response to injected particles. The influence of known inhibitors of thromboxane metabolism was not investigated.

The cardiovascular effect of DMP-115 was evaluated in both Rhesus and Cynomologus monkeys (study # SC 950020). Dr.Grauer and colleagues at Oregon Health Sciences center conducted the rhesus monkey study. The results indicated that at doses between 0.5-100 µl/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 Cynomologus monkeys 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. 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 possible 2 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, consistent with electrolyte imbalance in another. He concluded that the findings did not reveal any abnormalities attributable to treatment. DMP 115 did not induce hemolysis in the presence or absence of clinically relevant levels of ultrasound power.

PHARMACOKINETICS STUDIES:

MRI4490-F: In-vivo kinetics of the perfluoropropane component of MRX-115 in the dog. The study was carried out at GLP study. The lot number for the MRX-115 used was 744-71-0004. Vol.1.11 pp.6-60

Dr. Sadrieh reviewed this study for the IND.

The study examined the in-vivo kinetics of the perfluoropropane (PFP) component of MRX-115 following bolus intravenous injection to male Beagle dogs at three dose levels; 10, 100, 1000µl/kg of PFP (n=4) per group. The sponsor stated that PFP constitutes approximately 6.08% of the total dose translating to 0.608, 6.08, 60.8 µl/kg of PFP per dose. PFP concentration in blood and expired air collected at a total of 12 time points up to 30 minutes post-dose were determined by a gas chromatographic method.

It was not possible to determine pharmacokinetic parameters at 10 and 100µl/kg since concentration in the blood were below the detection limit. At the 1000µl/kg dose, the following parameters were obtained;

Cmax	0.0258-0.0452 µl PFP/mL.
T_{max}	10-45 seconds.
AUC	3.24µl.sec/ml
K_e	0.011/sec
T1/2	61 seconds
Cl	19ml/kg/sec
Vd	1.72 L/kg

PFP was eliminated in expired air following a single compartment model with a mean recovery of 117±42%. PFP eliminated from the lungs was detectable from as early as 5 second post-

dose. The levels in the expired air peaked at 20-36 seconds post-dose at the 1000µl/kg dose level. In 2 of the 4 dogs studied, PFP mean lung clearance at the high dose was estimated to be 24.2 ml/kg/sec.

It was concluded that when detectable, PFP was rapidly cleared from the blood and rapidly eliminated from the lung.

Reviewer's comments: Agree. The mean recovery of 117±42 is indicative of the difficulties encountered in the measurement of PFP concentration.

RDR 98-12: Pharmacokinetics, Distribution, metabolism and excretion of ^{14}C-DMP 115 following an intravenous dose to conscious Sprague Dawley rats. Study was conducted at DuPont Pharmaceuticals. Vol. 1.11 pp.61-88. GLP: No

Design: The study was designed to determine the pharmacokinetics, distribution, metabolism and excretion of total radioactivity following an intravenous dose of ^{14}C-DMP-115 in Sprague Dawley rats. DMP 115 is comprised of perfluoropropane (PFP) and three lipids, two of which are naturally occurring (DPPA and DPPC) and the third DPPE-MPEG. DPPE-MPEG was radiolabeled with carbon –14 to allow for the detection of the ^{14}C-DMP-115. Plasma pharmacokinetic was characterized in conscious rats following a 1 mL/kg (0.9µCi/kg) i.v. bolus injection of ^{14}C-DMP-115 (3 rats/sex/dose group). Plasma ^{14}C-DMP-115 was determined at 5 min, 15 min, 30min, 1, 2, 4, 6, 24, 48, and 72 hours post-injection. Tissue distribution and elimination of ^{14}C-DMP-115 were examined in another group of rats injected with the same dose as for the pharmacokinetic study (six rats per group, 5 groups). The animals were euthanized at 0.5, 4, 24, 48 and 72 hours post-injection. 23 tissues were collected from each animal at necropsy and the total radioactivity in each tissue determined by liquid scintillation counting. Metabolic fate of ^{14}C-DMP-115 was determined in plasma and urine by HPLC utilizing a flow through detector.

Results:

Pharmacokinetics Parameter[a]	
Cmax (µCi/ml)	0.02
Tmax (hr)	0.08
t1/2 Initial (hr)	0.3
t1/2 terminal (hr)	10.6
Vdss (L/kg)	0.17
CL (mL/min/kg)	0.25
AUC 0- ∞ (µCi•hr/mL)	0.07

[a] The time-activity data was fitted using JANA for windows software for statistical analysis.

^{14}C-DMP-115 was found to wash out from blood in a manner best fit by a 2 compartmental model (R=0.98). The half life of the initial and terminal phases were 0.3 and 10.6 hr. Volume of distribution at steady state was 0.17 L/kg and the systemic clearance was 0.25 ml/min/kg. The Cmax and Tmax were 0.2 µCi/mL and 0.08 hr, respectively. The area under the curve was 0.07µCi•hr/mL. (calculated from zero to infinity). Total radioactivity recovered at 0.5, 4, 24, 48 and 72 hours post-injection was 104%, 96%, 86%, 87& and 96% respectively. At 72 hours post

injection, the majority of radioactivity was in the urine (71.5%). 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% 0f the activity was in the form of ^{14}C-MPEG 5000 LPE. At 4hr plasma level are non detectable by HPLC. Urine metabolic profile showed 90% of the radioactivity in the form of ^{14}C-MPEG 5000. Fecal activity was below detection limit.

Conclusion: The sponsor concluded that DPPE [^{14}C]-MPEG5000 exhibits bi-phasic clearance from the blood and has a small volume of distribution. The kidney excreted 71% by 72 hours. It was metabolized into ^{14}C-MPEG 5000 LPE by the first hour post-injection.

Reviewer's comments: agree. The study was a repeat study. According to the sponsor, analysis of the test materials used in previous ADME studies under the direction of ImaRx Pharmaceutical Corporation (MRI 4312 & 4497) revealed radiochemical purity RCP's of < 50% and 79% for ^{14}C-labeled MRX-115 and ^{14}C-labeled MPEG5000 respectively. According to DuPont, the present study met acceptable RCP's criteria by the present standard.

MR1 4312: ^{14}C-PEG 5000 Products: Pharmacokinetics and disposition following intravenous administration to the rats. In-life phase February -March 1996. Vol.1.11. pp. 212-329. GLP: Yes

Dr. Sadrieh reviewed this study within the IND: However, results of radiochemical purity analysis showed RCP < 50% and 79% for labeled MRX-115 and ^{14}C-labeled MPEG5000 utilized for the study. These results indicated a need to repeat the ADME studies with ^{14}C-labeleled DMP 115 devoid of radiompurities. DuPont repeated the objectives of the study in study RDR98-12.

Despite this limitation, the results of the study assessing the pharmacokinetic and disposition of ^{14}C-labeledpolyethylene glycol 5000, dipalmityl phosphatidyl ethanolamine ^{14}C PED 5000 and ^{14}C-MRX 115 indicated no sex-related differences in handling and disposition of the labeled compounds. Peak plasma radioactivity was achieved within 5 minutes post-dose. Pharmacokinetic parameters including dose normalized AUC (DNAUC) estimated for the compounds were similar. Total recoveries ranged between 60% to 105 %. Principal route of elimination was the kidney completed by 24 hour. Biliary excretion was minimal.

MRI 4743: The effect of processing temperature on blood perfluoropropane levels.
This study was carried out at , The in-life phase of the study was May 12-16, 1997. GLP: Yes Lot No. 744-71-004

This study reviewed by Dr. Sadrieh for the IND was not submitted to the NDA. The major conclusion according to Dr. Sadrieh's review was that processing temperature of 37 °C or 45 °C did not affect the pharmacokinetics parameter of perfluoropropane in dogs. In her opinion, the fact that only two dogs were used for the study makes for difficulties in reaching conclusions, nevertheless, the results obtained were similar to those obtained in study T98-10-29.

PHARMACOKINETICS SUMMARY

An in vivo pharmacokinetic study of the PFP component of DMP-115 (study MR14490-F), demonstrated that PFP is rapidly eliminated from the lungs under 1 minute following a single compartment model. The mean recovery of 117±42 is indicative of the difficulties encountered in the measurement of PFP concentration.

Cmax	0,0258-0.0452 µl PFP/mL.
T_{max}	10-45 seconds.
AUC	3.24µl.sec/ml
K_e	0.011/sec
T1/2	61 seconds
Cl	19ml/kg/sec
Vd	1.72 L/kg

The pharmacokinetics, distribution and elimination of the ^{14}C-DMP-115 were characterized in rats. The following results best described by a 2 compartmental model were obtained:

Pharmacokinetics Parameter[a]	
Cmax (µl/ml	0.02
Tmax (hr)	0.08
t1/2 Initial (hr)	0.3
t1/2 terminal (hr)	10.6
Vdss (L/kg)	0.17
CL (mL/min/kg)	0.25
AUC 0- ∞ (µCi•hr/mL)	0.07

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 level are non detectable by HPLC. Fecal activity was below detection limit. The sponsor concluded that DPPE [^{14}C]-MPEG5000 exhibits bi-phasic clearance from the blood and has a small volume of distribution. Study MRI 4743 showed that PFP processing temperature 37 °C or 45 °C did not affect its pharmacokinetics parameters in dogs.

APPEARS THIS WAY ON ORIGINAL

TOXICOLOGY:

Acute Toxicology studies:

Study Title: Single dose IV toxicity study in rats with Aerosomes.
Study No: T95-07-25
Vol. 1:13, pages: 1-384
Conducting laboratory and location:
In-life phase: 7/27/94-8/11/94
GLP compliance: Yes
QA-Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary:

Male and female Sprague-Dawley rats, 10/sex/group were given a single intravenous bolus injection of 0.5, 5 and 10 ml/kg (X 4-80 MHD based on body surface area) Aerosomes™, degassed liposomes, or a solution without liposomes as controls followed by a 14-day observation period. Aerosomes was prepared by vigorous shaking for 1 minute using a Wig-L-Bug mixer. **All materials were administered within 4 hours of preparation.** Clinical signs were recorded 30-60 minutes after the last animal was dosed. **NOAEL was 5 ml/kg.** 5 males and 1 female rats died within 30 minutes post-dose at 10 ml/kg. At necropsy, histopathological examination revealed congestion of the lungs (four of five) and enlarged liver with congestion (five of five). Mild centrilobular vacuolization was noted in three of these animals. Similar findings were not noted in the female that died. No histopathological changes related to the administration of the test agent were observed in the surviving rats at necropsy performed at days 3 and 16.

