Methods Used in Pharmacokinetics Studies

Study	Methods
Ocular pharmacokinetics	

Results: No statistically significant differences between the ocular pharmacokinetics of Rescula® and MS-016 were identified. *[Reviewer's Comment: The sponsor provided diagrammatic data only. **In the NDA submission, line listings were provided for the ocular pharmacokinetic study.**]*

iv. Title: **0.15% unoprostone isopropyl ophthalmic solution. Ocular bioavailability after a single instillation into the right conjunctival sac of pigmented rabbits [Vol. 1.50; pp. 237-323]**
Study Identification:
Site:
Study Dates: May 25 – July 2, 1998
Formulation and Lot No.:
Certificate Analysis: Yes [X], p. 311-313
Final Report: October 15, 1998
GLP and QA Statements Signed: Yes [X]
Objective: "To compare the bioavailability of ...0.15% ...[UF-021] eyedrop formulation after a single instillation into the right conjunctival sac of pigmented rabbits."

Study Design – Radiolabeled UF-021[30 µl] was administered to the right eye of Dutch Belted male rabbits [app. 4 mos.; 2-2.5 kg; N=8/time point]. Body weight was obtained at study start and termination. Plasma samples and ocular structures from both eyes [conjunctiva, nictitating membrane, cornea, aqueous humor, iris, ciliary body, lens, vitreous, retina, choroid, sclera, optic nerve] were obtained at 15 and 30 min., 1, 2, 6, and 24 hours after dosing. Samples were combusted in a sample oxidizer, and radioactivity measured using liquid scintillation counting.

Results – Results were comparable in both pigmented and nonpigmented eyes, although in 1 study the concentrations were slightly greater in pigmented eyes than in the nonpigmented eyes. In general, the rank order of tissue concentrations [C_{max}] was cornea > nictitating membrane, anterior sclera, conjunctiva >> aqueous humor, iris, ciliary body>>choroid, optic nerve, retina, vitreous, lens. Peak concentration after dosing occurred [1] at 10-30 minutes in the cornea, conjunctiva, anterior sclera, nictitating membrane, and extraocular muscles; [2] at 30-40 minutes in the iris, ciliary body, retinochoroid and posterior sclera; and [3] at 30 min.-2 hours in the aqueous humor. Trace concentrations were detected in the other tissues. Corneal epithelial permeability was approximately 3-5 X 10^{-3} cm/hour in nonpigmented and pigmented eyes. Based on a 2-compartment model of the cornea and anterior chamber, the apparent elimination rate from the

aqueous humor was reported to range from approximately 0.2-0.9 hr^{-1} in nonpigmented and pigmented eyes. The apparent absorption rate was 1.3-1.8 hr^{-1} in nonpigmented and pigmented eyes. The AUC_{0-24h} values [formulation MS-016 and MS-028] in pigmented eyes are provided below. AUC_{0-24h} for the lens, vitreous humor, retina, choroid, and optic nerve was <570 ng-Eq•hr/g. Based on radioactivity, the plasma AUC_{0-24Hr}, C_{max}, and T_{max} for MS-028 [MS-016] was 45.04 [89.8] ng-Eq•hr/ml, 36.72 [67.29] ng-Eq/ml, and 15 [15] minutes, respectively. The table below compares the C_{max} and $AUC_{0-24\ hr}$ of MS-016 and MS-028 following a single application in the eyes of pigmented rabbits.

	Nictitating Membrane	Conjunctiva	Cornea	Aqueous Humor	Iris	Ciliary Process	Sclera
C_{max} [ng-Eq] (% of radioactive dose)							
MS-016	3606.99 [0.4%]	7948.52	15750.15 [2.87%]	993.74 [0.5%]	915.27 [0.13%]	1095.43 [0.02%]	2179.04
MS-028	2584.33 [0.23%]	4596.84	15264.87 [2.43%]	1039.44 [0.5%]	734.56 [0.1%]	389.05 [0.01%]	1748.08
AUC_{0-24h} [ng-Eq•hr/g] (% of radioactive dose)							
MS-016	3511.32 [0.28%]	6752.35	40399.05 [7.71%]	5128.82 [2.87%]	3914.66 [0.54%]	3055.89 [0.07%]	3543.75
MS-028	1595.28 [0.13%]	4215.97	44330.14 [7.17%]	4953.48 [2.49%]	3845.25 [0.49%]	1658.65 [0.04%]	2812.15

B. Systemic Pharmacokinetics

a. Ocular Administration - Single Dose

i. Title: Pharmacokinetics of UF-021[V] administration to rabbits by eyedrops [Vol. 1.50, pp. 71-107]
Study Identification:
Site:
Study Dates: April 2 – August 26, 1991
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following intraocular instillation in male rabbits"

Study Design – A single drop [app. 35µl/42µg] was instilled into the conjunctiva of the left eye of JW male rabbits [app. 12 weeks; 2.21-2.75 kg; N=3/group]. Radioactivity for determination of blood and plasma concentration and the hematocyte transport ratio was measured at 2, 5, 15, and 30 min., 1, 2, 4, 6, 8, 24 hours, and q24 hr for up to 168 hours following drug application. Radioactivity was measured at 4, 8, and 24 hours, and q24 hr for up to 168 hours in urine and q24 hr for up to 168 hours in feces.

Results – Systemic absorption of UF-021 was rapid. Plasma C_{max} was 73.9 ng-Eq/ml, t_{max} was 5 minutes, and $AUC_{0-\infty}$ was 62.5/135 ng-Eq•hr/ml [dry/wet method]. Plasma concentration was ≤1.1 ng/ml after 4 hours. RBC concentration of radioactivity [ratio] was ≤15% up to 8 hours. Parent compound was not identified in the plasma or aqueous humor. The major metabolite was M1 with a plasma and aqueous humor C_{max} of 61.7 ng-Eq/ml at 5 minutes and 535.9 ng-Eq/ml at 1 hour, respectively. Plasma concentration of M2, M4, and M6 was <2 ng-Eq/ml at all time points, but M3, M5, M7ab and M8 were not present at any time point. Aqueous humor C_{max} for M2 and M8 was 272.5 ng/ml at 2 hours and 89.5 ng/ml at 1 hour, respectively. M3 - M7 were not determined in the aqueous humor at any time point. The C_{max} of unidentified metabolites was 0.1-3.7 ng/ml in plasma and up to 39.7 ng/ml in aqueous humor. By 4 and 168 hours, 77% and 96% of the radioactivity had been eliminated in the urine, respectively. Only 1.2% of the radioactivity was eliminated in the feces at 168 hours. Neither parent compound nor M1 was found in the urine. The primary metabolite in the urine was M4 [app. 50% of total urine radioactivity] with lesser amounts of M6, M7a, and unidentified metabolites [<10% each of total urine radioactivity] present.

b. Parenteral Administration – IV

i. Title: UF-021 Pharmacokinetics; Unit dosage testing in rats [Vol. 1.51, pp. 1-115]
Study Identification:
Site:
Study Dates: June 26, 1990 - August 26, 1991
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following a single intravenous dose to rats."

Dr. Andrea Weir previously reviewed this study *The results of this study are summarized below.*

Results- Parent compound was not detected in the plasma at any timepoint following a single iv injection of radiolabeled UF-021 to rats. M1, M2, and M4 accounted for approximately 94% of the metabolites. The primary route of excretion was via the urine [approximately 50-60%], although fecal excretion [approximately 30-40%] was greater via the iv route than the ocular route. M4 was the major urinary metabolite with lesser amounts of M7ab, M6, M5, and M3. These metabolites accounted for approximately 95% of the urinary radioactivity for 24 hours. M4, M5, and M6 accounted for approximately 32%, 14%, and 16% of the fecal radioactivity for 24 hours. The major metabolites identified in the bile at 0-4 hours, in order of decreasing % of bile radioactivity, was M4 [~20%], M7ab and M5 [~12%], M2 [~10%], and M3, RB3 and RB4 [~6-8%]. At 4-8 hours post dosing only M4 and M5 were identified accounting for approximately 6% and 3% of bile radioactivity, respectively. Enterohepatic circulation was negligible with approximately ≤10% of an intraduodenally administered radiolabeled dose of UF-021 recovered in the bile at any time point.

