males died on day 22 (10ml/kg), and was noted to be prostrate before death. No major histopathological findings.
Cynomolgus monkeys (T98-5-2). ImaRx formulation	28-day repeat-dose 0.1, 0.3, 1 ml/kg	0.3 ml/kg	4.86	1/6 monkeys died, 4/6 exhibited an acute response after receiving 3.0 ml/kg on day 1. Clinical signs included abnormal respiration, ↓ heart rate, pale gum, dilated pupils, salivation, urination, and vocalization. Similar signs were observed beginning day 15 and 27 for 4/12 receiving 1 ml/kg. No major histopathological findings.
Rat (T95-07-25) ImaRx formulation	Single-dose 14-day observation 0.5, 5, 10 ml/kg	5 ml/kg	40	5/10 male and 1/0 female died at 10 ml/kg (X80 MHDbsa). At necropsy, congestion of lungs, enlargement of liver and mild centrilobular vacuolization was noted.
Dog (95-07-26) ImaRx formulation	Single-dose 14-day observation 0.1, 0.5, 1.5 5 ml/kg	0.1 ml/kg	2.7	Clinical signs include pale gum, rapid respiration, cold to touch, hyporeactivity and tremor. Similar effects were seen in animals given 2 ml/kg of degassed liposome. No significant histopathological changes reported
Cynomolgus monkeys (T97-9-51) Final Formulation	Single-dose 14-day observation 1, 10 ml/kg	1 ml/kg	16.2	1/sex/group studied. Slight elevation of GOT & GPT in male, and GOT in female that received 10 ml/kg. Abnormal alterations in QRS complexes and alteration prolongation of QRS interval suspected at 10 ml/kg. No significant histopathological changes.
Cynomolgus monkeys (T98-6-2) Final Formulation	Single-dose 14-day observation 1 ml/kg	1/ml/kg	16.2	Study conducted at 1 ml/kg only. No significant findings reported. Inadequate design need to see effects.
Cynomolgus monkeys (T98-7-1) Final Formulation	Single-dose 14-day observation 3 ml/kg	3 ml/kg	-	3/sex/group. No death reported. Clinical signs included decreased muscle tone, salivation, pale gums, vocalization, closed eyes. Yawning, dilated pupils, urination, defecation, and tremor. All monkey showed ST-T segment depression, ventricular extrasystole, ventricular tachycardia, first degree and complete atrioventricular block. Supplemental oxygen was administered.
Guinea pigs T97-02-53 & T96-10-23 Final Formulation	Antigenicity study Anaphylaxis			Study to determine ability of MRX 115 to induce acute systemic and passive cutaneous anaphylaxis. Slight evidence of acute systemic anaphylaxis.
Rat T98-10-29	The study examined role of PFP in DMP 115 toxicity			Intravenous injection of PFP led to animal death. Signs elicited prior to death included rapid breathing dyspnea, ataxia, decreased motor activity and loss of writhing reflex.
Rat T 97-02-54 Not Final Formulation	Study of fertility and early embryonic development in rats. Doses used were 0, 0.1.0 and 5 ml/kg.	5 ml/kg.	40	There was no incidence of embryo- toxicity. Two animals died one male and one female in the high dose group. Two additional females were sacrificed.

Rabbits T 96-02-25	Rabbit developmental toxicity study with MRX 115 Doses studied 0.5, 2.5, 7.5 ml/kg.	7.5 ml/kg	73	No treatment related embryotoxicity reported. Three high dose female died on days 16, 17 and 19. Tremor, muscle rigidity. Ataxia, erythema, convulsion, hyporeactivity, prostration and dyspnea were noted prior to death.
Rabbits T-97-09-52	Intravascular irritation study. 3 minutes retention in the retro-auricular vein prior to flow restoration for 7-8 days.			Non-irritant
Rabbits T97-09-53	Intramuscular irritation study. 1 ml dose into the right musculus vastus lateralis.			MRX-115 produced muscular irritation equivalent to that produced by 0.425% acetic acid. It resolved by 14 days post injection.
Rabbits T97-11-35	Eye irritation test. 100 µl injected into the left eyes. Observed from 1 hour to 8 days			Not an ocular irritant
Ames Bacteria Reversion T98-07-02	Salmonella/ E. Coli 60-1000µl/plate			Cytotoxicity was not observed at the highest dose tested. Not mutagenic at any dose with or without S-9 activation.
CHO T98-07-03	12.5-70µl/plate			Not mutagenic
Rat T98-7-4	0.25-1.0ml/kg Micronucleus assay			No increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of rats.
Dog RDR 98-20	3, 10, 30 µl/kg intravenously. Cardiovascular study	-	-	The study examined optimal dose of DMP 115 for assessment of ventricular opacification. There was dose-related increase in ventricular opacification. No effect on heart rate, mean arterial pressure or myocardial contractility.
Dog RDR 98-21	3, 10, 30 µl/kg intravenously. Cardiovascular study	-	-	Cardiovascular study examining ventricular opacification with DMP 115 prepared and used immediately after mixer activation, or used 4 days post preparation. Both were found to produce comparable ventricular opacification.
Dog RDR 98-23	0.3-10 µl/kg as bolus or infusion over two minutes. Infusion at rate of 9µl/kg for 30 minutes. Cardiovascular study	-	-	Bolus, two minutes infusion or infusion over 30 minutes produced quantifiable ventricular opacification.
Dog RDR 98-17	Dogs received 10 or 40 µl/kg intravenously. Cardiovascular study	40 µl/kg	1.1	In vivo cardiovascular study to examine whether intravenously administered DMP 115 in dogs exposed to echocardiography would lead to hemolysis. Results showed lack of hemolysis.
Pig	0.5, 1.0, 2.0, 5 and 10 µl/kg	2	X 0.03	The cardiovascular safety profile of DMP-115 The highest dose evaluated produced a transient