Reviewer's comments: The mild degenerative changes noted within the lungs and the liver are compatible with inadequate perfusion or oxygenation. These changes suggest a rapidly occurring alteration in the cardiopulmonary status of the animals affected by Aerosomes. It seems likely that other vital organs including the brain may also been damaged by the hypoxia but without adequate time for the development of observable morphologic manifestations. The rapidity with which all these animals died after dosing strongly implicates the dosing process in the deaths. Whether that might be the result of mechanical or physical properties of the injected material or due to some chemically mediated interference with oxygen delivery system to vital organs can not be determined. It is noted that animals receiving just the excipient or degassed liposomes were not affected

Study Title: Intravenous tolerance study in beagle dogs with Aerosomes™.
Study No: T95-07-26
Vol 1.14, pages: 1-54
Conducting laboratory and location:
Date of study initiation: 05/19/94
GLP compliance: NO
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary:

The study examined the highest dose of Aerosomes™ that can be administered as a single intravenous injection. Beagle dogs (2/sex/group) were given repeated i.v. injection of Aerosomes™ 0.1, 0.5,1.5 and 5 ml/kg (2.7 –135 MHD based on body surface area) on days 1, 5, 8 and 12 or control solutions (liposome without the gas, days 1 and 5 or vehicle, days 8 and 12). Aerosomes was prepared by vigorous shaking for 1 minute using a mixer. **All materials were administered within 4 hours of preparation.** The animals were observed for 14 days. Necropsy was performed on day 15. There was no mortality. NOEL was 0.1 ml/kg. Clinical signs including pale gums, rapid respiration and/or heart rate, cold to touch, hyporeactivity and slight tremors were seen at 0.5 ml/kg or higher. Similar effects were seen in animals given 2 ml/kg of degassed liposomes. There were no histopathological abnormalities related to the administration of the test agent. This study did not conform to the format of any of the recommended preclinical toxicological study.

Study Title: Single dose toxicity study in beagle dogs with Aerosomes™.
Study No: T95-07-27
Vol 1.14, pages: 56-251
Conducting laboratory and location:
Date of study initiation: 07/29/94
GLP compliance: Yes
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary:

Beagle dogs (3/sex/group) were administered a single IV injection of 0.1, 1 or 2 ml/kg (X 2.7-54 MHD) based on body surface area) DMP-115 via the cephalic vein. Two control groups received either the placebo formulation without liposomes or liposomes without the gas. Terminal necropsies were performed on day 15 post-dose. Clinical signs including pale gums, urinary or fecal incontinence, salivation, hypoactivity, dyspnea, polypnea, cold to touch, tremors, and/or abnormal respiratory sounds were observed in all treated animals. Urinary and fecal incontinence were also noted in the group of animals that received the degassed liposomes. All the effects were observed on day 1 only. No other compound-related effects were noted in body weight, food consumption, heart rate or clinical pathology. At necropsy, no changes in organ weights or gross and microscopic pathology were observed. **No NOEL** was established.

Study Title: Single intravenous dose toxicity study of MRX-115 in Cynomolgus monkeys.
Study No: T97-09-51
Vol 1.14, pages: 252-293
Conducting laboratory and location:
Date of study initiation: 12/10/96
GLP compliance: Yes (
QA- Report Yes (x) No ()

Dr. Sadrieh reviewed this study for the IND.

Study summary.

Cynomolgus monkeys (1/sex/group) were given MRX-115, 1 or 10 ml/kg (X16.2 or 162 MHD based on body surface area) or saline via intravenous injection into the sephanous vein at 5 ml/min. Animals were observed for 15 days post-dosing. Electrocardiograms were recorded 1 and 24 hours post-dosing and blood samples for clinical evaluation obtained 1, 3, 7, and 14 days post-dosing. Necropsy and histopathological examination were done on day 15. No death was reported during the study. Changes interpretable by the contract laboratory as left block (abnormal QRS complexes and prolongation of QRS interval, negative T waves in Lead 1) were reported on a few occasion in the female that received 10 ml/kg. The contract laboratory opined that such changes occur spontaneously in monkeys. Serum chemistry showed a slight elevation in plasma GOT and GPT in male and in GOT in female at 10 ml/kg on the day of dosing. No other changes in chemistry, hematology or urinalysis were noted. No significant treatment-related macroscopic or histopathologic findings were noted. It is noted that the timing of EKG recording was not optimal for observing acutely induced changes in EKG caused by MRX-115 administration.

Study Title: An acute intravenous injection toxicity study in Cynomolgus monkeys followed by a 14-day recovery period
Study No: T98-06-02
Vol. 1.15, and pages: 1-213
Conducting laboratory and location:
Date of study initiation: 06/23/98
GLP compliance: Yes
QA- Report Yes (x) No ()
Drug: DMP 115 lot: 4509Z

Methods:

Group	Dose (ml/kg)[a]	Males[b]	Females[b]
Saline ~~control~~	1	6	6
Formulation ~~control~~[c]	1	6	6
DMP-115	1	6	6

[a]**intravenous injection between 30 and 60 minutes after mixer activation of DMP115**
[b]Three monkeys per/sex/group were necropsied 48 h after dosing
[c]Formulation control = all components of DMP 115 without mixer activation

Ages: 2-4 years, weight: 2.3-3.8 kg

Observations and times: Mortality (twice daily throughout the study); clinical signs examination continuously for 5 minutes after initiation of dosing and a detailed clinical

examination at approximately 30-60 minutes post-dose; detailed clinical examination once daily, body weight twice daily; electrocardiography, twice pretreatment and on day 1, ≈ 0.5 hours after initiation of dosing, hematology and clinical biochemistry (once pretreatment and on day 3; necropsy days 3 & 15

Results:
Clinical signs: No significant findings

Body weights: No effect

Food consumption: no effect

Electrocardiography: No effect as certified by a veterinary cardiologist

Hematology: No effect

Clinical chemistry: No effect

Gross pathology: No treatment related effect

Histopathology: no treatment-related effect

Key Study Findings: No treatment-related adverse event reported.

Reviewer's comments: The study design is deficient. It examined the acute toxicity profile of DMP-115 at a single dose of 1 ml/kg whereas a minimum of three-dose level is incorporated into toxicity study design. The low dose should ideally produce no adverse effect. The high doses should be selected to produce overt signs of moderate to severe toxicity. This design enables one to determine potential target organs in clinical study. **NOEL for the study was 1 ml/kg.**

Study Title: Single dose intravenous injection tolerance study in cynomolgus monkeys
Study No: T98-7-1
Amendment # 4to IND page:144
Conducting laboratory and location:
Date of study initiation: 09/25/98
GLP compliance: No
QA- Report Yes (x) No ()

Methods:

Group	Dose (ml/kg)[a]	Males	Females
Saline control	3.0	3	3
DMP 115	3.0	3	3

[a]Intravenous injection (between 30 and 60 minutes after mixer activation of DMP 115 at a rate of 3 mL/min. Drug, lot#, 4509Z

Observations and times:
Clinical signs: Continuously for the first 30 minutes, 60 minutes and 24 hours after treatment initiation.

Body weights: Last week of acclimation and on day of dosing.
EKG: Twice during the pretreatment period. For the control group approximately 5 minutes prior to dosing, continuous for the first five minutes post dose followed by 10 seconds recording every three minutes from approximately 5 to 30 minutes. For the DMP115 group, continuous from about 5 minutes prior to dosing up to 15 minutes following dose initiation thereafter 10 seconds recording every three minutes till 30 minutes post dose. Systolic blood pressure was measured with brachial cuff.

Hematology: blood samples collected at approximately1, 3, 19 and 30 minutes post dose for hematology, plasma histamine, tryptase and complement levels.
Clinical chemistry: Not conducted

Urinalysis:	Not conducted
Organ weights:	Animals were not sacrificed
Gross pathology:	Animals were not sacrificed
Organs weighed:	Animals were not sacrificed
Histopathology:	Animals were not sacrificed

Results:
Clinical signs: There were no deaths. Clinical signs exhibited by animals that received 3.0 ml/kg DMP 115 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. No conclusion could be reached as to effect of DMP 115 on systolic blood pressure changes as there was a high degree of intra and inter animal variability.

Electrocardiography: All animals administered DMP 115 at 3ml/kg showed abnormal electrocardiograms. Each monkey demonstrated electrocardiographic evidence of ST-T segment depression within one minute of the start of infusion. This was followed by the development of ventricular extrasystoles, ventricular tachycardia, first degree and complete atrioventricular block and the transient development of right bundle branch block. 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.

Clinical Pathology: No DMP 115 related alterations of hematological parameters were observed although there was a high degree of variability among values for controls and DMP 115 treated monkeys. There were no DMP-related changes in histamine, tryptase and complement (SC5b-9) levels between control monkeys and treated monkeys.

Conclusions: Administration of DPM 115 to monkeys was characterized by abnormal respiration, pale gum, decreased muscle tone, salivation. The animals required supplemental oxygen. The ECG showed early ST-T segment depression followed by arrhythmias.

Reviewer's comments: Agree with the study conclusions. According to the consultant veterinary cardiologist, the acute development of ST-T segment depression followed by cardiac arrhytmias returning to normal sinus rhythm and gradual disappearance of ST-T segment changes is typical of myocardial ischemia.

Repeat-dose Toxicity Studies

Study Title: 7-Day intravenous toxicity study in rats with Aerosomes™.
Study No: T95-07-29
Vol 1.16, pages: 1-264
Conducting laboratory and location:
Date of study initiation: 07/29/94
GLP compliance: Yes
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary.

The study examined the acute toxicity of Aerosomes™ when administered as a slow bolus injection in rats for seven days. The animals (5/sex/group) were administered Aerosomes™ at doses of 0.5, 2.5 and 7.5 mL/kg. Two control groups received either the placebo formulation without liposomes or liposomes without the gas. Clinical observations and food consumption measurement were performed daily, body weights on days 1 and 8. Hematology and clinical chemistry were conducted on day 9 prior to necropsy. One male administered 7.5 ml/kg and one female treated with degassed liposomes died shortly after dosing on day 5 and 8 respectively. Clinical signs noted in the high dose male that died were dyspnea, pale body, and prostration. The dead animals exhibited no gross necropsy findings. In the 7.5 ml/kg group, one male and three females exhibited transient dyspnea, polypnea, pallor and /or prostration immediately after dosing on days 4-6. With the exception of incidental increases reported in lymphocyte counts as well as a decrease in serum chloride levels in male rats administered 2.5 ml/kg Aerosomes, no other effects were noted in any of the parameters assessed. The NOEL is reported to be 2.5 ml/kg.