The highest concentrations were observed 5 minutes after dosing with the greatest levels observed in the liver [~600 ng equivalents/gm tissue], kidney [~540 ng equivalents/gm tissue],

plasma [~120 ng equivalents/gm tissue], and blood, lung, brown fat, small intestine, and Harderian gland [~50-100 ng equivalents/gm tissue]. Tissue concentrations decreased with time with the exception of the large intestine. At all time points after 5 minutes, tissue concentrations were <10 ng equivalents/gm tissue with the exception of the liver [52 ng equivalents/gm tissue] and kidneys [600 ng equivalents/gm tissue]. The higher levels in the liver and kidney are attributed to fecal and renal excretion.

Plasma protein binding of M1 at concentrations ranging from 1 ng/ml – 1 µg/ml was [1] approximately 96-97% in the rat; [2] approximately 97-98% in the dog and rabbit; [3] approximately [redacted] in humans. *In vivo* protein binding in the rat post iv administration of 100 µg/kg of radiolabeled UF-021 was 60.2% and 15.1% at 5 minutes and 6 hours, respectively.

Placental transfer of radiolabeled UF-021 in pregnant dams on Gestation Day 18 resulted in tissue levels in the fetal liver [~50 ng equivalents/gm tissue], placenta [~25 ng equivalents/gm tissue], kidney, lung, heart, blood, fetus [~10 - 20 ng equivalents/gm tissue], and fetal brain and amniotic fluid [~5 ng equivalents/gm tissue]. Fetal tissue concentration was <6 ng equivalents/gm tissue at all other time points ≥30 minutes and <10% of the maternal dose at 5 minutes. The concentration of radioactivity in milk was <25 ng-Eq/ml at any time point post dosing.

ii. Title: <u>Pharmacokinetics of UF-021 [2]; Repeated administration to rats [Vol. 1.51; pp. 116-160]</u>
Study Identification: [redacted]
Site: [redacted]
Study Dates: January 6 - August 26, 1991
Formulation and Lot No.: [redacted]
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following repeated intravenous dosing in rats."

Study Design – Radiolabeled UF-021 was administered IV at 100 µg [2 ml]/kg to SD male rats [7-weeks, 246-307 g, N = 3] daily for up to 15 days. The following endpoints were measured: [1] blood concentration q24 hours after administration and at 2, 5, 15, 30 min., 1, 2, 4, 6, 8, and 24 then q24 hour for up to 168 hours after the 15th administration; [2] excretion ratios for urine, feces, and expired air, and measurement of whole body retention 24 hr after each dose and q24 hrs after the 15th for up to 168 hours; and [3] tissue concentrations 24 hr after the 5th and 10th administration, and 5 min, 6, 24, 168, and 335 hr after the 15th dose.

Results – [1] Blood concentration - Drug accumulated with a steady state achieved after the 9th dose. $AUC_{0-\infty}$ on Days 1 and 15 were 184 and 2410 ng-Eq•hr/ml, respectively. [Note: The values for 1 day are the same as those in the study described above: AE-1543: Pharmacokinetics; Unit dosage testing in rat]. C_{max} at 2 minutes was 75.6 ± 2.5 and 94.5 ± 9.6 ng-Eq/ml on days 1 and 15, respectively.

[2] Excretion – Cumulative excretion was 60.9, 26.4, and 7.3% in the urine, feces, and expired air with 3.8% of the radioactive dose present in the carcass 168 hours after the 15th dose.

[3] Tissue Distribution - Radioactivity accumulated in the tissues with an apparent steady state reached after approximately the 15th dose. The tissues with the greatest increase were white fat, cerebellum, and cerebrum with a concentration 15-16X the concentration following a single dose. However, at 5 minutes after administration, the concentration in these tissues was approximately 15-30% of the plasma concentration. Elimination from tissues was longer following repeated dosing compared to a single dose, especially in the white fat. Accumulation may reflect incorporation of ^3H_2O into tissues. The radioactivity in the white fat appeared to be associated primarily with tri and mono-glycerides.

iii. **Title:** Pharmacokinetics of UF-021 [IV]; Repeated administration to dogs [Vol. 1:51; pp. 218-260]
Study Identification: [redacted]
Site: [redacted]
Study Dates: January 6 - August 26, 1991
Formulation and Lot No.: [redacted]
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following a single dose in dogs." [Note: The title indicates that this was a repeat dose study. However, data for only a single IV dose were presented.]

Study Design - Radiolabeled UF-021 was administered IV at 100 µg [2 ml]/kg to male beagle dogs [9-weeks, 8.15-10.02 kg, N = 3]. The following endpoints were measured: [1] blood and plasma concentration at 2, 5, 15, 30 min., 1, 2, 4, 6, 8, and 24 then q24 hour for up to 168 hours after the 15th administration; [2] *in vivo* protein binding; [3] excretion ratios for urine, feces, and expired air for up to 168 hr after dosing; and [4] quantitative determination of metabolites by [redacted]

Results – [1] Blood and plasma concentration - Blood and plasma [dry] $AUC_{0-\infty}$ was 215 and 391, ng-Eq•hr/ml, respectively. C_{max} at 2 minutes was 174.2 ± 24.9 and 290.3 ± 18.8 ng-Eq/ml for blood and plasma, respectively.

[2] *In vivo* protein binding – Approximately 85 and 29% protein ratio binding was observed 5 minutes and 4 hours after dosing, respectively.

[3] Excretion – Cumulative excretion was 86.6, 12.7, and 1.0% in the urine, feces, and expired air, respectively.

[4] Metabolite quantitation – The primary metabolite in the plasma was M1 reaching a C_{max} of 184.5 ng-Eq/ml at 5 minutes. M3 and M4 concentrations increased with time reaching a C_{max} of 19.4 and 25.5 ng-Eq/ml at 30 and 60 minutes, respectively. All other metabolites, ^3H_2O, and UF-021 had a C_{max} of ≤11 ng-Eq/ml. Neither M6 nor M8 was detected. The C_{max} for unidentified metabolites was 31.1 ng-Eq/ml at 15 minutes. Urinary excretion of M3 and M4 accounted for approximately 11.2 and 21.7% of the urine radioactivity. All other identified metabolites accounted for ≤8% of the radioactivity with the exception of "others" which accounted for approximately 13% of the urine radioactivity. UF-021, M1, M2 and M8 were not detected in the urine. Each identified metabolite in the feces accounted for <2% of the radioactive dose.

iv. Title: Pharmacokinetics of UF-021 [III]; Single administration to rabbits [Vol. 1.51; pp. 183-217]
Study Identification:
Site:
Study Dates: June 26, 1990 - August 26, 1991
Formulation and Lot No.
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following a single intravenous dose to rabbits."

Study Design – Radiolabeled UF-021 was administered IV as a single dose of 100 µg/kg [2 ml/kg] to male rabbits [11-weeks, 2.27-2.4 kg, N = 3]. The following endpoints were measured: [1] blood and plasma concentration at 2, 5, 15, 30 min., 1, 2, 4, 6, 8, and 24 then q24 hour for up to 168 hours after dosing; [2] excretion ratios for urine, and feces for up to 168 hr after dosing; and [4] quantitative determination of metabolites by

Results – [1] Blood and plasma concentration - Blood and plasma [dry] $AUC_{0-\infty}$ was 139 and 261 ng-Eq•hr/ml, respectively. Plasma C_{max} at 2 min. was 198.7 ± 25.0 ng-Eq./ml.

[2] Excretion – Cumulative excretion was 94 and 1.2% in the urine and feces, respectively.

[3] Metabolite quantitation – The primary metabolite in the plasma was M1 reaching a C_{max} of 110.3 ng-Eq/ml at 5 minutes. M4 concentration increased with time reaching a C_{max} of 40.6 ng-Eq/ml at 15 minutes, respectively. C_{max} for M2 and M7ab at 5 min. was 15.6 and 11.4 ng-Eq/ml. All other metabolites, ^3H_2O, and UF-021 had a C_{max} of <5 ng-Eq/ml. M3, M5, and M8 were not determined. The C_{max} for unidentified metabolites was 18.6 ng-Eq/ml at 5 minutes. M4 and M7ab accounted for approximately 50 and 9.1% of the urine radioactivity. All other identified metabolites accounted for <8% of the radioactivity. UF-021, M1, M2, M3, M5, and M8 were not detected in the urine.

c. **Parental Administration – SC**

i. Title: *In vivo* kinetics of UF-021 with single subcutaneous administrations in rat [Vol. 1:51; pp. 161-182]
Study Identification: Not provided
Site:
Study Dates: November - December 1991
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: August 27, 1991
GLP and QA Statements Signed: No [X]
Objective: "To assess the pharmacokinetics of UF-021 following a single subcutaneous dose in rats."