	Cardiovascular study			increase in mean pulmonary pressure that returned to baseline within 8 minutes after dosing. The authors ascribed the elevation in pulmonary arterial pressure to thromboxane-mediated pulmonary hypertension caused by pulmonary intravascular response to injected particles.
Rabbits EB-ASO-10-94 & E-ASO-11-94	5, 15, 150, 225 μ l/kg Cardiovascular study	225 μ l/kg	5.6	Cardiovascular study in rabbits. No effect on systemic or pulmonary pressure. Although the report stated that arterial blood gases were unaffected, timing of arterial blood samples not optimal.
Cynomolgus monkey SC950020	0.05, 0.1 and 1 ml/kg. Cardiovascular study in monkey surgically implanted with radiotelemetric blood pressure and EKG transmitters.	0.1 ml/kg	1.62	The mean arterial pressure and heart rate were decreased by approximately 40 mm Hg (-38%) and 95 beats per minute (-46%) in one of the 4 animals at 1 ml/kg. The decreases rebounded within 10 minutes and returned to near baseline in 45 minutes. Continuous electrocardiographs were obtained from 24 hours before to 7 days after DMP-115 administration. Recording showed possible ventricular premature depolarization in one monkey and prolonged ST segments with alterations in the configurations of T waves that were consistent with electrolyte imbalance in another.
Dogs 2286	0.01, 0.1, 1 ml/kg. Cardiovascular studies	1 ml/kg	27	DMP-115 produced no change in respiration, blood pressure, heart rate, femoral blood flow, left ventricular pressure, LV dp/dtmax, or electrocardiogram PP-R and Q-T intervals. Furthermore, no marked change in pO_2 , pCO_2 or HCO_3^- concentration in blood or pH was seen.
Dog MR 14490	PK study Dose: 0.01, 0.1, 1 ml/kg Samples collected up to thirty minutes post-dose.			Pharmacokinetic evaluation of PFP in expired air and blood C_{max} 0.0258-0.0452 μ l PFP/mL. T_{max} 10-45 seconds. AUC 3.24 μ l.sec/ml K_a 0.011/sec $T_{1/2}$ 61 seconds Cl 19 ml/kg/sec Vd 1.72 L/kg
Rat RDR 98-12	PK study Rats administered 1 ml/kg (0.9 μ Ci/kg) 14 C-DMP 115			Pharmacokinetic, distribution, metabolism and excretion of 14 C-DMP 115 following intravenous administration. C_{max} (μ Ci/ml) 0.02 T_{max} (hr) 0.08 $t_{1/2}$ Initial (hr) 0.3 $t_{1/2}$ terminal (hr) 10.6 Vdss (L/kg) 0.17 CL (mL/min/kg) 0.25 AUC 0- ∞ (μ Ci \cdot hr/mL) 0.07

Appendix/attachments: Team Leader's Summary

CC:

Original NDA

DIVISION FILES

HFD-160/LANIYONU/SADRIEH

HFD-160/ZOLMAN/Jones

HFD-160/CHO

Team leader's review of NDA 21-064 (Definity):

Dr. Laniyonu has reviewed the pharmacology and toxicology sections of NDA 21-064. Most of the studies submitted to the NDA have been reviewed. The following is an overall assessment of the studies evaluated and described in Dr. Laniyonu's NDA review. For full details regarding those studies, please refer to the original NDA review. The team leader's review is not meant to replace the original primary review, rather it is intended to complement it. The opinions expressed are those of the pharmacology and toxicology team leader and are based on the evaluation of the data presented by the primary reviewer.