Study Title: 28-Day intravenous toxicity study of Aerosomes™, with a recovery period in rats.
Study No: T95-07-22
Vol. 1.17, pages: 1-321 & Vol. 1.18 pages 1-318
Conducting laboratory and location:
Date of study initiation: 07/29/94
GLP compliance: Yes
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary.

Male and female Sprague Dawley rats 15/sex/group were given i.v. bolus injection of Aerosomes™ at doses of 0.1, 1.0, and 5.0 ml/kg for at least 28 days. Two other groups of animals received either an excipient solution or a suspension of liposomes without the incorporated gas. Animals were observed daily for signs of toxicity. The terminal sacrifice and recovery sacrifice was performed on the surviving animals on day 29/30 and day 58 respectively. At 5.0 ml/kg 1 male and 3 females died prior on days 16-26. They exhibited the following clinical signs: prostration, dyspnea, convulsions, and pale body. Two of these animals had an enlarged liver, one animal had a liver mass and one animal had a pale area in the pituitary gland. The cause of death of one of the animals was reported to be infarction of the

hepatic lobe. The cause of death of the other animals could not be determined histopathologically. The surviving animals showed no clinical signs. No other adverse effects on any of the parameters measured are reported. It is however reported that increased lung weights were noted for the low dose (0.1 ml/kg) females at the end of the treatment period and in the low and high dose (0.1 and 5 ml/kg) at the end of the recovery period. Histopathological evaluation did not reveal morphological alterations. The NOEL was 1.0 ml/kg.

Study Title: DMP 115: 28-day intravenous dose toxicity study in Sprague Dawley Rats
Study No: T97-9-15
Vol :19, pages: 1-58
Conducting laboratory and location:
Date of study initiation: 09/23/97
GLP compliance: No
QA- Report Yes (x) No ()
Methods:

	Dose Volume mL/kg/day		Number of animals	
Group	Day 1	Beginning Day 2[a]	Males	Females
1 Vehicle cont[b]	3.0	1.0	15	15
2 DMP 115	0.3	0.3	15	15
3 DMP 115	1.0	1.0	15	15
4 DMP 115	3.0	0.1	15	15

Dosing:
[a] Dose volumes for groups 1 and 4 were changed prior to dosing on day 2 as a result of mortality in group 3 (one male and one female following dosing on day 1). Group 4 rats were not dosed on day 1. Group 1 terminated on day 8 due to excessive mortality. Entire study was terminated on study day 17 due to excessive death.
[b]Vehicle control =0.9% sodium chloride in water:propylene glycol: glycerol (80:10:10, v/v/v).

DMP 115 was administered intravenously within 30 minutes of mixer activation at approximate rates of 0.6, 1.8 and 6.0 ml/min at doses of 0.1, 0.3, and 1.0 ml/kg respectively

Observations and times:

Clinical signs: continuous observation first 30 minutes post-dose followed by a single observation 30-60 minutes post-dose daily.
Mortality checks: once daily
Body weights: twice weekly
Food consumption: qualitative evaluation twice weekly
Ophthalmoscopy: pretreatment period only

Clinical chemistry & hematology: Not performed
Necropsy: upon death. Entire study terminated on study day 17 due to excessive death
Histopathology: Not conducted, however protocol-specified tissues were archived although no histological evaluation was performed.

Results:

Mortality: 10 of 15 males and 8 of 15 females given 1.0 mL/kg/day of DMP 115(with mixer activation) died after dosing with most of the death occurring within 6 minutes of dosing. The study date that death occurred is given below

Study day	No of death
1	2
3	2
5	2
6	2
7	4
10	1
11	2
13	1
15	1
16	1

Clinical signs observed immediately prior to death included abnormal respiration, ataxia, decreased motor activity, and loss of righting reflex. The sponsor considered the death to be related to DMP 115

Clinical signs: None observed at either 0.1 or 0.3 mL/kg/day. At 1.0 ml/kg/day, clinical signs similar to those observed in the animals that died were noticed in 15 of 15 males and 14 of 15 females. The animals either recovered 30-60 minutes post-dose or died.

Body weights: No effect
Food consumption: No effect
Hematology: Not conducted
Clinical chemistry: Not conducted
Urinalysis: Red urine was observed in one male and 3 females given 1.0 ml/kg/day on not more than 3 occasions. Urinalysis was not done

Organ Weights: Not stated
Gross pathology: No macroscopic changes.
Histopathology: Not conducted

Conclusion: The sponsor concluded that daily injection of DMP 115 to Sprague Dawley rats resulted in a high incidence of mortality and clinical sign of toxicity. The occurrence of death immediately after dosing was suggestive of an acute reaction to the test article.
The sponsor suggested that because the rats were injected within 30 minutes of DMP 115 activation, the toxicity might be associated with the level of perfluoropropane gas present in the product at the time of use compared to the time of activation.

Reviewer's comments: This non-GLP study is similar to the next study (T97-11-05) to be reviewed. The only reason a written review was done was to document the significant mortality observed and correlate the study findings with that of T97-11-05.

Study Title: DMP115 28-day intravenous injection toxicity study in Sprague Dawley rats
Study No: T97-11-05
Vol.1.19, 20, pages: 59-326, 1-295
Conducting laboratory and location:
Date of study initiation: 11/12/97
GLP compliance: Yes
QA- Report: Yes (x) No ()
Methods:

Group	Dose volume mL/kg/day	Males	Females
Vehicle control[a]	1.0	15	15
Formulation control[b]	1.0	15	15
DMP115 (low dose)[c]	0.1	15	15
DMP115 (mid dose)	0.3	15	15
DMP115 (high dose)	1.0	16[d]	15

[a]Vehicle control ==0.9% sodium chloride in water:propylene glycol:glycerol (80:10:10)
[b]Formulation control+all components of DMP 115 without mixer activation.
[c]DMP 115 with mixer activation. [d]An additional male was added as replacement for a death on day 4.

Dosing: DMP 115 was administered intravenously (tail vein, slow bolus injection) within 30 –60 minutes of mixer activation.

Observations and times:

Mortality:	Twice daily
Clinical signs:	Continuous for the first ten minutes post-dose, thereafter twice daily
Body weights:	wice weekly
Food consumption:	Qualitative evaluation thrice weekly
Ophthalmoscopy:	Pretest and week 4
EKG:	Not done
Hematology & Clinical chemistry:	Terminal necropsy
Urinalysis:	week 4
Gross pathology & Histopathology:	Terminal necropsy

Results:

Mortality: 7 of 16 males and 5 of 15 females given 1.0 mL/kg/day of DMP 115 died after dosing with death occurring immediately (within 6 minutes) after dosing. The study date that death occurred is given below. Clinical signs observed immediately prior to death included abnormal respiration (↓ rate, labored, stopped, 5/10), loss of righting reflex (5/10), convulsion (4/10) and loss of consciousness (3/10). The contract laboratory considered the death to be related to DMP 115

Study day	No of death
4	1
7	1
8	1
11	1
14	1
16	2
17	2
24	1
26	1
29	1

Clinical signs: None observed at either 0.1 or 0.3 mL/kg/day. At 1.0 ml/kg/day, transient clinical signs similar to those observed in the animals that died were noticed in all the animals.

Body weights:No effect

Food consumption: No effect

Ophthalmoscopy: No DMP-11 related effect

Hematology: DMP-115 related increases (2.6-3.8 fold) in absolute mean eosinophil count occurred in male and female rats administered 0.3 or 1.0 ml/kg/day.

Clinical chemistry: Mean chloride concentration for both formulation control and DMP-115 groups were at or above the upper limit of the reference range 73-93% of individual males and 80-90% of individual females compared to the vehicle controls (20-27%).

Urinalysis: No DMP 115 related effects.

Organ Weights: The marginal changes noted were not considered to be of toxicological significance.

Gross pathology: Macroscopic enlargement of pulmonary-associated lymph nodes observed in 0.3 and 1.0 ml/kg/day dose groups. Other macroscopic findings I in the lungs and trachea included dark areas, mottled and/or uncollapsed lungs and presence of pale frothy fluid in the trachea.

Histopathology: Lesions were seen in the lungs of rats given DMP-115 at 0.1, 0.3,and 1.0ml/kg/day with severity increasing with dose. They were described as hemorrhage, alveolar macrophage accumulation, perivascular and peribronchiolar eosinophilic infiltration, bronchiolar goblet cell hypertrophy/hyperplasia and interstitial pneumonia. Extramedullary hematopoiesis in the spleen of males 4/15 but not females dosed with 1.0 ml/kg/day. **No NOEL** was established as pulmonary changes were seen in all dose groups.

Conclusion: Daily intravenous injection of activated DMP-115 to Sprague-Dawley rats resulted in mortality at 1.0ml/kg/day (8X MHDbsa). Death occurred immediately after dosing and was preceded by signs suggestive of an acute reaction to the test substance. Pulmonary lesions were the primary histologic-related findings.

Reviewer's comments: Agree with the study conclusions. No NOEL was established.

Study Title: DMP 115: Acute/28-Day intravenous injection toxicity study in Sprague-Dawley rats followed by a 4-week recovery period
Study No: T98-3-46
Vol: 1.21-22, and page:1-387, 1-353
Conducting laboratory and location:
Date of study initiation: 4/6/98
GLP compliance: Yes
QA- Report Yes (x) No ()

Methods:

Group	Dose volume mL/kg/day	Males	Females
1Saline control	0.3	40	40
2 Formulation control	0.3	40	40
3 DMP115 (CMP)	0.03	40	40
4 DMP115 (CMP)	0.1	40	40
5 DMP115 (CMP)	0.3	40	40
6 DMP115 (PMP)	0.3	40	40

CMP= current manufacturing process; PMP= prior manufacturing process

Dosing: DMP 115 was administered intravenously (tail vein, slow bolus injection) within 30 –60 minutes of mixer activation.

Ten rats/sex/group were necropsied 48 hours and 2 weeks after being given a single dose of DMP 115.
Ten rats /sex/group were necropsied after 28 daily doses of DMP 115 and after approximately 1 month recovery period.
Formulation control = all components of DMP 115 without activation

Observations and times:
Mortality: Twice daily

Clinical signs: 30-60 minutes after dose administration. For rats, necropsied 48 hours after a single dose, detailed examination was performed prior to necropsy. For the 14- and 28-Day recovery study, examinations were conducted once weekly.