Dr. Andrea Weir previously reviewed this study ***The results of this study are summarized blow.***

Results: No parent compound was detected in either group at any timepoint. The major metabolite in both groups was M1, followed by M4 and M2. Radioactivity was detected in the plasma up to 12 (2 mg/kg) to 24 (20 mg/kg) hours after treatment, but no metabolites were detected after 8 hours (20 mg/kg) and 4 hours (2 mg/kg). Pharmacokinetic parameters for M1 are shown in the table below.

	Dose	
Parameter	**2 mg/kg**	**20 mg/kg**
Cmax (ng equiv./mL)	388	1042
Tmax (hr)	1	1
t1/2 (hr)	—	2.9
AUC ng equiv•hr/mL)	551	4825

C. Metabolism

a. *In vitro* Studies - Rabbit

i. Title: *In vitro* corneal permeation of unoprostone isopropyl [UF-021] and it metabolization in the isolated pig eye [Vol 1.50, pp.21-41]
Study Identification:
Site:
Study Dates: Not provided
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: January 6, 1999
GLP and QA Statements Signed: No [X]
Objective: "To assess the *in vitro* corneal permeation of ...isopropyl unoprostone in the isolated pig cornea and to characterize its metabolization in pig eye tissues and aqueous humor"

Study Design - The methods are the same as used above under A.a.i. [Bioavailability Studies]. The following endpoints were evaluated using pig cornea [1] corneal permeation of both UF-021 and its free acid; [2] corneal accumulation kinetics of UF-021 and free acid; [3] localization of enzymatic activity within the cornea. Metabolism of free acid by conjunctiva, trabeculum, iris-ciliary body or in aqueous humor was assessed.

Results [Note: Sponsor provided only graphic representation of data] - The free acid metabolite, M1, does not appear to cross an intact epithelial barrier. UF-021 accumulates in the corneal epithelium in a linear fashion over time. The free acid metabolite accumulation plateaus after app. 50 minutes indicating an equilibrium between production and diffusion from the cornea and that cleavage of UF-021 is the rate-limiting step.

Enzymatic activity [>85%] was localized in the corneal epithelium and not endothelium or stroma. Physostigmine partially inhibited UF-021 metabolism suggesting that esterases, specifically butyrylcholinesterase, are involved in the metabolism of this drug. Failure to totally

inhibit metabolism suggests that other enzymes are also involved. M1 is not further metabolized by conjunctiva, trabeculum, and iris-ciliary body or in aqueous humor. [Note: No data presented.]

b. *In vivo* Studies

i. Title: Isolation and identification of UF-021 metabolites: Metabolic pathway of UF-021 [Vol 1.52, pp.30-69] – The Sponsor proposes that M1 is generated by the hydrolysis of the isopropyl group of UF-021 and M2-M7 are generated by either β- or ω-oxidation. This would suggest that UF-021 is metabolized and excreted by the same pathway as that for $PGF_{2\alpha}$ metabolism. [Note: The schema for the metabolic pathway of UF-021, as suggested by the Sponsor, is provided in the Summary section.]

D. Drug-Interactions

i. Title: Effect of UF-021 on drug metabolism enzymes [Vol. 1.52; pp. 100-116]
Study Identification: Not provided
Site: [redacted]
Study Dates: November 1991
Formulation and Lot No.: [redacted]
Certificate Analysis: No [X]
Final Report: Not provided
GLP and QA Statements Signed: No [X]
Objective: "To investigate UF-021 as to its indirect interactions pertaining to drug metabolism"

Study Design - [1] Male SD rats [N=5] were administered UF-021 SC at 5, 100, and 500 μ/k for 7 days. Phenobarbital served as a positive control. Twenty hours after the final dose, livers were harvested and microsomal preparations were generated. The following endpoints were measured [i] body weight, liver weight, and liver microsomal protein; [ii] cytochromes b_5 and P-450; [iii] NADPH-cytochrome P-450 reductase activity; and [iv] p-hydroxylation, N-demethylation, and O-deethylation activity. [2] The potential for direct drug interaction with UF-021 was assessed *in vitro*. UF-021 at 1 X 10^{-7}, 1 X 10^{-6}, and 1 X 10^{-5} M was added directly to liver microsomal preparations and p-hydroxylation, N-demethylation, and O-deethylation activity was measured. Metyrapone was the positive control.

Results and Conclusions: In general, here were no significant changes that were considered to be potentially drug-induced in any of the parameters evaluated at any of the concentrations or doses used. The exception was a12% in N-demethylation activity at 10 μM of UF-021. These data suggest that UF-021 should not demonstrate significant drug interaction.

D. SUMMARY OF PHARMACOKINETICS/TOXICOKINETICS – *In vitro* corneal permeation of unoprostone isopropyl [Rescula® or MS-016] was less than 1% of the dose. Biodistribution and kinetic analysis conducted with 0.12% [4 studies] and 0.15% [1 study] UF-021 was generally comparable between pigmented and nonpigmented rabbits. In general, the rank order of tissue concentration [C_{max}] based on radioactivity was cornea > nictitating membrane, anterior sclera, conjunctiva >> aqueous humor, iris, ciliary body>>choroid, optic nerve, retina, vitreous, lens. Contralateral passage of UF-021 to the untreated eye was low. Corneal epithelial permeability was approximately 3-5 X 10^{-3} cm/hour in nonpigmented and pigmented eyes. Based

on a 2-compartment model of the cornea and anterior chamber, the apparent elimination rate from the aqueous humor was reported to range from approximately 0.2-0.9 hr^{-1} in nonpigmented and pigmented eyes. The apparent absorption rate was 1.3-1.8 hr^{-1} in nonpigmented and pigmented eyes. The ocular pharmacokinetics following administration of a single dose of MS-028 [the clinical formulation] to pigmented rabbits are provided in the table below. $AUC_{0\text{-}24h}$ for the lens, vitreous humor, retina, choroid, and optic nerve was <570 ng-Eq•hr/g. [Note: The findings with 0.12% UF-021 were comparable, although the ng equivalent for ciliary process was approximately 2X the 0.15% value.]

	Nictitating Membrane	Conjunctiva	Cornea	Aqueous Humor	Iris	Ciliary Process	Sclera
C_{max} [ng-Eq] (% of radioactive dose)							
MS-028	2584.33 [0.23%]	4596.84	15264.87 [2.43%]	1039.44 [0.5%]	734.56 [0.1%]	389.05 [0.01%]	1748.08
$AUC_{0\text{-}24h}$ [ng-Eq•hr/g] (% of radioactive dose)							
MS-028	1595.28 [0.13%]	4215.97	44330.14 [7.17%]	4953.48 [2.49%]	3845.25 [0.49%]	1658.65 [0.04%]	2812.15

The systemic absorption of radiolabel following ocular administration of UF-021 to pigmented rabbits was rapid with a C_{max} that represented approximately 10% of the radioactive dose. The table below compares plasma $AUC_{0\text{-}\infty}$, C_{max}, and t_{max} [based on total radioactivity] following a single administration by either the iv, sc or ocular routes to rats, rabbits, and/or dogs.

	Intravenous [0.1 mg/kg]						Subcutaneous [20 mg/kg]		Ocular
	Rat		Rabbit		Dog		Rat		Rabbit*
	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Plasma
$AUC_{0\text{-}\infty}$ [ng-Eq•hr/ml]	184	-	139	216	215	391	-	19849	45.04
C_{max} [ng-Eq/ml]	75.6	-	121.7	198.7	174.2	290.3	1668.1	2568.1	36.72
t_{max} [min]	2	-	2	2	2	2	120	120	15

*Plasma level at 4 hours was <1.1 ng Eq/ml – nonpigmented rabbit

In the rabbit, excretion was comparable following either ocular or iv administration of UF-021 with approximately 96% and 1.2% of the radioactivity recovered in the urine and feces, respectively. Although urine excretion predominated in the rat and dog following iv administration [approximately 60% and 86% of the radioactivity, respectively], the level of fecal excretion in rats and dogs [approximately 30% and 13% of the radioactive dose, respectively] exceeded that observed in the rabbit.