DMP 115 (MRX115, Definity) is an ultrasound imaging agent intended to enhance contrast in echocardiographic images. DMP 115 is a gas-filled (perfluoropropane), lipid-encapsulated (DPPA, DPPC and MPEG 5000 DPPE) microbubble suspension that is prepared on-site by shaking with a mechanical shaker provided by the sponsor.

The drug is to be administered intravenously. The clinical dose is 10-20 $\mu\text{l}/\text{kg}$. The maximum human dose tested to date is 50 $\mu\text{l}/\text{kg}$. The following is an overall summary of the pre-clinical program conducted in support of the safety of NDA 21-064.

The efficacy of DMP 115 as an ultrasound-imaging agent was characterized in several pharmacology studies. In an in vitro study, as bubble concentration increased (4000 particles/ml), the video intensity increased. Additionally, the same study (RDR-98-26) suggested that image acquisition in the second harmonic mode was significantly better (higher video intensity) than image acquisition in the fundamental mode.

The optimal dose for opacification of the left ventricle in dogs was reported to be 10 $\mu\text{l}/\text{kg}$ (RDR-98-20). The method of production of DMP 115 was not found to impact the assessment of left ventricular opacification and second harmonic imaging in the anesthetized dog (RDR-98-22).

The stability of DMP 115 in vivo was assessed in several studies. An in vitro study (RDR-98-15) showed that DMP 115 is more stable in blood (longer $t_{1/2}$ for signal decay) than in saline. Interestingly, image efficacy (as measured by video intensity) was not affected when DMP 115 was prepared 4 days prior to use, as compared to 5 minutes prior to use (RDR-98-21). In fact, the peak video intensity was higher when the 4-day old preparation of DMP 115 was used, even though the number of particles was significantly lower (in all size ranges) after 4 days. This puts into question the optimal dose of DMP 115 recommended by the sponsor. It would seem that if video intensity is not affected after 4 days of preparation, while bubble concentration has decreased, then perhaps the optimal dose selected for imaging may actually be unnecessarily too high.

Three methods of drug administration were assessed for optimal image intensity (RDR-98-23). A bolus, a 2-minute infusion and a 30-minute infusion in a 50 ml IV saline bag were compared. At the optimal dose selected (10 µl/kg), the image intensity lasted 20 minutes for the bolus, 11 minutes for the 2-minute infusion and 30 minutes for the 50 ml saline bag infusion. It should be pointed out however that the peak video intensity for left ventricular opacification with the 50 ml saline bag infusion was one half that for the other modes of administration.

Safety pharmacology studies were conducted in dogs, pigs, rhesus monkeys, cynomolgus monkeys (single and multiple doses) and chinchilla rabbits.

In three anesthetized dogs dosed with up to 10 µl/kg DMP 115, contrast enhancement was produced without reported changes in systemic blood pressures, pulmonary artery pressure or heart rate. However, in a study conducted with MRX 115 at doses up to 10 µl/kg in dogs, post mortem examination of the animals showed slight congestion of the posterior dorsal lobe of the lungs. In 12 open-chest dogs (J. Am. Soc. Echocardiography, 11:36-46; 1998), the size of a perfusion defect (coronary occlusion) measured during intermittent harmonic imaging correlated with technitium autoradiography ($r=0.83$) and postmortem infarct tissue staining ($r=0.92$). However, hemodynamic parameters and pulmonary gas exchange were not reported to be affected. In a safety pharmacology study, anesthetized dogs were dosed once with up to 1 ml/kg DMP 115. No changes in respiration, blood pressure, heart rate, femoral artery flow, left ventricular pressure and ECG tracings were reported. In the same study, no changes in pO_2 , pCO_2 , HCO_3 concentration or blood pH was reported.

In pigs a transient increase of 13 and 16 mm Hg in mean pulmonary artery was reported at 5 and 10 µl/kg (0.5 X maximal human dose based on body surface area). This was attributed to the presence of intravascular macrophages leading to thromboxane-mediated pulmonary hypertension. The origin of this statement is not clear.

In rhesus monkeys, doses of 0.5-100 µl/kg were used in transthoracic echocardiography and 20-50 µl/kg were used in open-chest epicardial echocardiography. These doses were not reported to affect heart rate, pulmonary artery pressure, systemic arterial pressure and pO_2 . In cynomolgus monkeys fitted with radiotelemetric transmitters for blood pressure and ECG measurements, doses of DMP 115 up to 1 ml/kg (16.2 X maximal human dose based on body surface area) were administered by intravenous injection. At the 1 ml/kg dose, a transient but significant decrease in mean arterial pressure (40 mm Hg or 38%) and heart rate (95 beats or 46%) were reported in one out of 4 animals. ECG tracings did not reveal abnormalities. When the same animals were used for a multiple dose study (1ml/kg DMP 115 followed by 3 consecutive

doses of 500 $\mu\text{l}/\text{kg}$ at 10 minute intervals), no changes in blood pressure, heart rate or ECG tracings were reported.