Body weights: Pre-dose. Thereafter twice weekly

Food consumption: Qualitative evaluation thrice weekly

Electrocardiography:	Not examined
Hematology & Clinical chemistry:	Terminal necropsy
Urinalysis:	week 4
Gross pathology & Histopathology:	Terminal necropsy

Results:

Mortality: 1 male administered 0.3 ml/kg/day (prior manufactured process) died within one minute on day 17 following dosing. The animal was uncoordinated and lost consciousness. 1 male in the saline control group was found dead on day 50. There were no clinical signs exhibited prior to death.

Clinical signs: One male in the 0.3 ml/kg/day (CMP, single dose study) exhibited transient signs of weakness, labored respiration and increased heart rate that returned to normal within 30 sec. No other clinical signs were apparent in rats given formulation control or dosed ≤ 0.1 ml/kg/day.

Body weights: No effect

Food consumption: No effect

Electrocardiography: Not examined

Hematology:

Single dose followed by 48 hours or 14 day observation period: No treatment-related changes observed.

28 days dosing:
Mean eosinophil counts that were 1.8-3.1 folds greater than that of the saline control group were noted for groups 4, 5 and 6. No treatment related changes were noted for the 0.03ml/kg/day group.

28 days dosing followed by 28 days recovery period:
No treatment related changes.

Urinalysis: Not reported

Organ Weights: Not reported

Gross pathology:

Single dose followed by 48 hours or 14 day observation period: No treatment-related changes observed.

0.03 ml/kg/day: No treatment-related changes

0.3 ml/kg/day: enlargement of the bronchial lymph nodes

Histopathology:

0.1 and 0.3 ml/kg/day: Microscopic lung lesion observed with both manufacturing process. Lesion was dose-dependent including a combination of the following; hemorrhage, alveolar macrophage accumulation, eosinophil infiltration, bronchiolar goblet cell hypertrophy/hyperplasia and interstitial pneumonia. Following 4 weeks of treatment and 4 weeks recovery, DMP-related changes were seen only in one 0.3 ml/kg/day (original manufacturing process) male treated rat.

Conclusions: The sponsor concluded that no lung or lymph node changes were seen in rats following a single intravenous dose at ≤ 0.3 ml/kg/day. Lungs and lymph node changes were observed following 28 days of DMP-115 administration at doses ≥ 0.1 ml/kg/day ( ). No difference in the histopathology profile between the two manufacturing processes. NOEL for the lung effect was 0.03ml/kg/day. **Histopathological changes were reversible following a one-month recovery period.**

Reviewer's comments: Agree.

Study Title: 7-Day intravenous toxicity study in dogs with Aerosomes™.
Study No: T95-07-28
Vol 1.23, pages: 1-266
Conducting laboratory and location:
Date of study initiation: 07/29/94
GLP compliance: Yes
QA- Report Yes () No (x)

Dr. Dundore reviewed this study for the IND.

Study summary.

All materials were administered within four hours of preparation.
Dogs (3/sex/group) were administered intravenous injection of 0.1, 1, 2 ml/kg DMP115, the placebo formulation without liposomes or liposomes without the gas via the cephalic vein daily for 8 consecutive days. Animals were observed for toxicity. Blood samples were obtained for hematology and clinical chemistry prior to study initiation and at necropsy. Terminal necropsies were performed on day 9 post-dose. All animals survived the study. Clinical signs observed in all the groups included pale gums, urinary or fecal incontinence, salivation, hypoactivity, prostration, polypnea, cold to touch, tremors, lacrimation, miosis and/or abnormal respiratory sounds. One high dose female required epinephrine and steriod for recovery. The clinical signs were noted within one hour after dosing beginning on day 1 in the high dose group, day 2 in the low and mid dose groups and day 4 in the group of dogs given a suspension of liposomes without the incorporated gas. Animals appeared to be normal within 6 hours. There appeared to be statistically significant decreases in circulating platelets in the high dose males with a trend towards dose-dependent decrease in platelet counts observed in all animals that received the test agent. Histomorphological abnormalities noted included peribronchial/perivascular lymphoid infiltrates and foci of pneumonitis, foci of mononuclear cells in the liver and early chronic progressive nephropathy. **No NOEL** was established.

Study Title: One-week intravenous toxicity study in the dog.
Study No: T95-06-34
Vol 1.24, pages: 1-76
Conducting laboratory and location:
Date of study initiation: 12/8/94
GLP compliance: No
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary.

Dogs (3/sex/group) were administered intravenous injection of MRX-115 0.1 ml/kg/day or saline 0.1ml/kg/day for 7 consecutive days and the animals were sacrificed on day 8. The animals did not manifest any clinical signs on days 1 and 2 of treatment. Beginning on day 3, clinical signs characterized by cyanosis, depression, defecation and staggering gait were exhibited by the animals. The signs occurring within 30 minutes post-dose. Additionally, erythema of the ear pinna and sporadic instances of urination and hyperventilation were also noted. Clinical pathology data showed decreased platelets, WBC and neutrophils beginning on day 2. Increases in lymphocytes, reticulocyes and eosinophils were also noted, but without a consistent pattern. Organ weights were not affected except for a decrease in spleen weights in the males where histopathological examination showed low numbers of erythrocytes and higher number of leukocytes in the vessels.

Study Title: 7-Day repeated toxicity study in Beagle dogs with MRX-115.
Study No: T95-08-42
Vol. 1.24, pages: 77-251
Conducting laboratory and location:
Date of study initiation: 2/21/95
GLP compliance: Yes
QA- Report Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary:

Doses used were 0.01, 0.03 and 0.1 ml/kg injected in the cephalic vein once daily for 7 days. Control dogs received either saline, placebo for MRX-115 or liposome. Following a two-week wash out period, the dogs on MRX-115 were given a single challenge dose of 0.1 ml/kg. The other animals received appropriate control. The day following the challenge dose, the animals were sacrificed and subjected to necropsy. Regular toxicology measurements were assessed as well as serum protein studies including electrophoresis and plasma histamine. All animals appeared normal after dosing on days 1-3. Beginning on day 4 of treatment, clinical signs were noted following dosage administration in all animals receiving MRX-115, including degassed liposomes. These were similar to signs observed in the previous study. Following the administration of the challenge dose, animals in the group receiving the study drug showed post dose ataxia, dyspnea, red coloration of peioral area, ears or entire body, and injected sclera. These signs disappeared within 6 hours of dosing. Similar clinical signs were noted 30 minutes after administration of the challenge dose of MRX-115 on day 22 in the MRX-115 treatment groups, but not in the control group given degassed liposomes. Red blood cell count,

hemoglobin concentration and hematocrit were significantly lower in dogs treated with MRX-115 when compared to control animals on day 8. An increase in histamine was seen at day 4, and after the challenge dose. A NOEL was not established. It is possible that an immunologic mechanism may be responsible for some of the findings.

Study Title: 28-Day intravenous toxicity study in Cynomolgus monkeys with MRX 115
Study No: T95-07-24
Vol.1: 25, and pages: 1-334
Conducting laboratory and location:
Date of study initiation: 11/22/94
GLP compliance: Yes
QA- Report: Yes (x) No ()

Dr. Dundore reviewed this study for the IND.

Study summary:

All materials were administered within four hours of preparation.

MRX 115 (0.15,1, or 10 ml/kg), or saline (10ml/kg) or excipients for MRX-115 10 ml/kg) was administered intravenously to Cynomolgus monkeys (3/sex/group) daily for 28 days. Animals were observed daily for signs of toxicity. Blood samples were obtained periodically before and after the initiation of the dosing regimen for hematology, coagulation, clinical chemistry and plasma histamine analysis. Opthalmoscopic and electrocardiographic examination were performed prior to and at the end of treatment. At the end of treatment animals were sacrificed and subjected to necropsy and gross examination. Selected tissues and tissues were preserved for histomorphological examination. One of the 6 animals given MRX-115 at 10 ml/kg died after dosing on day 22. The animal was found prostrate prior to death. Remaining animals survived to study termination. No other signs of toxicity were observed. No treatment related effect on hematology, coagulation clinical chemistry, body weight or electrocardiography. Although it is noted that the timing of EKG evaluation may have precluded observing acute changes in the EKG occurring during or immediately following the administration of MRX-115. MRX-115 did not affect plasma histamine level. The cause of death for the animals that died after receiving MRX-115, 10 ml/kg was not evident from the histopathological analysis.

Study Title: DMP-115: A 28-Day intravenous injection toxicity study in Cynomolgus monkeys followed by a 28-Day recovery period.
Study No. 98-5-2
Vols: 1.26-27, and page: 1-271, 1-314
Conducting laboratory and location:
Date of study initiation: 06/16/98
GLP compliance: Yes
QA- Report: Yes (x) No ()

Methods:
All materials were administered between 30-60 minutes after mixer activation.

Group	Dose volume mL/kg/day[a,b]	Males[c]	Females[c]
1Saline control	1.0	6	6
2 Formulation control	1.0	6	6
3 DMP115	0.1	6	6
4 DMP115	0.3	6	6
5 DMP115	1.0	6	7[d]

[a]Intravenous injection at a rate of 3 ml/min
[b]Dosing was initiated on consecutive days with 3 monkeys/sex/group dosed on each day. The dose for groups 1, 2, and 5 was changed from 3.0 to 1.0 mL/kg/day prior to dosing on the second day of the study as a result of the death of one female given 3.0 ml/kg on the first day of dosing. The dose of group 4 was changed from 0.5 to 0.3 ml/kg/day on the second day of the study.
[c]3 monkeys/sex/group were necropsied after 28 days of treatment (Day 29); the remaining monkeys were necropsied following a 28-day recovery period with the exception of one female animal in the 1.0 ml/kg group sacrificed on day 17 due to broken bones (fracture of the.
[d]An additional female was added as a replacement for a death on the first day of dosing.
Lot No:4509Z. For formulation control, vials not mixed before administration.

Observations and times:
Mortality: Twice daily

Clinical signs: Continuous for about 5 minutes post-dose, and at 30-60 minutes post-dose

Body weights: Twice weekly

Food consumption: Qualitative assessment only

Ophthalmoscopy: Once prior to study initiation and at week 4.

EKG: Twice during acclimation and once during weeks 1 and 4 at approximately 0.5 hours after initiation of dose

Hematology &Clinical chemistry: Twice during the acclimation period, and once prior to dosing during weeks 2 and 4 of the treatment period.