There was accumulation in plasma, blood, and tissues following IV administration for 15 days in rats with steady state achieved on Day 9 of dosing. Tissue elimination in the rats was generally comparable to plasma with the exception of white fat from which elimination was delayed.

Based on pharmacokinetic studies in rats, rabbits, and dogs administered UF-021 by either the IV, SC or PO route, the Sponsor proposes the following metabolic pathway.

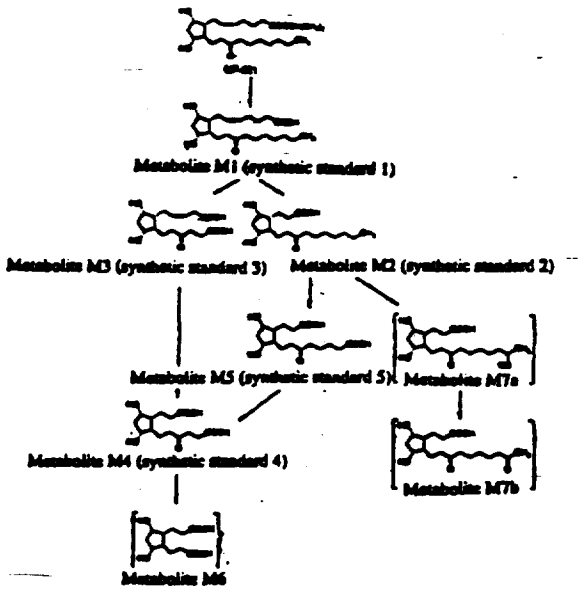

Figure 17. Proposed metabolic pathway for UF-021 (proposed structures shown in brackets)

Regardless of route [iv, sc, or ocular] essentially no parent compound was found in plasma with the major metabolite being M1. Other identified metabolites were found to a lesser extent including [1] M2 in the rat (iv, sc) and rabbit (iv, ocular) which increased over time; [2] M3 in the dog (iv); [3] M4 in the rat (iv, sc), rabbit (iv, ocular) and dog (iv); and [4] M6 in the rabbit (ocular). Plasma levels of M2, M4 and M6 following ocular instillation were <2 ng Eq/ml.

Free acid [M1], but not parent compound, was detected in the aqueous humor of [redacted] rabbits following ocular administration of 0.12% UF-021 [C_{max} at 1 hour = 535.9 ± 152.7 ng equiv/ml]. Free acid metabolite did not cross the corneal epithelium. The data indicate that the parent compound is completely hydrolyzed by corneal esterases to the free acid, M1. The enzymatic activity appeared to be localized in the corneal epithelium with no apparent further metabolism in other ocular structures. The data suggest that butyrylcholinesterase or several similar enzymes were responsible, in part, for metabolism of UF-021. The other identified major metabolites in the aqueous humor were M2 [C_{max} at 2 hours = 272.5 ± 152.7 ng-Eq/ml] and M8 [unknown structure, C_{max} at 1 hour = 89.5 ± 15.9 ng equiv/ml]. M3 - M7 were not found in the aqueous humor at any time point.

The table below provides a comparison of exposures to the identified metabolites in male rats, rabbits, and dogs following a single dose by either the iv, sc, or ocular routes.

Metabolite	C_{max} [ng-Eq/ml] AUC [ng-Eq•hr/ml]					
	Rat – IV – 0.1 mg/kg	Dog – IV - 0.1 mg/kg	Rabbit – IV - 0.1 mg/kg	Rat – SC – 20 mg/kg	Rabbit – ocular – 0.042 mg/eye	Human – Ocular –5 µg/kg
UF-021	ND	9.1	-	-	-	
M1	39.5 -	184.5	110.3	1042.2 4825	61.7	0.53 0.292*
M2	50.5	6.4	15.6	346.9 1516	0.9	
M3	-	19.4	-	-	Not determined	
M4	25.6	25.5	30.6	613.8 2973	1.7	
M5	3.8	7.6	-	-	Not determined	
M6	5.4	-	4.3	96.3 277	0.4	
M7ab	9.7	9.2	11.4	-	Not determined	
M8	-	-	-	-	Not determined	
Unidentified	≤2.1	17.4	9.2	-	1.2-3.7	
^3H_2O	43.3	4.5	2.0	-	0.4	
Others	10.8	31.1	18.6	-	1.3	

- Not detected
*Values obtained from Study #C99-UIOS-018; AUC values are for 0-24 hours

The major metabolite excreted in the urine in all species and in the bile of the rat following either iv or ocular administration of UF-021 was M4. Lesser or no levels of M3, M5 M6, and/or M7ab were identified in the urine depending on route and/or species. Essentially no M1, M2, M8, or parent compound were detected in the urine regardless of species or route. These data suggest that the metabolic pathway of UF-021 in these species resemble that for endogenous $PGF_{2\alpha}$.

In vitro protein binding of the free acid [e.g. de-esterified ^{3}H-UF-021; M1] was [redacted] in rats, rabbits, dogs, and humans. *In vivo* protein binding at 5 minutes was 60 and 85% in rats and dogs, respectively and 15 and 29% at 6 hours.

There did not appear to be any significant interactions with hepatic microsomal enzymes evaluated nor was enzyme induction demonstrated in rats at the doses tested [e.g. up to 0.5 mg/kg X 7 days].

IV. Toxicology:

A. Ocular Studies

1. Repeat Dose Studies with UF-021

a. Rabbits - Irritation Studies

i. Title: UF-021/MS-019 eye-drops dose-ranging effects on intraocular pressure after a single topical administration in ocular normotensive New Zealand Rabbits [Vol. 1.9, pp. 45-69]
Study Identification:
Site:
Study Dates [In-life]: Not provided
Formulation and Lot No.:
Vehicle:
Certificate Analysis: Yes [X]
Final Report: December 18, 1996
GLP and QA Statements Signed: No [X]
Objective: "To study the dose-ranging effects of a single topical treatment ...UF-021/MS-019 formulations on the ... IOP ... in ocular normotensive New Zealand rabbits"

Since this is a non-GLP dose-ranging study, the results will be summarized. In addition, the composition of MS-019 was not provided.

Study Design and Results – A single administration of 30 µl of 0.06%, 0.09%, 0.12%, 0.15%, and 0.18% UF-021/MS-019 was instilled into one eye each of ocular normotensive New Zealand White rabbits [F; N=5/group with each group treated at 2 concentration levels; several week washout period between treatments]. Saline was instilled into the contralateral eye. Ocular tolerability and IOP was assessed at 15, 30, 45 min., 1, 2, 3, 4, 5, and 6 hours after instillation. Ocular irritation, characterized by conjunctival redness, uveitis, and miosis, was observed at all concentrations although the incidence was somewhat dose-dependent. A transient increase at 30-45 minutes post-instillation preceded the drop in IOP, which was noted from approximately 2-6 hours post drug administration. The Sponsor states that the IOP lowering effects should be interpreted with consideration of the IOP effects of uveitis.

ii. Title: Test of 14 days continuous topical UF-021 application using rabbits [Vol. 1.13: pp. 1-25]
Study Identification:
Site:
Study Dates [In-life]: October 8 – 22, 1987
Formulation and Lot No.:
Excipient Vehicle
Certificate Analysis: No [X]
Final Report: Date not provided
GLP and QA Statements Signed: No [X]

Objective: To assess the potential ocular toxicity of UF-021 following 14-day continuous administration in rabbits.

The results of this study will be summarized.

Study Design: Either 50 µl/eye of VH or UF-021 at 0.05% [25 µg/eye], 0.1% [50 µg/eye], or 0.2% [100 µg/eye] was instilled into one eye of male albino rabbits [N=5] BID for 14 days. Physiological saline was instilled into the contralateral eye. Clinical observations, body weight, food consumption, Draize scoring, tear volume [redacted], and histopathology of ocular tissues and adnexa were evaluated.