In anesthetized chinchilla rabbits dosed with DMP 115 at up to 225 $\mu\text{l}/\text{kg}$ (5.6 X maximal human dose based on body surface area), no hemodynamic effects (characterized by changes in systemic or pulmonary artery pressure) were reported. In conscious chinchilla rabbits dosed with DMP 115 at 75 $\mu\text{l}/\text{kg}$, no hematologic effects (characterized by effects on platelets, white blood cells, granulocytes and lymphocytes) were reported.

A safety pharmacology study conducted in mice and rats at up to 1 ml/kg was reported not to affect hexobarbital sleeping time in mice. Similarly, no changes in temperature, water and electrolyte balance were reported in rats.

As with all microbubble agents, there is a significant safety concern regarding the effects of DMP 115 to cause pulmonary emboli, via either physical (damming or occlusion) or biochemical (release of vasoconstrictive mediators) effects on the pulmonary microcirculation. With regards to the latter (the release of vasoconstrictive mediators), based on the study in pigs (where an increase in pulmonary artery pressure was reported) and the conclusions presented by the sponsor, pulmonary hypertension can result as a consequence of activated macrophages. Pulmonary artery pressure was assessed in dogs, rhesus monkeys, chinchilla rabbits and pigs. Increases in pulmonary artery pressure were not reported in these species. However, congestion of the posterior lobe of the lungs was reported in dogs dosed with 10 $\mu\text{l}/\text{kg}$ MRX115. In the case of pigs, the sponsor concluded that the effects on the pulmonary artery pressure were species-specific, however, these effects were reported at 0.5 times the maximal human dose, based on body surface area. It should be emphasized that the studies in monkeys and dogs used doses that ranged from 0.27 to 1.6 times the maximal human dose based on body surface area. This hardly constitutes an adequate safety margin for the prediction of potential pulmonary effects in humans, especially in those with preexisting pathological conditions, or infants with immature lungs. This matter will again be addressed in the conclusion of this review (effects on the microvasculature), and the adequacy of the dog as a model species in safety pharmacology studies will be argued, especially in light of the findings in toxicology studies (anaphylactoid response). The study in rhesus monkeys, which showed that DMP 115 did not increase pulmonary artery pressure, is considered an informative study. However, the doses used in that study were again too low to provide an adequate margin of safety for possible pulmonary artery pressure changes.

The kinetic profile of DMP 115 was studied in vivo in dogs and rats. The levels of PFP in the blood of dogs were evaluated after administration of 1000 $\mu\text{l}/\text{kg}$ IV. The $t_{1/2}$ was reported to be 61 seconds, the CL was 19 ml/kg/sec and the Vd was 1.72 L/kg. In the expired air, PFP was rapidly eliminated and measurable levels were detected at 5 seconds after dosing and peaked at 20-36 seconds. In rats,

the disposition of ^{14}C -DMP 115 was assessed after a 1 ml/kg IV bolus dose (radiolabel was on DPPE-MPEG). The $t_{1/2\alpha}$ was estimated at 18 minutes, while the $t_{1/2\beta}$ was estimated at 10.6 hours. The CL of ^{14}C -DMP 115 was 0.25ml/min/kg. At 72 hours post-dose, 71.5% of the drug was eliminated from the urine (90% of which was in the form of ^{14}C -MPEG 5000) and 10.5% was eliminated via the feces. At 4 hours post-dose, similar amounts of radioactivity were detected in the liver and plasma (17.8% and 17.2%), however, at 72 hours post-dose, the levels in the liver had declined to 3.7% of the initial dose.

Acute and repeat-dose toxicology studies were conducted in rats, dogs and monkeys.