Urinalysis: Once during acclimation and once during week 4

Organ weights, Gross pathology & Histopathology: At necropsy

Results:

Mortality: One female given 3.0 ml/kg of DMP 115 on day 1 died within 10 minutes post-dose. Clinical signs consisted of decreased muscle tone. Abnormal respiration (decrease rate and gasping), decrease heart rate, pale gums, dilated pupils, urination, vocalization and salivation. No abnormalities found upon gross and histological examination. Three of the surviving monkeys also exhibited similar symptoms that subsided within 60 minutes post-dose. One female had supplemental oxygen.

Clinical signs: The animals dosed with 1.0ml/kg did not exhibit any clinical sign on the first day of dosing. However between days 15-27 signs including abnormal respiration, incoordination, pale gums, dilated pupils and salivation were observed immediately after dosing occurred in two males and two females. Three of these monkeys lost consciousness. One required supplemental oxygen. The 0.3 and 0.1 ml/kg/day groups did not exhibit any clinical sign.

Body weights: No effect

Food consumption: Qualitative evaluation only. No effect

Ophthalmoscopy: No effect

Electrocardiography: No effect.

Hematology: No effect attributable to DMP-115.

Clinical chemistry: No effect attributable to DMP-115.

Organ Weights: No effect attributable to DMP-115.

Gross pathology & Histopathology: No gross or histologic findings attributable to DMP-115.

Key Study Findings: NOEL for the study was 0.3 ml/kg/day

Reviewer's comments: Overall, I agree with the study conclusions, however, however it is Noted that electrocardiography was done twice during acclimation and once during weeks 1 and 4 at approximately 0.5 hours after initiation of dose probably missing the critical period when some of the animals were exhibiting clinical symptoms.

TOXICOLOGY SUMMARY

Acute toxicity studies:

The acute toxicity of DMP-115 was evaluated in Sprague- Dawley rats, beagle dogs and cynomolgus monkeys. Study T95-07-25 was a single dose acute intravenous study in rats at dose range of 0.5 –10 ml/kg (4 X – 80X MHDbsa). All materials were administered within 4 hours of preparation. Clinical signs were recorded 30-60 minutes after the last animal was dosed. NOAEL was 5 ml/kg (40X MHD). 5 males and 1 female rats died within 30 minutes post-dose at 80X MHD. At necropsy, histopathological examination revealed congestion of the lungs (four of five) and enlarged liver with congestion (five of five). Mild centrilobular vacuolization was noted in three of these animals. These changes suggest a rapidly occurring alteration in the

cardiopulmonary status of the animals affected by Aerosomes. The mild degenerative changes noted within the liver are compatible with inadequate perfusion or oxygenation. It seems likely that other vital organs including the brain may also been damaged by the hypoxia but without adequate time for the development of observable morphologic manifestations. The rapidity with which all these animals died after dosing strongly implicates the dosing process in the deaths. Whether that might be the result of mechanical or physical properties of the injected material or due to some chemically mediated interference with oxygen delivery system to vital organs can not be determined. It is noted that animals receiving just the excipient or degassed liposomes were not affected. No histopathological changes related to the administration of the test agent were observed in the surviving rats at necropsy performed at days 3 and 16. However, in contrast to the results obtained in this study, 1/15 males and 1/15 females died on the first day after receiving a single 1 ml/kg dose of DMP-115 (X 8 MHD) in a chronic toxicity study sponsored by DuPont (T97-9-15). This study was terminated early due to excessive mortality. Clinical signs observed prior to death included abnormal respiration, ataxia, decreased motor activity and loss of righting reflex. than that reported in study 95-07-25. In a combined acute/28-day intravenous injection study in rats, (98-3-46), 1/40 rats showed labored respiration and increased heart rate immediately following dosing 0.3 ml/kg (X2.4MHD). These signs were no longer observed within ≈30 minutes post-dose. No DMP-115 related lesions were seen in the lungs of rats following a single dose at X2.4 MHD. The NOEL was 0.1 ml/kg (X 0.8 MHD) which is 50 times lower than that reported in study T95-07-25.

MRX-115 0.01, 1 and 2 ml/kg (X2.7, X27 or X 54 MHD), was administered to beagle dogs in a single dose toxicity study, and the animals were sacrificed on day 15 (T95-07-27). Clinical signs including pale gums, urinary or fecal incontinence, salivation, hypoactivity, dyspnea, polypnea, cold to touch, tremors, and/or abnormal respiratory sounds were observed in all treated animals. Urinary and fecal incontinence were also noted in the group of animals that received the degassed liposomes. The clinical signs were observed on day 1 only. No other compound-related effects were noted in body weight, food consumption, heart rate or clinical pathology. At necropsy, no changes in organ weights or gross and microscopic pathology were observed. No NOEL was established. A non-GLP study (T95-07-26) established NOEL in dogs as 0.1ml/kg (X2.7 MHD). Clinical signs were similar for the two studies.

The acute toxicity of a single intravenous administration of MRX-115 in Cynomolgus monkeys was investigated in two studies (T97-9-51 and T98-6-2). Doses employed were 1 and 10 ml/kg (X16.2, X162 MHD). NOEL was established in T98-6-2 to be X16.2 MHD. In study 97-9-51, which was conducted in one cynomologus monkey per sex per group,electrocardiograms were recorded 1 and 24 hours post-dosing. Changes interpretable by the contract laboratory as left block (abnormal QRS complexes and prolongation of QRS interval, negative T waves in lead 1 were reported on a few occasion in the female that received 10 ml/kg (X162 MHD). The contract laboratory opined that such changes occur spontaneously in monkeys. Serum chemistry showed a slight elevation in plasma GOT and GPT in one female monkey at 10 ml/kg. In a more recent study in Cynomololgus administered a single doe of DMP 115, 3ml/kg (studyT98-7-1), submitted midway through the review process, There were no deaths. Clinical signs exhibited by animals that received consisted of 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. 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 the 60 minutes post dose.

All six animals administered DMP 115 at 3ml/kg showed abnormal electrocardiograms with electrocardiographic evidence of ST-T segment depression within I 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.

Repeat-dose toxicity studies:

Five repeat-dose toxicity studies were performed in rats. Two (T95-7-29, T95-7-22) by ImaRx Pharmaceutical and the remaining (T97-9-15, T97-11-5, T98-3-46) by DuPont Pharmaceutical. The major differences between the studies were; (1) the timing of injection after mixer agitation (within four hours for ImaRx studies, and within 60 minutes for the DuPont studies), (2) the dose that produced lethality and the severity of the adverse events. The DuPont studies were conducted using to be marketed formulation. T95-7-29 was a 7-day intravenous study, the remaining studies were 28-day studies although T97-9-15 was terminated earlier due to excessive mortality. 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 for T95-7-29 and T95-7-22 and T97-9-15. For 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 and 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 formulation. The study established similar NOEL for the two preparations and there were no significant differences in profile between the two formulations.

Species	Study type	NOEL (ml/kg)	MHD	Major findings
Rat (T95-7-29) ImaRx study.	7-day	2.5	20	1/5 male at X60 MHD, 1/5 female control died
Rat (T95-7-22) ImaRx study	28-day	1.0	8	3/15 male, 1/15 females died at X40MHD
Rat (T97-9-15) DuPonT study	28-day but terminated due to unexpected mortality	0.3	2.4	10/15 males and 8/15 females died at X8MHD
Rat (T97-11-5) DuPont study	28	No NOEL established as there were evidence of lung lesions at all doses examined	-	7/16 males and 5/15 females died at X8MHD
Rat (T978-3-46 **DuPont study. This study used both ImaR formulation and DuPont's new formulation for comparison**	28-day	0.1	0.8	No significant difference in the results obtained for the two manufacturing processes. Enlargement of bronchial lymph nodes seen in most males and a few females from each manufacturing process. Pulmonary changes were as described .

Three repeat-dose toxicology studies were performed in dogs (T95-7-28, T95-6-34, and TT95-8-42)

Species	Study type	NOEL (ml/kg)	MHD	Major findings
Dog (T95-7-28) ImaRx study.	7-day	None established doses studied X2.7-X54 MHD	-	Clinical signs beginning on day 1 were observed: Pale gums, polyuria, salivation, abnormal respiratory sound, emesis, hypoactivity. No major histopathological findings
Dog (T95-6-34) ImaRx study.	7-day Non GLP	None established Dose studied was X2.7MHD	-	Clinical signs that became prominent beginning day 3. Cyanosis of oral or conjuctiva mucosa, depression, changes in the coagulation system.
Dog (T95-8-42) ImaRx study	7-day	None established. Doses studied X0.27-X 2.7	-	Clinical signs beginning post-dose day 4 became apparent. Dyspnea, ataxia, red discoloration of the perioral area, increase in histamine level.

Two repeat-dose toxicology studiess were performed in cynomolgus monkeys (T-95-7-24, T98-5-2

Species	Study type	NOEL (ml/kg)	MHD	Major findings
Cynomolgus monkeys (T95-7-24) ImaRx study.	28-day	1 ml/kg	16.2	1/3 males died on day 22 (10ml/kg), was noted to prostrate before death. No major histopathological findings.
Cynomolgus monkeys (T98-5-2).	28-day	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 including loss of consciousness were observed beginning day 15 and 27 for 4/12 receiving 1 ml/kg. No major histopathological findings.

CARCINOGENICITY: None conducted.

APPEARS THIS WAY ON ORIGINAL

IMMUNOTOXICOLOGY:

Two immunotoxicology studies were submitted as part of the IND. Dr. Sadrieh reviewed them, her review is reproduced in full.

T97-02-53: Antigenicity study of MRX-115 in guinea pigs. The study was carried out at The study was carried out in accordance with GLP regulations. The report date is October 8th, 1996. The in-life phase is March 20-May 1, 1996. The study is located in serial No. 014 page 731. The lot number for the test substance was 744-71-002.

Experimental design: The purpose of the study was to assess whether MRX-115 induces antigenicity in male albino guinea pigs (Crl: (HA)BR strain) utilizing active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA). Thirty guinea pigs were used for each phase of the study with 5 animals per group.