Results – There was a dose-dependent increase in the frequency of conjunctival and iridic congestion, which was described in all test article groups. [Note: Conjunctival reddening was not considered congestion unless vasodilation or vascular congestion was evident.] Iridic congestion was more commonly described than conjunctival congestion. At a concentration of 0.05% the findings were sporadic. At a concentration ≥0.1%, these signs persisted through the 14-day treatment period. The incidence and duration of signs, however, tended to decrease with time. Although tear volume was decreased in 1/5 animals in each group, a treatment-related effect was not clearly identified. The incidence of histopathology findings, including mild conjunctival edema and mild corneal hypertrophy, was comparable across treatment groups. Excipient VH treated eyes served as the control. A saline control group would have been more appropriate. Ethanol was used to fix the eyes, which can introduce artifact during processing. A NOAEL was not determined in this study.

iii. Title: 30-Day ocular irritation study in rabbits [Vol. 1.13; pp. 26-171]
Study Identification: [redacted]
Site: [redacted]
Study Dates [In-life]: January 14 – February 13, 1997
Formulation and Lot No.: [redacted]
Certificate Analysis: No [X]
Final Report: September 23, 1997
GLP and QA Statements Signed: Yes [X]
Objective: "To determine the irritation potential of Isopropyl Unoprostone Ophthalmic Solution [IUOS] administrated four times daily for 30 days to Dutch Belted Rabbits"

Study Design

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	µg/eye/ dose	µl/eye /dose	route	Tx regimen			
Vehicle Control	0	30	ocular OD	30 days QID [q3.5-4 hrs]	M/F	8	Dutch Belted rabbits – [redacted] App. 24-30 weeks old M – 1.8-2.2 kg, F – 1.9-2.4 kg
UF-021 - 0.06%	18						
UF-021 - 0.12%	36						
UF0-21 - 0.15%	45						
UF0-21 - 0.18%	54						

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	Prior to the 1st dose and just prior to sacrifice
Body weight	Days 0, 7, 14, 21, and 30
Food consumption	Daily
Ocular Observations - Draize scoring	1 hour after the last dose of the day and prior to sacrifice
Ophthalmology [Baldwin scoring] – direct and indirect, slit lamp	Prior to randomization, Days 7 and 30

Results – Mortality/Clinical observations – There were no premature decedents and no treatment-related effects.

- **Body weight and food consumption** - There were no treatment-related effects.

- **Ocular observations** – One vehicle control male and female exhibited slight [Score of 2] conjunctival redness [vessel injection] on a single day. There tended to be a dose dependent increase in the frequency and animal incidence of conjunctival redness, although incidence at 0.12 and 0.15% UF-021 was comparable. The Draize score was generally 2. However, 3 males and 2 females at 0.18% had a score of 4.0 on ≥1 day. One male at 0.18% exhibited "diffuse, beefy red conjunctivae and chemosis".

- **Ophthalmology** – On Day 7, 4/8 males at 0.15% exhibited slight fluorescein epithelial staining of up to 25% of the cornea and corneal erosions. Slight conjunctival redness was observed in 1 animal. Similar findings were observed in 1/8 males and females at 0.18% UF-021. On Day 30, corneal epithelial staining [area up to 25%] and palpebral and bulbar conjunctival ulceration were observed 1/8 males and 1/8 females at 0.12 and 0.15%, respectively. Moderate corneal epithelial staining [area up to 25%] and corneal opacity was noted in1/8 males at 0.18% UF-021.

Reviewer's Comment – Study Design and Data Presentation –

1. Overall Draize scores were provided. It would have been preferred had the Sponsor provided a breakdown of the scoring [e.g. individual changes observed in the conjunctiva, cornea, iris, etc.]

Sponsor's Conclusion [numbered] and Reviewer's Comments –

1. The NOAEL for ocular irritation was 0.06%. Slight ocular irritation was observed 0.12 and 0.15% and slight to moderate ocular irritation was observed at 0.18%.

b. Rabbits - Toxicity Studies

i. Title: Eye mucous membrane irritation tests in repeated instillation of UF-021 for albino rabbits [Vol. 1.13; pp. 172-382]
Study Identification:
Site:
Study Dates: February 13, 1990 – June 7, 1991
Formulation and Lot No.:
Excipient Vehicle
Certificate Analysis: No [X]
Final Report: June 7, 1991
GLP and QA Statements Signed: Yes [X]

Objective: To determine the ocular irritation and systemic toxicity potential of UF-021 administered for 13-weeks in albino rabbits.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [redacted] ***The clinical formulation was not used in this study. Study design and results will be summarized.***

Study Design - Groups of 6 male New Zealand white rabbits received ocular instillations [100 µl] of either physiological saline and VH BID, or 0.12% BID [0.16 mg/kg] or QID [0.32 mg/kg]. The treatments were administered for 13 weeks. Clinical observations, body weight and food consumption, clinical pathology, ophthalmologic examination, necropsy, organ weights and histopathology were conducted. [Note: Eyes were fixed with [redacted] instead of either Davidson's or Bouin's. Histopathology for only the ocular tissues was provided].

Results - Rabbits that received 2 or 4 instillations of UF-021 per day exhibited a transient, slight hyperemia of the iris during the treatment period. In all instances the hyperemia resolved before the first administration on the following day. In the group that received 2 instillations per day, the effects were essentially absent from Week 4 forward. Two and 5 animals exhibited anterior segment irritation (score of 2.5-7, generally 5) in the BID and QID treated groups, respectively. There were no more than 3 animals affected at any of the given time points. Essentially no effects were observed in the conjunctiva. Histopathology revealed vasodilation of the ciliary body in 2 rabbits in the group that received 4 instillations of UF-021. Due to the hyperemia that was observed in the irises of both treatment groups, a NOAEL was not identified for this study.

ii. Title: <u>90-day ocular toxicity study in rabbits [Vol. 1.14 – 1.16]</u>
Study Identification: [redacted]
Site: [redacted]
Study Dates [In-life]: July 9 – October 8, 1996
Formulation and Lot No.: [redacted]

Excipient Vehicle Control

[redacted]

Certificate Analysis: The Sponsor provided results of an [redacted] analysis [App. 5] of formulations from Days 1 and 90, which indicated that UF-021 was not present in the vehicle and that drug substance was within specifications [e.g. 1.08-1.32 mg/ml]
Final Report: October 28, 1997
GLP and QA Statements Signed: Yes [X]
Objective: "To compare the ocular toxicity of two test article formulations administered either twice or four times daily for 30 and/or 90 days to New Zealand White Rabbits"

Study Design:

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	µg/eye/ dose	µl/eye /dose	route	Tx regimen			
1- Saline Control			OD	90 days	M/F	8	New Zealand White Rabbits - [redacted] App. 16-18 weeks M – 1.6-2.2 kg; F – 1.5-2.1 kg
2 – VH Control							
3 – Rescula Eyedrops	120			BID			
4 – Rescula Eyedrops				QID			
5 – MS-016				BID			
6 – MS-016				QID			

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	Prior to dosing and sacrifice
Body weight	Weekly
Food consumption	Daily
Ocular observations - Draize	Prior to dosing and 1-hr after dosing on Days 1, 3, 5, 7, 8, 13, 16, 20, 23, 27, 30, 44, 51, 58, 65, 72, 79, 86, and 90 or prior to sacrifice on Days 30 and 90
Ophthalmology - direct and indirect ophthalmoscopy, slit lamp	Prior to dosing, during Weeks 4 and 8 and prior to sacrifice on Day 90
Hematology [fasted, central ear artery] - Hb, Hct, RBC and WBC counts, RBC morphology, WBC differential, plt count, MCV, MCH, MCHC	Prior to dosing and sacrifice on Days 30 [N=3] and 90 [N=5]
Serum chemistry [fasted, central ear artery] – gluc., alb., glob, total prot., A/G ratio, total bili. Cl, Ca, Na, P, K, AST, ALT, ALP, BUN, creat. CPK, chol.	Prior to dosing and sacrifice on Days 30 [N=3] and 90 [N=5]
Necropsy	At sacrifice on Days 30 [N=3] and 90 [N=5]
Organ weights – liver, adrenals, brain, heart, kidneys, ovaries, testes, pituitary*	At sacrifice on Days 30 [N=3] and 90 [N=5]
Histopathology – adrenals, aorta, brain, cecum, sacculous rotundus, appendix, colon, duodenum, epididymides, esophagus, lacrimal gland/Harder's gland, ocular adnexa, eyes with optic nerve**, fallopian tubes, femur, gross lesions, heart, ileum, jejunum, mesenteric lymph node, ovaries, pancreas, peripheral nerve, pituitary gland, prostate/seminal vesicles, rectum, salivary gland, skin, spinal cord [cervical, mid thoracic, and lumbar], spleen, sternum with bone marrow, stomach, testes, skeletal muscle, thymus, thyroid/parathyroid, kidneys, liver/gallbladder, lungs, mammary gland, tongue, trachea, urinary bladder, uterus, vagina	At sacrifice on Days 30 [N=3] and 90 [N=5]

*weighed after fixation
**fixed in Davidson's solution

Results – Mortality/Clinical observations – There were no premature decedents and no treatment-related clinical observations.