In a single-dose toxicology study in rats (T 95-07-25), animals were dosed with up to 10 ml/kg (80 x maximal human dose based on body surface area), and within 30 minutes of dosing, 6 animals (out of 20) died in the high-dose group. Upon necropsy, the animals had congested lungs and an enlarged and congested liver. Animals receiving the degassed liposomes were not reported to show adverse effects. This indicated that the gas-filled microbubbles were responsible for the death of the animals. The acute NOEL was reported to be 5 ml/kg in rats. In dogs treated with a single IV dose of DMP 115 at up to 2 ml/kg (T 95-07-27), clinical signs of an anaphylactoid reaction (characterized by pale gums, urinary or fecal incontinence, salivation, hypoactivity, dyspnea, polypnea, cold to touch, tremors and/or abnormal respiratory sounds) were noted in all tested animals (no NOEL). In cynomolgus monkeys (1 animal/sex/group), a single dose of up to 10 ml/kg (162 x maximal human dose based on body surface area) resulted in an abnormal ECG in one female (abnormal and prolonged QRS with negative T wave). Additionally, the same female monkey had an elevation in serum GOT (AST), indicating possible liver damage (study T 97-07-51) or damage to the cardiac tissue (which is consistent with the ECG changes). In the high dose male monkey, there were increases in both SGOT (AST) and SGPT (ALT). The sponsor reported however that there were no histological lesions in the liver. In another single dose acute toxicity study in cynomolgus monkeys (T98-7-1), 3 monkeys per sex per group were administered either saline or DMP 115 at 3 ml/kg (48 times the maximal human dose based on body surface area). Abnormalities in the ECG profiles (transient ST-T segment depression, followed by ventricular extrasystoles, ventricular tachycardia, first degree and complete atrioventricular block and transient right bundle branch block) were noted in all animals within one minute of dosing with DMP 115. These changes were attributed to myocardial ischemia, by the veterinary cardiologist, since immune mediators such as histamine, tryptase and complement (C5b) were not elevated. Clinical signs (abnormal respiration, pale gums, and symptoms of parasympathetic stimulation) were noted and supplemental oxygen was administered for 3-6 minutes to all animals. Clearly, DMP 115 appears to cause significant cardiotoxicity at a single dose of 48 times the maximal human dose, based on surface area. The sponsor is recommended to determine the highest dose at

which these effects are not seen. Additionally, it should be stated that histopathologic analysis was not conducted, therefore it is not known if pulmonary lesions, such as microemboli, were formed in the cynomolgus monkey study described above. Moreover, one cannot conclude whether the cardiotoxicity is directly due to DMP 115 administration, or whether it is a secondary result in response to pulmonary lesions.

In a 7-day repeat-dose toxicology studies in rats (T 95-07-29), one rat died at 7.5 ml/kg on day 5 of dosing, however, in the same study, a control rat that received degassed liposomes died on day 8 of dosing. The NOEL was set at 2.5 ml/kg (one half the NOEL of the single-dose toxicology study in rats). In the high-dose group (7.5 ml/kg), the clinical signs observed in 4 animals, as well as the one that died, were dyspnea, pale body and prostration.

Four 28-day repeat-dose toxicology studies were conducted in rats. In those studies, the highest dose of DMP 115 used was 5 ml/kg (T 95-07-22). At 5 ml/kg, 4 rats died on days 16-26, exhibiting prostration, dyspnea, pale body and convulsions. In the animals that died, 2 had an enlarged liver and one had a liver mass. At least one of the deaths was attributed to an infarction of the hepatic lobe. Effects on the liver (congestion) had also been observed in the single dose toxicity study in rats. Additionally, an increase in lung weight (without corresponding histological findings) was noted in the low-dose group (0.1 ml/kg). In another 28-day study in rats (T 97-9-15), 10 out of 15 males and 8 out of 15 females died between days 1 and 16, within 6 minutes of dosing with 1 ml/kg DMP 115. In this study, rats that died on the first day of dosing died at one tenth the dose used in the single-dose toxicity study (1 ml/kg vs. 10 ml/kg). It should be stressed that the latter study did not evaluate many of the parameters usually assessed in toxicology studies, such as hematology, clinical chemistry and histopathology. In a follow-up study (T-97-11-05), 7 of 16 males and 5 out of 15 females dosed with 1.0 ml/kg died within 6 minutes of dosing, starting from day 4 through to the end of dosing (day 29). In the mid and high-dose groups, (0.3 and 1.0 ml/kg) and enlargement of the pulmonary lymph nodes was associated with macroscopic findings. However, microscopically, pulmonary changes characterized by hemorrhage, alveolar macrophage accumulation, perivascular and peribronchiolar eosinophilic infiltration, bronchiolar goblet cell hypertrophy and interstitial pneumonia were observed at all doses (including low-dose animals). In the final 28-day repeat-dose toxicology study in rats (T-98-3-46), lower doses of DMP 115 were administered for 28 days. The highest dose tested was 0.3 ml/kg. In this study, one male rat died on day 17 following dosing with 0.3 ml/kg (manufactured by a prior manufacturing process) and another male rat exhibited weakness, labored respiration and increased heart rate. Interestingly, the microscopic lung lesions seen in study T-97-11-05 were reproduced in the present study and at the same doses (0.1 and 0.3 ml/kg). Therefore, it can be concluded that the microscopic lung lesions seen at or above doses of 0.1 ml/kg DMP 115, were reproducible. In this study however, a lower dose-group was also included (0.03 ml/kg) and at that dose, no lung lesions were