The ASA study was performed as follows:

	INDUCTION (subcutaneous injection) Groups 1, 2, 3, 5 and 6:Days 1, 8 and 15 Group 4: Days 1, 2, 3, 4, 5, 8, 9, 10, 11 and 12		**CHALLENGE** (intravenous injection in dorsal metatarsal vein) All groups: Day 30	
	Formulation	Dose Volume	Formulation	Dose Volume
1	saline/FCA	0.2 ml/animal	saline	2 ml/kg
2	saline/FCA	0.2 ml/animal	MRX-115 (1 mg/ml)	2 ml/kg
3	saline/FCA	0.2 ml/animal	BSA	0.5 ml (5 mg/animal)
4	MRX-115 (1 mg/ml)	0.2 ml/animal	MRX-115 (1 mg/ml)	2 ml/kg
5	MRX-115/FCA (1 mg/ml)	0.2 ml (0.1 mg MRX-115/animal)	MRX-115 (1 mg/ml)	2 ml/kg
6	BSA/FCA (10 mg/ml)	0.2 ml (1 mg BSA/animal)	BSA	0.5 ml (5 mg/animal)

On day 28, blood was collected from each animal and serum harvested and pooled. The pooled serum samples were then diluted with 0.9% saline and used for the PCA phase as

described below. Daily clinical observations were conducted and after challenge, observations for anaphylaxis were carried out. Body weight was determined at the initiation and termination of the ASA phase of the study.

The PCA phase of the study was carried out in 30 naïve guinea pigs according to the following design:

GROUP	TREATMENT	DILUTIONS ADMINISTERED BY INTRADERMAL INJECTION OF 0.05 ml (v/v in saline)
7	group 1 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
8	group 2 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
9	group 3 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
10	group 4 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
11	group 5 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
12	group 6 serum	1:100; 1:1000; 1:10,000

Four to six hours after the intradermal injections, each animal received an intravenous injection of 1% Evan's Blue in MRX-115. The animals in group 12 received an intravenous injection of 0.5 ml BSA. Thirty minutes after the intravenous injections, the diameter of the reaction at each intradermal injection site was measured.

Results: All animals gained weight throughout the ASA phase of the study. No significant clinical signs were noted during the induction phase of the study. During the challenge phase of the study, no signs of anaphylaxis were noted in the animals in groups 1, 2 and 3 (animals induced with saline/FCA). However, in one animal in group 2, (induced with saline/FCA and challenged with MRX-115), retching was seen. In group 4, (animals induced with MRX-115), hypoactivity was noted in one animal and retching was detected in 2 animals (severity score 1) within 15 minutes of dosing. These clinical signs were not considered to be a definitive indication of anaphylaxis, since retching was also noted in one group 2 animal (induced with saline/FCA and challenged with MRX-115). In group 5 animals (induced with MRX-115/FCA and challenged with MRX-115), all animals exhibited signs of mild anaphylaxis following the challenge dose with MRX-115. The clinical signs included head shaking, retching and/or ear scratching, pawing at the nose, hypoactivity, dyspnea and staggered gait. In group 6 animals (induced and challenged with BSA), grade 5 anaphylactic response was noted in all animals within 6 minutes after the challenge dose. Gross necropsy of these animals did not reveal any visible lesions in 3 of the animals. One of the group 6 guinea pigs exhibited an enlarged bladder and another guinea pig exhibited dark red foci on all lobes of the lungs.

Passive cutaneous anaphylaxis: Two animals in group 9 (group receiving serum from saline/FCA animals) and one in group 10 (receiving serum from MRX-115-treated animals) died immediately after intravenous administration of the 1% Evan's Blue in MRX-115. Additionally, difficulties in the intravenous administration to one animal in each of the following groups was found: group 7, 10, 11 and 12. These difficulties were thought to be related to the dosing mixture since the Evan's Blue did not seem to be uniformly dispersed in the MRX-115 formulation. It is therefore thought that intravenous blockages may have occurred and been the cause of death in the 3 animals. No PCA reactions were noted in the remaining animals treated with the serum from saline/FCA-treated guinea pigs. One of the 3 animals in group 10

exhibited a 2 mm in diameter reaction to the 1:1 serum dilution from the animals receiving MRX-115 during the ASA phase of the reaction. No PCA reactions were noted in the 4 group 11 animals treated with serum from the guinea pigs receiving MRX-115/FCA during the induction period of the ASA phase. In the positive control group 12 (treated with serum from the animals given BSA/FCA), PCA reactions ranging from 11 to 15 mm in diameter were observed in all 4 animals. These PCA reactions were seen at sites treated with the 1:100 and in 3 animals the 1:1000 dilution.

Conclusions: In the ASA phase of the study, all group 5 animals (induced with MRX-115/FCA and challenged with MRX-115), exhibited signs of mild anaphylaxis following the challenge dose with MRX-115. The clinical signs included head shaking, retching and/or ear scratching, pawing at the nose, hypoactivity, dyspnea and staggered gait. In the positive control group 6 animals (induced and challenged with BSA), grade 5 anaphylactic response was noted in all animals within 6 minutes after the challenge dose. No signs of anaphylaxis were noted in the negative control groups (saline/FCA). Additionally, the animals treated with MRX-115 alone (without FCA) did not exhibit notable signs of an anaphylactic response.

No PCA reactions were noted in the animals treated with the serum from saline/FCA-treated guinea pigs. One of the 3 animals in group 10 exhibited a 2 mm in diameter reaction to the 1:1 serum dilution from the animals receiving MRX-115 during the ASA phase of the reaction. However, no PCA reactions were noted in the 4 group 11 animals treated with serum from the guinea pigs receiving MRX-115/FCA during the induction period of the ASA phase. In the positive control group 12 (treated with serum from the animals given BSA/FCA), PCA reactions ranging from 11 to 15 mm in diameter were observed in all 4 animals. These PCA reactions were seen at sites treated with the 1:100 and in 3 animals the 1:1000 dilution.

Therefore, it is concluded that in the PCA phase of the study, no PCA reaction was noted in the animals treated with the serum from saline/FCA and MRX-115-treated animals. However, in the ASA phase of the study, a mild anaphylactic response was noted in all animals treated with MRX-115/FCA. The results of this study demonstrate that the capacity of MRX-115 to cause an anaphylactic response cannot be ruled out at this point.

T96-10-23. Antigenicity study of MRX-115 in guinea pigs. The study was carried out at The study was carried out in accordance with GLP regulations. The report date is February 28th, 1997. The in-life phase is October 21-December 4, 1996. The study is located in serial No. 021 page 1. The lot number for the test substance was 744-71-004 (the certificate of analysis for the test substance is not included in the study report).

Experimental design: The purpose of the study was to assess whether MRX-115 induces antigenicity in male albino guinea pigs (Crl: (HA)BR strain) utilizing active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA). This study is a follow-up study of the previous antigenicity study (CHW 6721-100) using a lower dose of MRX-115 in the induction and challenge periods of the ASA phase. Forty five guinea pigs were used in the ASA phase and 29 guinea pigs were used in the PCA phase.

The ASA study was performed as follows:

GROUP	TREATMENT			
	INDUCTION (subcutaneous injection)		**CHALLENGE** (intravenous injection in dorsal metatarsal vein) (day 31)	
	Formulation	Dose Volume	Formulation	Dose Volume
1	saline/FCA	0.1 ml/animal (n=5)	saline	1 ml/kg
2	saline/FCA	0.1 ml/animal (n=10)	MRX-115 (1 mg/ml)	1 ml/kg (n=5) 0.1 ml/kg (n=5)
3	saline/FCA	0.1 ml/animal (n=5)	BSA	0.5 ml (5 mg/animal) (n=5)
4	MRX-115 (1 mg/ml)	0.1 ml/animal (n=10)	MRX-115 (1 mg/ml)	1 ml/kg (n=5)
5	MRX-115/FCA (1 mg/ml)	0.1 ml (0.05 mg MRX-115/animal) (n=10)	MRX-115 (1 mg/ml)	1 ml/kg (n=5)
6	BSA/FCA (10 mg/ml)	0.1 ml (1 mg BSA/animal) (n=5)	BSA	0.5 ml (5 mg/animal) (n=5)

On day 29, approximately 2 ml of blood was collected from each animal in groups 1, 4, 5 and 6 and serum harvested and pooled. The pooled serum samples were then diluted with 0.9% saline and used for the PCA phase as described below. Daily clinical observations were conducted and after challenge, observations for anaphylaxis were carried out. Body weight was determined at the initiation and termination of the ASA phase of the study. Animals dying during the ASA phase were subjected to an abbreviated gross necropsy examination and the surviving animals were discarded without necropsy examination.

The PCA phase of the study was carried out in 29 naïve guinea pigs according to the following design:

GROUP	TREATMENT	DILUTIONS ADMINISTERED BY INTRADERMAL INJECTION OF 0.05 ml (v/v in saline)
7 (n=5)	group 1 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
8 (n=9)	group 4 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
9 (n=10)	group 5 serum	1:1; 1:10; 1:100; 1:1000; 1:10,000
10 (n=5)	group 6 serum	1:10; 1:100; 1:1000; 1:10,000

Four hours after the intradermal injections, each animal in group 7, 8 and 9 received an intravenous injection of 0.1% Evan's Blue in MRX-115. Animals is group 10 received 0.5 ml of an injection of 10 mg/ml BSA in 0.1% Evan's Blue. Thirty minutes after the intravenous injections, the diameter of the reaction at each intradermal injection site was measured. No

other clinical observations were done.