- **Body weight/food consumption** – There were no treatment-related effects. The statistically significant changes were considered incidental due to the direction of the change, the magnitude of the change, a lack of a dose-dependent response, and/or the sporadic occurrence.
- **Ocular observations** – Very slight redness [e.g. vessels injected above normal] was observed sporadically in both control groups. There was an increased incidence and frequency of this finding in all treated groups regardless of formulation. In general, the score was 2. However, in 2 males in Group 5 and 2 females in Group 6 on 1 day, the score was 4, which consisted of "more diffuse, crimson red and individual vessels not easily discernible". [Note: There is a discrepancy between the text on p. 16 and Table 1A. The text indicates that 2 females were affected and the table indicates 1 female was affected.] Frequency of irritation tended to decrease with time. The following table delineates the number of animals affected and the total number of observations for males and females combined.

DOSE GROUP	INCIDENCE OF OCULAR IRRITATION			
	PRIOR TO DOSING [# animals/total observations]		1 HOUR POST DOSING [# animals/total observations]	
	Days 1-30*	Days 37-90**	Days 1-27*	Days 30-86**
Saline Control				
VH Control				
Rescula Eyedrops - BID	4/4	0	15/41	6/14
Rescula Eyedrops - QID	4/4	1/1	14/42	4/7
MS-016 –BID	4/6	1/1	11/48	5/10
MS-016 - QID	3/3	1/1	13/50	313

*n=16
**n=10

- **Ophthalmology**

There were no treatment-related effects.

- **Hematology/Serum chemistry** - There were sporadic, statistically significant changes in both males and females. These were not considered treatment-related due to the magnitude of the change, lack of a consistent pattern, and/or lack of a dose-dependent response.
- **Necropsy** – There were no treatment-related effects.
- **Organ Weights** – There were no treatment-related effects.
- **Histopathology – Ocular Findings** – Tissues from the 30-day sacrifice time point were not evaluated based on the 90-day evaluation. There were no effects in either ocular or non-ocular tissues that were considered to be treatment-related. Observed effects occurred either sporadically or at a comparable incidence rate across treatment groups or in the untreated eye.

Reviewer's Comments – Data Presentation and Study Design – These were adequate for the stated objective of the study.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. There was no systemic toxicity observed with the administration of either formulation of UF-021. **Reviewer's Comment** – The Reviewer concurs.

2. There were no "biological differences" between the formulations. Very minimal ocular irritation [e.g. conjunctival vessels injected above normal] was observed in all groups. However, the incidence was greater in the groups administered either formulation of UF-021. **Reviewer's Comment** – In general the Reviewer concurs.

iii. Title: 90-day ocular toxicity study in Dutch Belted rabbits [Vol. 1.17-1.19]
Study Identification:
Site:
Study Dates [In-life]: April 17-July 17, 1997
Formulation and Lot No.:

Excipient Vehicle Control

Certificate Analysis: The Sponsor provided results of an analysis [App. 5] which indicated that UF-021 was not present in the vehicle and that drug substance was within specifications [e.g. 1.08-1.32 mg/ml]
Final Report: February 24, 1998
GLP and QA Statements Signed: Yes [X]
Objective: "To compare the ocular toxicity of two test article formulations administered either three or four times daily for 90 days to Dutch Belted rabbits"

Study Design:

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	µg/eye/ dose	µl/eye /dose	route	Tx regimen			
1- VH Control			OD	90 days	M/F	8	Dutch Belted Rabbits –
2 – INN - 0.12%	36			QID			App. 12-16 weeks
3 – INN - 0.15%	45			TID			M – 1.6-2.1 kg; F – 1.7-2.3 kg
4 – INN - 0.15%	45			QID			
5 – IUOS –0.12%	36			QID			

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	Prior to dosing and sacrifice
Body weight	Weekly
Food consumption	Daily
Ocular observations - Draize	Prior to dosing on Days 1-9, 12, 13, 15, 16, 20, 22, 23, 26, 27, 29, 30, 36, 37, 43, 44, 50, 51, 57, 58, 64, 65, 71, 72, 78, 79, 85, 86, 90 and 91 and 1-hr after dosing on Days 1, 3, 5, 7, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50, 57, 64, 71, 78, and 85
Ophthalmology - direct and indirect ophthalmoscopy, slit lamp	Prior to dosing, Day 7, during Weeks 4 and 8 and prior to sacrifice
Hematology [fasted, central ear artery] - Hb, Hct, RBC and WBC counts, RBC morphology, WBC differential, plt count, MCV, MCH, MCHC Coagulation parameters - PT, APTT	Prior to dosing and sacrifice
Serum chemistry [fasted, central ear artery] – gluc., alb., glob, total prot., A/G ratio, total bili. Cl, Ca, Na, P, K, AST, ALT, ALP, BUN, creat. CPK. chol., triglyc., LDH	Prior to dosing and sacrifice
Necropsy	At sacrifice

Parameter Evaluated	Time Point(s)
Organ weights – liver, adrenals, brain, heart, kidneys, ovaries, testes, pituitary*, epididymides, prostate, spleen, thymus, uterus	At sacrifice
Histopathology – adrenals, aorta, brain, cecum, sacculous rotundus, appendix, cervix, colon, duodenum, epididymides, esophagus, lacrimal gland/Harder's gland, ocular adnexa, eyes with optic nerve**, fallopian tubes, femur, gross lesions, heart, ileum, jejunum, mesenteric lymph node, ovaries, pancreas, peripheral nerve [sciatic], pituitary gland, prostate/seminal vesicles, rectum, salivary gland, skin , spinal cord [cervical, mid thoracic, and lumbar], spleen, sternum with bone marrow, stomach, testes, skeletal muscle, thymus, thyroid/parathyroid, kidneys, liver, lungs, mammary gland, tongue, trachea, urinary bladder, uterus, vagina	At sacrifice

*weighed after fixation
**fixed in Davidson's solution

Results – [Note: Ocular lesions on Days 90 and 91 were not considered for 2, 1, and 3 animals from the placebo, 0.12% INN [QID] and 015% INN [TID] since eyes were inadvertently rinsed with [redacted] contaminated solution following fluorescein staining.]

- **Mortality/Clinical observations** – There were no premature decedents and no treatment-related clinical observations.
- **Body weight/food consumption** – There were no treatment-related effects.
- **Ocular observations** – When observed, ocular irritation [vessels injected above normal, Draize score = 2] was very minimal in all treatment groups with the exception of a single male administered 0.15% INN [QID] which exhibited moderate ocular irritation [Draize score = 4] on 3 days. No irritation was observed in the VH control animals. The number of animals exhibiting irritation prior to dosing as well as the total number of observations prior to dosing increased with time from the value on Days 1-16 to that on Days 19-50 and Days 51-91. There was no apparent change over time in the number of animals exhibiting irritation. The total number of observations 1 hour after dosing was either unchanged or decreased with time. The number of animals exhibiting irritation was comparable across treatment groups, but the total number of observations varied, with the greatest number of observations observed in the 0.15% INN [QID] group. The foliowing table delineates the number of animals affected and the total number of observations for males and females combined.