reported. This low dose (0.03 ml/kg) corresponded to 0.2 times the human dose based on body surface area and is considered to be the NOEL for the repeat-dose rat toxicity studies. Therefore, in rats, significant mortality was noted at 1 ml/kg (8 times the maximal human dose based on body surface area) and microscopic pulmonary lesions were reproducibly seen in several studies at doses at and above 0.1 ml/kg (0.8 times the maximal human dose based on body surface area). It is expected that the larger bubbles would be filtered out in the lungs, therefore the microscopic lesions reported in the lungs may be an indication of possible occlusion of microvasculature due to damming by the larger microbubbles. Whether this would happen in humans, particularly those with preexisting pulmonary pathologies, and whether this would lead to noticeable adverse events remains to be determined. Nevertheless, the findings in rats (and to some extent pigs and dogs) indicate that a certain degree of caution is warranted when administering DMP 115 to subjects with preexisting pulmonary pathology. The lowest dose that killed rats after a single dose was 1ml/kg and the lowest dose that killed rats after multiple dosing was 0.3 ml/kg.

In dogs, studies that exceeded 7 days were not conducted. The highest dose tested was 2 ml/kg (T-95-07-28). In all studies, when dogs were given multiple doses of DMP 115 at 0.1 ml/kg and above, clinical signs of an anaphylactoid response were noted. A dose-response was seen, since the high-dose groups showed signs on day 1 of dosing, whereas the lower dose groups showed clinical signs on day 2. It should also be noted that control dogs dosed with degassed liposomes were reported to show clinical signs on day 4. The reader is reminded that in a 7-day repeat-dose toxicology study in rats (T 95-07-29), a control rat that received degassed liposomes died on day 8 of dosing. This may indicate that the PFP may enhance the toxicity, but that the liposomes themselves possess innate toxicity. However, in the single-dose toxicity study in rats, animals dosed with degassed liposomes did not die. In either case, the drug product is a combination of PFP and the liposomes, therefore results with the degassed liposomes provide useful information, however, the results obtained from administration of the complete drug product will be considered most relevant to the overall evaluation of the safety of DMP 115. In the dogs, a dose-dependent decrease in circulating-platelet counts was seen and histological examination showed pulmonary, hepatic and renal lesions. The decrease in circulating platelet may have resulted from margination of platelets, possibly in response to histamine release. Similar findings were seen in a study where the dogs were dosed with ~~0.1 ml/kg~~ DMP 115 for 7 days (T-95-06-34). In another study in dogs (T-95-08-42), the dose of DMP 115 was lowered to 0.01 ml/kg-0.1 ml/kg for 7 days and again an anaphylactoid response was reported after the 4th dose. The animals were challenged with 0.1 ml/kg DMP 115 after a 2-week washout period, and interestingly, clinical signs of an anaphylactoid response were accompanied with an increase in serum levels of histamine. Clearly, the dog seems to be particularly sensitive to an immune-mediated response to DMP 115. In conclusion, dogs did not appear to tolerate DMP 115 as evidenced by the anaphylactoid response elicited and the accompanying increases in serum

histamine levels. These effects reported in dogs occurred at doses as little as 0.01 ml/kg DMP 115, which is a dose that corresponds to 0.27 times the maximal human dose based on body surface area.

In cynomolgus monkeys dosed for 28 days with up to 10 ml/kg DMP 115, one out of 6 animals was found prostrate and died on day 22 of treatment (T-95-07-24). No histological findings were, however, reported in the animal that died. In a subsequent 28-day repeat-dose toxicology study in cynomolgus monkey (98-5-2), it was initially planned that the animals would be dosed with up to 3.0 ml/kg DMP 115. However, after the death of 6 females, the highest dose was lowered from 3.0 ml/kg and reset to 1.0 ml/kg. Prior to death, the animals that died exhibited similar clinical signs as those given a single dose of 3 ml/kg DMP 115 (study T98-7-1). Interestingly, these clinical signs were also reported in dogs, but in dogs, they were attributed to histamine release, whereas in the cynomolgus monkeys, neither histamine, nor complement (C5b) levels were elevated. Even though no other animals died in the multiple dose monkey study after the dose reduction, between days 15 and 22, 2 males and 2 females dosed with 1.0 ml/kg exhibited abnormal respiration, incoordination, pale gums, dilated pupils and salivation, immediately after dosing. At the 0.3 and 0.1 ml/kg doses, no clinical signs were reported, therefore the multiple dose NOEL in monkeys was set at 0.3 ml/kg (5 times the maximal human dose based on body surface area).

The sponsor conducted several special toxicology studies. An intracarotid administration of 0.1 ml/kg DMP 115 in anesthetized rats (RDR-98-05) was most likely aimed at addressing safety concerns in people with a right to left shunt. In the opinion of the reviewer (Dr. Laniyonu), the study was deemed inadequate because the animals were sacrificed only 5 minutes after dosing, therefore not allowing enough time for the development of damage which could be detected histologically. I agree with the primary reviewer's evaluation. In fact, the sponsor did not examine biochemical markers of neuronal cell damage (such as enzyme release), nor include a positive control group (such as agents known to cause disrupt the blood brain barrier, or induce lesions in the brain) to validate the study, gives little safety predictive value to this study.