Results: All animals gained weight throughout the ASA phase of the study. The reported clinical signs are as follows:

During the induction phase, the most common clinical finding was the presence of subcutaneous masses at the sites of injection of the animals in groups 1,2, 3, and 5, which had all received FCA. It is reported that this is a normal finding when FCA is injected subcutaneously (in the previous antigenicity study CHW 6721-100, scabs at the site of injection were noted in the animals that received FCA). Twenty two animals, distributed between all groups, exhibited soft stool at various at times throughout the study. One animal in group 1 (induced with saline/FCA and challenged with saline), exhibited watery stool on day 15 and was found dead on day 16 of the study. No visible lesions were reported upon an abbreviated gross necropsy examination. The incidence of soft stool and the death of the animal in group 1 is attributed to a high bacterial (pseudomonas) level in the automatic watering system of the animal housing units. It is concluded by the study director that these findings would not directly affect the findings of the study. *Reviewer's comment: since the study is an antigenitcity study aimed at determining the immune response of animals to a foreign agent, it is preferable that all the animals be healthy and not stressed by an infection that may compromise the immune response. Since this study is a follow-up study to a previous antigenicity study, there is room for comparison between the 2 studies. Therefore, I will agree with the study director that the bacterial infection of the water supply should affect the outcome of the study. However, under normal circumstances and in the absence of a previous study, it is not acceptable to have an infection in the animals during the study.*

During the challenge phase of the study, all animals treated with saline/FCA during the induction phase (groups 1, 2, and 3) and challenged with saline, MRX-115 and BSA, appeared normal. Three out of 5 group 4 animals (treated with MRX-115 during the induction phase) and challenged with 1 ml/kg MRX-115 showed slight signs of an ASA response characterized by retching and pawing at the nose/mouth within 15 minutes but appeared normal at one hour after the challenge dose. One out of 4 animals in group 4 animals that were challenged with 0.1 ml/kg MRX-115 showed slight signs of an ASA response (retching and head shaking) within 15 minutes after the challenge dose, but appeared normal at one hour after the challenge dose. Three out of 5 group 5 animals (treated with MRX/FCA during the induction phase) showed slight signs of an ASA response such as head shaking, retching and/or pawing at the nose/mouth, within 15 minutes of dosing with the challenge dose of 1 ml/kg MRX-115. Within a hour after the challenge dose, the animals appeared normal again. Only one out of 5 animals from group 5, when challenged with the lower dose of MRX-115 (0.1 ml/kg) showed slight signs of anaphylaxis (pawing at the nose/mouth) within 15 minutes following the challenge injection. *Reviewer's comments: It appears that there is a dose-dependent ASA response to a challenge dose of MRX-115, in animals previously induced with MRX-115 or MRX-115/FCA. The ASA response is slight (severity score of 1 in most cases), nonetheless, the observed effect cannot be denied, in particular since in a previous antigenicity study, similar ASA responses were noted in guinea pigs induced with MRX-115 and then challenged with the same drug.* In the positive control group (group 6 which was induced with BSA) 4 out of 5 animals showed grade 5 anaphylactic reactions including retching, pawing at the nose/mouth, cyanosis, staggered gait, convulsions, gasping, coughing and death (the latter happened within 5 minutes after the challenge injection). The remaining animal in the positive control group 6 exhibited a grade 4 anaphylactic reaction. Upon gross necropsy, the 4 animals that died following the challenge dose exhibited lungs that appeared uncollapsed, which is attributed to bronchoconstriction associated with anaphylaxis in guinea pigs.

During the passive cutaneous anaphylaxis phase of the study, no PCA reactions were noted in the animals from groups 7, 8 and 9 (treated with serum from saline/FCA, MRX-115 and MRX-115/FCA-treated animals). Group 10 animals treated with serum from the positive control group animals, positive PCA reactions were noted in all animals.

Conclusions and reviewer's comments: Active systemic anaphylaxis (ASA) reactions characterized as "slight" were noted in several animals subcutaneously treated with MRX-115 or MRX-115/FCA and then challenged with an intravenous injection of mRX-115. The effect was present but less severe in animals challenged with 0.1 ml/kg MRX-115 as compared to animals challenged with 1 ml/kg MRX-115.

In the PCA phase of the study, no positive reactions were noted other than in the positive control group.

The sponsor concludes that the slight anaphylactic reaction observed during the ASA phase is not confirmed by the PCA phase. This reviewer thinks that since the above-described study confirms the findings in a previous antigenicity study where positive ASA reactions were noted, it must be concluded that MRX-115 has the potential to be antigenic. Therefore, it is recommended that labeling for the drug indicate the potential for an allergic response.

Summary of immuntoxicology studies:

Two antigenicity studies were carried out by the sponsor in order to determine the ability of MRX-115 to induce acute systemic and passive cutaneous anaphylaxis. The first study was carried out in 2 phases: an active systemic anaphylaxis (ASA) phase and a passive cutaneous anaphylaxis (PCA) phase. Thirty animals were used for each phase. The animals were immunized with 200 µl/animal and challenged with 2 ml/kg DMP115. Signs of anaphylaxis occurred in the drug/FCA group as well as the positive control BSA group of the ASA phase of the study. In the PCA phase of the study, 2 deaths occurred as well as some technical difficulties in the administration of Evan's Blue. A PCA reaction was noted in one animal receiving serum from an animal treated with the study drug. The study results were not very clear therefore the experiment was repeated. Therefore, it should be noted that signs of a slight anaphylactic response were observed in the first antigenicity study.

Due to the reaction observed in the first study, a second study was performed to assess whether DMP115 induces antigenicity in guinea pigs utilizing active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) methods. However, for the second study, lower doses of the study drug were used for both the immunization and challenge phases of the study. In the ASA phase of the second study, the animals were immunized with 100 µl/kg and challenged with 1 ml/kg of DMP115. The results of this study showed that subcutaneous injection of DMP115 to guinea pigs followed by a day 31 intravenous challenge injection of DMP115 produced "very slight" active systemic anaphylactic reactions in 3 of 5 animals (retching in one animal, pawing at the nose/mouth in 2 animals). Additionally, a "very slight" systemic anaphylactic reaction (retching and head shaking) was noted in one of five similarly induced animals following a day 31 intravenous challenge injection of the study drug. Similar responses were seen in the group of animals receiving DMP115 in combination with FCA. In the PCA phase, no PCA reactions were seen to the serum collected from the control, DMP115 or DMP115/FCA-treated animals. Positive PCA reactions were observed in the BSA positive control group.

It was concluded by the sponsor that the acute systemic anaphylaxis reactions observed in both experiments were mild in nature compared to the positive control groups. This was confirmed by the lack of passive cutaneous reactions in the second antigenicity study.

REPRODUCTIVE TOXICOLOGY:

Dr. Nakissa Sadrieh reviewed the reproductive toxicology studies under the IND. Dr. Sadrieh's review is reproduced in full.

Study T97-02-54: Study of fertility and early embryonic development to implantation in rats with MRX115. The study was carried out at [redacted]. The report date is February 4th, 1997. The study was performed in compliance with GLP regulations (21 CFR part 58). The study is located in Vol.1-28 pps: 1-228. The protocol was designed in accordance with ICH 4.1.4. The in-life phase of the study was April 4th to July 31st, 1996. The lot number of MRX-115 used in the study was 744-71-0002. The control material used was 0.9% saline.

Experimental Design: Eight week old male and female Sprague-Dawley Crl:CD BR rats were used for this study. The rats weighed between 267-315 gm for the males and 179-228 gm for the females. The experimental protocol was as follows:

Group	Dosage level (mg/kg/day)	Number of males	Number of females
1 (control)	0	25	25
2 (low)	0.1	25	25
3 (mid)	1.0	25	25
4 (high)	5.0	25	25

MRX-115 was injected intravenously in the tail vein. The males were dosed for at least 28 days prior to mating and throughout the mating period until the day prior to sacrifice (10 weeks total). The females were dosed at least 14 days prior to mating, throughout the mating period, and through gestation day 7.

The animals were observed for mortality and moribundity twice daily. Body weight were recorded twice weekly for the males and unconfirmed females. Females that were conformed to mate were weighed on gestation days 0, 3, 7, 10 and 13. Food consumption was measured weekly prior to breeding. Each pair of animals was given 21 days to mate. Vaginal smears were assessed for the stage of estrus.

On day 13 of gestation, all females were weighed, sacrificed by CO2 asphyxiation and exsanguination. The uterus from each gravid female was excised, weighed and examined for the number and placement of implantation sites, live and dead fetuses, early resorptions and abnormalities of the uterus. All abnormal tissues were preserved in 10% neutral-buffered formalin. Following at least 10 weeks of treatment, the males were sacrificed by CO2 asphyxiation and exsanguination and examined grossly. Total sperm number and morphology

were evaluated from the right epididymis of the first 10 surviving males/group. Sperm motility was evaluated from the right vas deferens. Statistical analyses were performed by One-Way ANOVA.

Results: Two animals died during the course of this study; one male in the high dose group died on day 54 and one female in the high dose group on day 17 of treatment. Upon necropsy, the lungs of the female had a mottled appearance and in the male a pale spleen was noted. Other that these findings, no other adverse signs were noted in the animals that died. On day 35, one female in the control group was sacrificed even though she was pregnant, however no confirmed-mated. Additionally, in the low dose group, another female was sacrificed on day 45 because she failed to show signs of mating. All females in gestation survived until their scheduled cesarean section.

The majority of the animals mated in the first 4 days of mating. The estrous cycle patterns were similar amongst the groups.

The mean body weight values were similar in all animals in the control and treated groups. In the conformed-mated females, the mean body weight change values were also similar in the control and treated groups. Food consumption was not significantly different between the groups.

Mean organ weight values were similar in the control and treated groups.

Mean percent motility , total sperm count and morphology were not affected by treatment with MRX-115 at any dose level.

Treatment-related effects on fertility were not observed in any group. The percent of animals mated (copulation index) were 100%, 96%, 100% and 96% in the control, low, mid and high groups, respectively. The percent of animals successfully mated (male/female fertility) was 92%, 92%, 88% and 96% in the control, low, mid and high dose groups. None of the confirmed-mated females aborted, died or delivered early.

The mean gravid uterine weight, corrected weight, and net weight change (from day 0) values were similar in the control and treated groups.

The mean number of corpora lutea and implantation sites were similar among the groups. Preimplantation losses were 19.4, 13.3, 7.6 and 19% for groups 1-4, respectively. Postimplantation losses were 15.4, 10.6, 5.9 and 13.7% in groups 1-4, respectively.

Conclusions: The males were dosed for at least 28 days prior to mating and throughout the mating period until the day prior to sacrifice (10 weeks total). The females were dosed at least 14 days prior to mating, throughout the mating period, 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**.

Study T95-08-40: Dose range-finding developmental toxicity study in rats with MRX115. The study was carried out at The report date is August 31st, 1995. The study was performed in compliance with GLP regulations (21 CFR part 58). The study is located in Vol. 1.28 pps: 230-349. The in-life phase of the study was March 27th-April 19th, 1995. The lot number of MRX-115 used in the study was 744-71-0001. The control material used was 0.9% saline.

Experimental Design: The study was a pilot study to determine the doses to be used in the definitive developmental toxicity study. Five female Sprague-Dawley rats per group were injected with either saline (5 ml/kg/day), 1, 3, or 5 ml/kg/day MRX-115 between days 6-17 of gestation. The animals weighed between 212 and 256 gm. The animals were observed for mortality and moribundity twice daily. Body weight values were recorded on gestation days 0, 6, 8, 12, 16 and 20. Food consumption values were measured during gestation for days 0-6, 6-8, 8-12, 12-16 and 16-20. On day 20 of presumed gestation, all surviving females were weighed, sacrificed by carbon dioxide asphyxiation and exsanguination. The animals were examined grossly and the uterus from each gravid female was excised, weighed and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions and abnormalities of the uterus and embryonic sac. Fetuses were weighed, sexed and examined for external abnormalities. Following the external examinations, all fetuses were discarded without necropsy. All data were statistically evaluated.