DOSE GROUP	INCIDENCE OF OCULAR IRRITATION					
	PRIOR TO DOSING [# animals/total observations]			1 HOUR POST DOSING [# animals/total observations]		
	Days 1-16	Days 19-50	Days 51-91	Days 1-16	Days 19-50	Days 51-91
0.12% IUOS QID	1/1	10/14	5/9	7/9	5/8	4/9
0.12% INN QID	2/2	5/13	11/14	6/17	8/20	6/9
0.15% INN TID	5/6	8/21	7/22	9/18	8/17	6/13
0.15% INN QID	3/4	7/10	12/30	11/31	8/21	10/18

- **Ophthalmology** [redacted]

There were 3 animals, [1 each in the placebo, 0.15% INN (TID) and 0.15% INN (QID)], that exhibited an erosion involving up to 25% of the cornea on Day 7 only. [redacted]

[redacted] indicated that the erosions might have been secondary to trauma but that a drug and VH effect could not be ruled out.

- **Hematology/Serum chemistry** - There were no treatment-related effects compared to placebo control and/or baseline. In females administered 0.15% INN QID, there was a statistically significant increase in BUN compared to control and baseline values [26 ± 2.5, 22 ± 2.7, and 31 ± 3.2 mg/dL for control animals and 0.15% INN QID animals at baseline and terminal sacrifice, respectively]. There was a statistically significant increase in creatinine compared to control values but not compared to baseline values for that group. The Sponsor indicates that the values were within historical control values, but historical controls were not provided. This finding is not considered treatment-related based on results from the other toxicity studies. In addition, it was not observed in males.

- **Necropsy** – Cloudy corneas were observed in several of the animals that had their eyes flushed inadvertently with [redacted] contaminated rinse following fluorescein staining. Other findings occurred sporadically [e.g. 1 animal] and were not considered to be treatment-related.

- **Organ Weights** – There were no treatment-related effects. There was a statistically significant increase in absolute and organ:brain ovary weight vs. placebo control. A treatment-related effect should be considered based on findings from other toxicity studies [nonocular administration of UF-021]. However, due to the differences in exposure, the magnitude of the change and a lack of histopathological correlates in this study, this finding is considered most likely an incidental finding.

- **Histopathology** – Acute keratitis and stromal fiber swelling observed in 1 male each in the control group and in the 0.15% INN [TID] groups was attributed to rinsing the eye with [redacted] contaminated solution. Other ocular lesions were graded as slight involved the adnexa [e.g. Harder's gland adenitis], occurred at a low rate of ≤2 animals/group, and were observed in both treated and untreated eyes. Non-ocular lesions either occurred sporadically [e.g. generally in 1 animal/group] or at a frequency that was comparable across treatment groups. The conclusion that there were no treatment-related effects is based on the assumption that the VH did not induce any histopathological changes.

Reviewer's Comments – Data Presentation and Study Design – A saline control would have been a more appropriate control than the VH control. However, data presentation and study design were adequate for the stated objective.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. There was no evidence of systemic toxicity or treatment-induced changes in the histopathology of the eyes and adnexa.

2. Slight ocular irritation was observed in all drug-treated groups.

Reviewer's Comments – In general, the Reviewer concurs. The overall incidence of the ocular irritation tended to be slightly greater in the animals instilled with 0.15% INN QID.

iv. Title: <u>1 Year Ocular Toxicity Study of 0.15% Unoprostone Isopropyl Ophthalmic Solution [UIOS] in Dutch Belted Rabbits [Vol. 1.20-1.23]</u>
Study Identification: [redacted]
Site: [redacted]
Study Dates [In-life]: Feb. 18, 1998 – Feb. 19, 1999

Formulation and Lot No.: [redacted]
Excipient Vehicle Control
Saline Control

Certificate Analysis: Yes [X] – Appendix V; Vol. 1.23; Analysis on Days 1 and 365 indicated that the actual concentration of US-021 was approximately 100% of the anticipated concentration

Final Report: November 10, 1999

GLP and QA Statements Signed: Yes [X]

Objective: "To determine the ocular toxicity of 0.15% Unoprostone Isopropyl Ophthalmic Solution [UIOS] when administered two or four times daily for 1 year to Dutch Belted rabbits."

Study Design:

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	µg/eye	µl/eye	route	# of days			
1- VH Control			OD	1 year	M/F	8	Dutch Belted Rabbits – [redacted] App. 15 weeks at study start M – 1.5-2.0 kg; F – 1.5-2.0 kg Individually housed
2 – Saline Control							
3 – UIOS - 0.15%	45			BID			
5 – IUOS –0.15%	45			QID			

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	Prior to dosing and sacrifice
Body weight	Weekly
Food consumption	Daily
Ocular observations - Draize	Weekly for 30 days, then q2wk until sacrifice – 1 hour after the last dose of the day and prior to 1st dose of the day
Ophthalmology - direct and indirect ophthalmoscopy, slit lamp	Prior to dosing, Day 7, during Weeks 12, 26, 40 and 52 [prior to sacrifice]
Hematology [fasted, central ear artery] - Hb, Hct, RBC and WBC counts, RBC morphology, WBC differential, plt count, MCV, MCH, MCHC Coagulation parameters - PT, APTT	Prior to dosing, at 6 mo., and prior to sacrifice
Serum chemistry [fasted, central ear artery] – gluc., alb., glob, total prot., A/G ratio, total bili. Cl, Ca, Na, P, K, AST, ALT, ALP, BUN, creat. CPK, chol., triglyc., LDH	Prior to dosing, at 6 mo., and prior to sacrifice
Urinalysis [collected overnight] – color, appearance, pH, protein, urobil, gluc. ket., occult blood, spG, bili, microscopic examination	Prior to dosing, at 6 mo., and prior to sacrifice
Necropsy	At sacrifice [Day 366]
Organ weights – liver, adrenals, brain, heart, kidneys, ovaries, testes, pituitary and thyroid/parathyroid*, epididymides, prostate, spleen, thymus, uterus	At sacrifice

Parameter Evaluated	Time Point(s)
Histopathology – adrenals, aorta, brain, cecum, sacculous rotundus, appendix, cervix, colon, duodenum, epididymides, esophagus, lacrimal gland/Harder's gland, ocular adnexa, eyes with optic nerve**, femur including articular surface, heart, ileum, jejunum, kidney, liver, lungs, mammary gland[s], mesenteric lymph node, ovaries, pancreas, peripheral nerve [sciatic], pituitary gland, prostate/seminal vesicles, rectum, salivary gland, sciatic nerve, skin , spinal cord [cervical, mid thoracic, and lumbar], spleen, sternum with bone marrow, stomach, testes, skeletal muscle, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina	At sacrifice

*weighed after fixation
**fixed in Davidson's solution

Results

Mortality - There were 4 premature decedents: 1 Group 4 male on Day 209; 1 Group 3 male on Day 243; and 2 Group 2 females sacrificed on Days 184 and 232 in moribund condition. Death was attributed to hairballs. Trichobezoars were found in 3/4 of these animals. Scant contents in the GI tract and a thickened pylorus was observed in the 4th animal.

Clinical Observations - Signs in the premature decedents included salivation, poor grooming, prominent inner eyelid, labored breathing, decreased activity, abnormal gait and stance, decreased respiration, weight loss, and/or white mucousy, dry, scant or no feces. Other signs [e.g. soft or scant stool, ocular discharge, hair loss, skin lesion] occurred sporadically, generally in 1 animal per group, and were not considered treatment related. There was brown mucous on cage paper in 4/8 females in Group 4. This finding was observed in 3 of these animals between Days 309-312. The relationship to treatment is not known. [Note: There were several inaccuracies in the data entry. These inaccuracies did not impact the interpretation of the findings.]

Ocular Observations [Draize Scoring] – Irritation, generally minimal [Draize score of 2] and characterized by increased conjunctival vessel injection, was observed in all groups. The Sponsor attributed all episodes of irritation to mechanical and not treatment effects since it was sporadic and had resolved overnight. The frequency of irritation was greater in the drug-treated groups compared to the saline and VH control groups. Therefore these findings are considered to be treatment-related. During approximately the first 6 months, the overall incidence of irritation tended to increase with an increased frequency of drug administration. There were 1-2 animals in the VH control and drug-treated groups that sporadically had a Draize score of 4-6. There was not always a correlation between the animals that exhibited irritation 1 hour after the last dose and those exhibiting irritation prior to the 1st dose. The table below outlines the number of animals affected and the total incidence of irritation for both males and females.