The hemolytic potential of ultrasound energy was assessed in three studies. In an anesthetized dog model, animals were infused with 10 or 40 μ l/kg DMP 115 over 2 minutes and imaged using a 1.6/3.34 MHz echocardiographic probe. Hemolysis, characterized by an increase in plasma Hb, was not reported in blood collected from the femoral artery and left ventricle (R-98-17). It should be noted however, that the doses used in the latter study corresponded to 0.27 and 1.1 times the maximal human dose, based on body surface area, therefore not allowing for a margin of safety in assessing the hemolytic potential of DMP 115. A similar study conducted in vitro and in water (not blood), was published in radiology (32:10, 728-734, 1997). The study indicated that when DMP 115 microbubbles were exposed to ultrasound energy of 25 W/cm², bubble destruction resulted. In the published report, the bubbles were counted using an

accusizer, and ultrasound reflectivity was measured in both the standard and the harmonic modes. Declines in bubble reflectivity were associated with longer exposure to ultrasound, with continuous rather than intermittent imaging and with use of higher frequencies. The third study was also an in vitro hemolysis study, however, the study was deemed unacceptable. DMP 115 was mixed in vitro with human blood with a ratio of 10:1 (DMP 115: blood) and visually inspected for signs of hemolysis.

Two immunotoxicology studies were conducted to determine DMP 115's potential to induce active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) in guinea pigs. The first study (T-97-02-53) indicated that animals treated with DMP 115 and FCA (Freund's complete adjuvant) exhibited mild anaphylaxis in the ASA assay, but the PCA reaction was negative. The study was repeated (T-96-10-23) and the induction and challenge doses of DMP 115 were halved (0.1 ml for induction and 1 ml/kg or 0.1 ml/kg for the challenge). The results in the second study were similar to the first study, however, a dose response was noted. When the challenge dose was 1 ml/kg, 3 out of 5 animals showed mild signs of anaphylaxis. However, if the challenge dose was lowered to 0.1 ml/kg, 1 out of 4 animals showed mild signs of anaphylaxis. In conclusion, both immunotoxicology studies indicated that DMP 115 was positive in the ASA assay, but the PCA assay was negative.

The developmental toxicology studies consisted of one segment I (fertility and early embryonic development to implantation in rats) and 2 segment II (developmental toxicity) studies in both rats and rabbits. No significant findings were reported regarding the effects on fertility, early embryonic development and fetal malformations.

Irritation studies consisted of IV irritation, IM irritation and eye irritation studies. No perivascular irritation studies were conducted to assess potential toxicity resulting from extravasation.

The battery of genotoxicity studies consisted of an Ames assay with and without microsomal activation, a chromosomal aberration in CHO cells, an in vivo rat micronucleus assay and a TK⁺/ mouse lymphoma forward mutation assay. All genotoxicity assays were negative.

In conclusion, the preclinical development of DMP 115 consisted of animal studies aimed at determining the imaging efficacy and safety of the drug. In several animal species, such as dogs and guinea pigs, significant symptoms of an immunologic response were seen. These findings were reproducibly reported in multiple studies.

In rats, a high mortality rate was noted in multiple dose studies, however, death was reported in 30% of the animals after a single dose administration of 10 ml/kg DMP 115. In one of the multiple-dose rat toxicity studies, 2 rats died at 1 ml/kg after the first dose. This was at one tenth the dose of the single dose toxicology study in rats. In the rat toxicology studies, pulmonary lesions were reported at nearly all doses used. The NOEL for the pulmonary effects in rats was reported to be 0.03 ml/kg, which constitutes approximately 0.2 times the maximal human dose based on body surface area. In the single-dose toxicity study in rats, where mortality was reported at 10 ml/kg, the cause of death was attributed to hepatic lobe congestion. This indicates possible effects on the hepatic microcirculation and or Kupffer cell uptake capacity. However, pulmonary congestion was also reported in the same rat toxicology studies. The pulmonary congestion may also have contributed to the liver congestion, due to a backup in the circulatory system, resulting from right ventricular failure.