Results: Two animals in the 3 ml/kg/day group (days 6 and 16) and 3 animals in the 5 ml/kg/day group (days 6, 12 and 13) were found dead. In each of the groups, one animal had pale lungs upon necropsy. Examination of the uterus of the dead animals revealed that all the animals were pregnant with normally developing fetuses. The post-dose observations for these animals as well as those that survived to the scheduled day 20 sacrifice included a pale appearance, prostration, hypoactivity and labored and/or rapid breathing. Additionally, the 3 animals in 3 ml/kg/day group were observed to have post-dose convulsions prior to their death. The mean body weight and body weight change values of the treated groups were generally similar to the concurrent controls.

The mean food consumption values of the treated groups were generally similar to those of the concurrent controls.

At necropsy, gross pathology showed bilateral hydrometra in one non-pregnant female treated with 1 ml/kg/day. In one animal in the 5 ml/kg/day group, red amniotic fluid was noted in the uterus and this was attributed to the presence of 4 late resorptions and one dead fetus in the litter.

The mean gravid uterus weights and corrected terminal body weight values of the treatment groups were similar to those of the concurrent control group.

The pregnancy rates were 100% for all groups except for the 1 ml/kg/day group which was 60%. The number of pregnant females and litters evaluated at term (gestation day 20) was 5, 3, 3, and 2 in the control, 1, 3, and 5 ml/kg/day groups, respectively.

The mean number of corpora lutea and implantation sites were similar in all groups. The mean preimplantation loss values were slightly, however nonsignificantly, higher in the treated groups, as compared to the control groups (4.5, 10.1, 13.5 and 13.5 in groups 1-4, respectively). It is stated that these values were within the historical control ranges.

The mean postimplantation loss values for the 3 and 5 ml/kg/day groups were also higher, (however nonsignificantly), when compared with the concurrent controls (7.1, 6.5, 12.6 and 28.1 for groups 1-4, respectively). These values are stated to be above those of the historical controls for the 5 ml/kg/day group. This increase in the mean postimplantation loss value is reported by the sponsor to reflect the higher occurrence of early (in the 3 and 5 ml/kg/day groups) and late (in the 5 ml/kg/day group) resorptions. Additionally, the mean percent of late and early resorptions for the 5 ml/kg/day group is reported to exceed the historical control data ranges. Finally, the only dead fetus found was in the 5 ml/kg/day group. As a result, the mean number of live fetuses per litter in the 5 ml/kg/day group was lower than that of the control group.

The mean covariate fetal weight values of the 3 and 5 ml/kg/day groups were nonsignificantly lower than the respective control values and the historical control data ranges. Sex ratios were comparable between the control and treated groups.

There were no external fetal variations or malformation noted.

Conclusions: Doses of 1, 3 and 5 ml/kg were used by IV administration 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.

Study CHV T96-02-24: Rat developmental toxicity study in rats with MRX115. The study was carried out at The report date is February 6th, 1996. The study was performed in compliance with GLP regulations (21 CFR part 58). The study is located in Vol.1-29 pps:1-284. The in-life phase of the study was May 9-June 6, 1995. The protocol was designed according to ICH 4.1.3. The lot number of MRX-115 used in the study was 744-71-0001. The control material used was 0.9% saline or a placebo containing all inert ingredients of MRX-115.

Experimental Design: The study was designed to evaluate the maternal and embryo/fetal toxicity and teratogenic potential of MRX-115 when administered intravenously to Sprague-Dawley rats at doses of 0.5, 1.0, and 2.0 ml/kg/day during the period of organogenesis (days 6-17).

The study protocol was as follows:

Group	Dosage level (ml/kg/day)	Number of females
1 (Saline control)	2.0	25
2 (Placebo)	2.0	25
3 (Low)	0.5	25
4 (Mid)	1.0	25
5 (High)	2.0	25

The body weight of the animals on day 0 of gestation ranged from 225 to 284 gm.

The animals were observed for mortality and moribundity twice daily. Body weight values were recorded on gestation days 0, 6, 8, 10, 12, 14, 16, 18 and 20. Food consumption values were also recorded. On day 20 of gestation, all females were weighed and sacrificed. The animals were observed for gross abnormalities. The uterus from gravid females was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and abnormalities of the uterus and embryonic sac. The ovaries were examined for the number of corpora lutea.

Fetuses were sexed, weighed, examined for external abnormalities, identified and sacrificed via intraperitoneal injection of sodium pentobarbital. Half the fetuses from each litter were processed for visceral examination and the remaining fetuses were eviscerated and processed for skeletal examination. All data were statistically analyzed.

Results: All females survived until cesarean section on day 20.

The mean body weight and body weight change values for the treated and controls were similar.

Food consumption values were similar in the treated and the control groups except for the mean consumption value of the 1.0 ml/kg/day group from days 8-10 which was statistically significantly lower than the concurrent placebo value.

At necropsy, no significant observations were made other than a mass in the cervical region of a control female in the placebo group, an irregular shaped spleen in a control group male, a pale liver lobe in a 1 ml/kg/day group and a dilated pelvis of the left kidney in one 1 ml/kg/day group.

No changes were seen in the mean gravid uterine weights, the corrected terminal body weights and the net body weight changes.

The pregnancy rates were 100% for all groups except for the placebo group which had one nonpregnant female. One female in each of the 1 and 2 ml/kg/day groups had no viable fetuses. There were no dead fetuses in any of the groups and the ratio of males to females were similar amongst the groups.

The mean number of corpora lutea , implantation sites, preimplantation loss, early and late resorptions, number of live fetuses and covariate fetal weights for males females and sexes combined were similar amongst the treated and control groups.

The teratology results are as follows:

No fetal external variations were noted. Fetal external malformations consisted of an edematous fetus in the saline control group and in the 0.5 ml/kg/day group. One fetus had gastrochistisis in the 2 ml/kg/day group.

Soft tissue variations consisted of dilatation of the lateral and third ventricles with a litter incidence of 4.2% in all groups except the saline control group. Renal pelvic cavitation was seen with a litter incidence of 4-13% in all groups. Dilated ureters were seen in all treated groups with a litter incidence of 4-8%. No pattern was present for any of these effects. No fetal soft tissue malformation s were noted.

Skeletal malformations consisted of one fetus with vertebral anomaly with/without associated rib anomaly in the 0.5 ml/kg/day group and one fetus with fused ribs in the 2 ml/kg/day group.

Conclusions: 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 DMP115). 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. The high dose did not result in maternal toxicity. Based on the dose ranging finding study, the high dose group should have been dosed with 3ml/kg

Study T 95-08-41: Dose range-finding developmental toxicity study in rabbits with MRX115. The study was carried out at The report date is August 31st, 1996. The study was performed in compliance with GLP regulations (21 CFR part 58). The study is located in Vol. 1.29 pps. 286-377. The in-life phase of the study was April 10th-May 1st, 1995. The lot number of MRX-115 used in the study was 744-71-0001. No control was used.

Experimental Design: The purpose of this study was to set dosage levels for an expanded developmental toxicity study. This study was designed to evaluate the maternal and in utero toxicity potential of MRX-115 when administered intravenously at doses of 0.5, 2.5, 7.5, and 10.0 ml/kg/day to 4 groups of 6 mated Hra: (NZW)SPF rabbits over the period of organogenesis (days 7-20). The study protocol was as follows:

Group	Dosage level (mg/kg/day)	Number of females
1	0.5	6
2	2.5	6
3	7.5	6
4	10.0	6

On day 21 of presumed gestation, surviving females were weighed and sacrificed. The parameters evaluated were: survival, clinical signs, body weight, body weight changes, food consumption, gross pathology of the dams, and cesarean section data including implantations, corpora lutea, resorptions and an evaluation of the uterus. No statistical analysis was carried out die to the lack of control animals.

Results: Four animals died during this study. One animal from the 7.5 ml/kg/day group was found dead on day 10 of gestation and three animals from the 10 ml/kg/day group were found dead on days 7, 9 and 10 of gestation. Two of the animals found dead in the 10 ml/kg/day had a pale appearance, convulsions, hyperactivity, prostration and/or labored breathing on the day of their death.

Total body weight change values for day 0-21 tended to decrease with increasing dose level. Food consumption values also tended to decrease in a dose-dependent fashion during the period of administration.

Gross pathology findings were limited to the 3 animals found dead. These animals had mottled and pale lungs, a pale spleen and enlarged and/or pale kidneys. The uterus of the animals found dead on day 10 showed normally developing fetuses.

Mean gravid uterine weights for the 7.5 and 10 ml/kg/day groups were slightly higher than the 0.5 and 2.5 ml/kg/day groups. The mean net body weight change from day 0 for the test groups tended to decrease in a dose-dependent manner.

Pregnancy rates were 100% for all dose groups. No developmental toxicity findings were noted in any of the fetuses. The mean number of corpora lutea, implantation sites, and live fetuses were slightly higher in the 7.5 and 10 ml/kg/day groups than the 0.5 and 2.5 ml/kg/day dose groups. No abortions or dead fetuses were found in any of the groups. The number of resorptions were generally similar among the groups. The mean percent preimplantation loss values were slightly lower for the 2 high dose groups when compared to the 2 low dose groups. The mean percent postimplantation loss values for the 0.5, 2.5 and 7.5 ml/kg/day groups (11, 7.6 and 10%, respectively) were higher than those in the 10 ml/kg/day group (0%).

Discussion: 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.

Study T96-02-25: Rabbit developmental toxicity study in rats with MRX115. The study was carried out at The report date is February 6th, 1996. The study was performed in compliance with GLP regulations (21 CFR part 58). The study is located in Vol. 1.30 pps.1-237. The in-life phase of the study was June 26-September 7, 1995. The study was carried out according to ICH 4.1.3. The lot number of MRX-115 used in the study was 744-71-0001, 744-71-0001A, 744-71-0002. The control material used was 0.9% saline or a placebo containing all inert ingredients of MRX-115.

Experimental Design: The study was designed to evaluate the maternal and embryo/fetal toxicity and teratogenic potential of MRX-115 when administered intravenously to Hra: (NZW)SPF rabbits at doses of 0.5, 2.5, and 7.5 ml/kg/day during the period of organogenesis (days 7-20). The study protocol was as follows:

Group	Dosage level (mg/kg/day)	Number of females
1 (Saline control)	7.5	20
2 (Placebo)	7.5	20
3 (Low)	0.5	20
4 (Mid)	2.5	20
5 (High)	7.5	20