As with all microbubble agents, there is significant concern regarding the capacity of the echogenic microbubbles to cause pulmonary emboli. This would occur as a result of a blockage of the microcirculation, by bubbles trapped in small capillaries, leading to pulmonary lesions (microemboli) and increased pulmonary artery pressure. Preclinical results indicated that pulmonary artery pressure was increased in pigs dosed with 0.01 ml/kg DMP 115 (0.5 times the maximum human dose based on body surface area). The sponsor however ruled out the pig as an adequate model. At the same dose as that used in the pig study, no effects on pulmonary artery pressure were reported in dogs. However, in one study in dogs dosed with 10 µl/kg MRX 115 (0.25 times the maximal human dose based on body surface area), postmortem examination showed congestion of the posterior dorsal lobe of the lungs. However, due to the anaphylactoid response reproducibly seen in dogs, the dog may not have been an adequate model for studies with DMP 115, and it is likely that the anaphylactoid response would have contributed to the pulmonary congestion. In either case, the doses used in the studies assessing effects on the pulmonary artery pressure are considered to be too low to accurately provide an adequate margin of safety for the above-mentioned safety parameter. Clearly, rats showed a significant sensitivity to pulmonary pathology resulting from exposure to DMP 115 with a NOEL of 0.03 ml/kg (0.2 times the maximal human dose based on surface area). Perhaps monkeys may have provided a better model system to conduct a rigorous safety pharmacology study at higher dose multiples and with an adequate number of animals per group. It should however be noted that in multiple-dose toxicology studies, 6 monkeys died at doses above 1 ml/kg (16 times the maximum human dose based on body surface area). No lethalties were reported in single-dose toxicology studies in monkeys, at doses up to 10 ml/kg (160 times the human dose based on body surface area). However, at 3 ml/kg, ECG abnormalities (arrhythmias) were reported in all cynomolgus monkeys dosed, and these were attributed to myocardial ischemia. Whether the myocardial ischemia reported was a consequence of direct cardiotoxicity, or whether it was a secondary response resulting from pulmonary lesions, was not determined. The

use of special animal models, such as animals with induced pulmonary hypertension, would have been useful in assessing possible pulmonary effects of DMP 115 in target populations. If such an animal model were to be used, appropriate positive controls (such as microspheres) should be considered, in order to validate the model.

As with all microbubbles, there is concern regarding the ability of DMP 115 to adversely affect the microcirculation. This concern is tightly linked to the previously mentioned concern regarding effects of microbubbles on the pulmonary vasculature. Once the microbubbles pass the pulmonary circulation, they are systemically available and thus capable of getting trapped in other microvessels. No studies were conducted to address the effects of DMP 115 on the microcirculation. In light of the effects of DMP 115 in rats (congestion of lungs and liver), and in one dog study (congestion of the posterior lobe of the lung after dosing with 10 µl/kg), a study such as a hamster cheek pouch or cat mesenteric assay, would have elucidated possible effects of DMP 115 on the microcirculation.

No studies were conducted in immature animals in order to address safety issues in pediatric populations. Concern is raised by the findings of pulmonary lesions in rats (NOEL=0.03 ml/kg, or 0.2 times the maximal human dose based on body surface area), because infants are expected to have immature lungs and thus be potentially at greater risk of toxicity than adults. If DMP 115 is to be contraindicated in infants, this should be clearly stated and reasons provided for this decision.

An intra-arterial toxicity was conducted. The purpose of this study is thought to be to assess the toxicity of DMP 115 in patients with right to left shunts and who might be at higher risk to toxicity to the CNS. After intra-arterial DMP 115 was administered intra-arterially, however, the rats were terminated after 5 minutes of dosing and subsequently evaluated for histopathologic changes. The study was inadequate due to the short interval between dosing and termination. Therefore, an adequate intra-arterial study, using a positive control group, needs to be conducted. People with a right to left shunt should not be given DMP 115.

The immunotoxicology potential of DMP 115 was studied in guinea pigs and the drug was found to be positive in the ASA (acute systemic anaphylaxis) assay. However, it ^{SHOULD} be kept in mind that microbubbles are taken up by the cells of the RES (reticuloendothelial system) and the RES is considered to be part of the immune system. Evidence of the involvement of the RES was suggested by liver congestion reported in rats. No studies were conducted to assess the effects of DMP 115 on phagocytic cell function or on cytokine production by RES cells (macrophages). The only immune mediator measured was histamine, which was found to be increased in the serum of dogs undergoing an anaphylactoid response.

The sponsor has however adequately addressed the issue of bubble stability after activation, as it relates to imaging capacity. Even after 4 days, DMP 115 was shown to produce adequate imaging. After 4 days, the number of bubbles over 10 μm had not increased. This suggests that bubble coalescence may be minimal for DMP 115.

On the whole, the preclinical program for DMP 115 is rather comprehensive and a significant number of studies with a wide scope have been conducted. Several deficiencies in study design and conduct are noted. Additionally, certain areas of concern have not been addressed. All the deficiencies have been clearly listed in the pharmacology and toxicology review written by Dr. Laniyonu. If these deficient areas are addressed, the application is considered to be approvable.

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