	Days 1-58		Days 71-156		Days 169-254		Days 267-366	
	1 Hour Post*	Prior to Dose 1	1 Hour Post	1 Hour Prior	1 Hour Post	1 Hour Prior	1 Hour Post	1 Hour Prior
Saline **								
VH Control								
UIOS BID	12/30	3/5	9/15	4/6	10/30	10/14	7/19	9/18
UIOS QID	16/45	9/18	11/33	9/21	13/30	9/19	8/16	8/18

*1 hour after the last dose

** 1 female receiving saline exhibited OU ocular discharge and was not included in this table, it also does not include the scores for animals whose eyes were inadvertently rinsed with

- **Ophthalmic Examination** - There were no treatment-related effects [e.g. Baldwin Score was 0 for all animals.]

- **Body Weight, Body Weight Gain, Food Consumption** - There was a decrease in all three parameters in 3/4 premature decedents. There were no treatment-related effects on body weight, body weight gain, or food consumption.

- **Hematology** – There were no effects that were considered treatment-related. The Sponsor indicates that the statistically significant change in platelet counts [20% ↓] in Group 4 females and in lymphocyte numbers in Groups 2 and 4 females [~30% ↑] values were within historical controls, but the historical control data were not provided.

- **Serum Chemistry** – There were no treatment-related effects. The statistically significant increase in ALT in Group 4 males at 6 months but not at 12 months was considered unrelated to treatment due to the magnitude of change compared to baseline and a lack of histopathological correlates.

- **Urinalysis** – There was a slight increase in the frequency of blood in the urine in Groups 3 and 4 females [4 each] compared to Group 1 [1]. The relationship to treatment is considered unlikely.

- **Necropsy** – There were no treatment-related effects. Three of the four premature decedents had trichobezoars. The fourth premature decedent had scant contents in the GI tract, thickened pylorus and multi red areas in the nonglandular region, and red fluid in the bladder.

- **Organ Weights** – There were no treatment-related effects.

- **Histopathology** – Moderate to marked hepatic lipidosis was noted in 3/4 premature decedents. Harderian gland inflammation was noted in the right eye in 0, 2, 3, and 2 animals from Groups 1, 2, 3, and 4, respectively. It was also noted in the untreated eye of 2 animals each from Groups 2 and 3. The relationship to treatment is questionable. All other lesions occurred sporadically [e.g. ≤2 animals/group] and/or at the same incidence as in the saline control animals. In general, the severity of lesions was minimal to mild.

Reviewer's Comment –Study Design and Data Presentation – These were adequate for the stated purpose. The Sponsor indicates that all values for serum chemistry were within historical controls. However, they did not provide any historical control data.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. UIOS at 0.15% did not induce any systemic or ocular toxicity when administered BID or QID. **Reviewer's Comment** – The Sponsor attributes the ocular irritation, based on Draize scoring, to mechanical effect. The incidence was greater in the drug-treated groups and is considered likely to be treatment-related. However, the irritation was minimal to mild.

c. Dogs

i. Title: Chronic toxicity study of repeated topical administration of UF-021 to the eyes of beagles [Vol. 1.23-1.24]
Study Identification: [redacted]
Site: [redacted]
Study Dates: March 19, 1990 – January 28, 1992
Formulation and Lot No.: [redacted]
Excipient Vehicle
Certificate Analysis: No [X] Analyses were conducted on March 19, June 18, September 4 and December 5, 1990, and February 25, 1991
Final Report: January 28, 1992
GLP and QA Statements Signed: Yes [X] [redacted]
Objective: "To conduct a nonclinical study concerning the safety of UF-021 by means of 1 year repeated topical administration ...to the eyes of beagles."

Dr. Terry Peters reviewed this study for [redacted] [Review completed April 10, 1997; pp. 4-5]. The results are summarized here.

Results – There was no anterior chamber flare in dogs administered 35 µl/eye of either normal saline, excipient VH, or 0.12% UF-021 [redacted] BID-QID. Conjunctival congestion was random among all groups. It should be noted that the control group received excipient VH, which was found to be mildly irritating in rabbits. Miosis was observed in UF-021 treated animals only and was a dose-dependent effect. The UF-021 miotic effect is specific to dogs and cats. This is supported by *in vitro* data cited by the Sponsor that indicated that $PGF_{2\alpha}$-ester induced a strong contractile response of the pupillary sphincter muscles in cats and dogs but not in humans and rabbits. There were no significant differences in clinical condition, body weight, food consumption, EKGs, audiology, hematology, clinical chemistries, organ weights, or histopathology, in animals receiving test article when compared to control animals.

d. Nonhuman Primates

i. Title: UF-021/MS-019 Dose ranging effects on intraocular pressure during a single topical administration in ocular normotensive cynomolgus monkeys [Vol. 1.9, pp. 70-102]
Study Identification: [redacted]
Site: [redacted]
Study Dates: Not provided
Formulation and Lot No.: [redacted]
VH and Saline control
Certificate Analysis: Yes [X]
Final Report: February 14, 1997
GLP and QA Statements Signed: No [X]
Objective: "To assess the dose-ranging effects of a single topical treatment with ... UF-021/MS-019 eye-drop formulations on the ... IOP in ocular normotensive cynomolgus monkeys"

Since this is a non-GLP dose-ranging study, the results will be summarized. In addition, the composition of MS-019 was not provided.

Study Design and Results – A single administration of 30 µl of 0.06%, 0.09%, 0.12%, 0.15%, and 0.18% UF-021/MS-019 was instilled into one eye each of ocular normotensive cynomolgus monkeys [M; N=10 for VH; N=8/group with each group treated at all concentration levels; washout period duration not indicated]. Saline was instilled into the contralateral eye. Ocular tolerability and IOP was assessed, under anesthesia, at 30 min., 1, 2, 3, 4, and 6 hours after instillation. Moderate ocular irritation [incidence not indicated], characterized by discharge, lid edema, and repeated blinking was observed at 0.15% and 0.18% only. The Sponsor indicates that there was "slight discomfort" observed at 0.12%. Response to IOP effects was variable with some monkeys not responding. Decreases in IOP in the contralateral eye were inconsistent and not related to dose.

ii. Title: <u>**104-week ocular chronic toxicity study in the cynomolgus monkey [Vol. 1.25 (pp. 60-244) – Vol. 1.26]**</u>
Study Identification:
Site:
Study Dates: April 16, 1996 – April 22, 1998
Formulation and Lot No.:
Saline control:
Certificate Analysis: Yes [X]; Addendum 4; Analyses were conducted on Jan. 19, 1996 and Oct. 6, 1998
Final Report: October 6, 1999
GLP and QA Statements Signed: Yes [X]
Objective: "To evaluate the ocular toxicity of ...(Rescula 0.12% Eye Drops) with special emphasis on possible iris color changes, when administered twice daily...to the Cynomolgus monkey for at least 104 weeks."

Study Design:

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	µg/eye/ dose	µl/eye /dose	route	# of doses			
1 - Saline Control					M	10**	Cynomolgus monkeys – purpose bred – Best Engineering Co. App. 5-8 years 3.4-7.0 kg
2 - Rescula –0.12%	60			104 wk			

*1 drop
**p. 71 – It is stated that 19 animals were obtained – it is assumed that the 20th monkey was already in house.

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	BID
Body weight	Weekly
Food consumption	Estimated daily and evaluated weekly

Parameter Evaluated	Time Point(s)
Ophthalmology [blinded evaluation]- direct and indirect ophthalmoscopy, slit lamp	Prior to dosing then monthly
Iris Examination [blinded evaluation] – iris colorimetry	Prior to dosing then monthly
Necropsy	At sacrifice [Day 366]
Organ weights – liver, adrenals, brain, heart, kidneys, testes, thyroid/parathyroid, spleen, thyroid	At sacrifice
Histopathology –eyes with optic nerve* -standard H&E, electron microscopy, densitometry	At sacrifice

*fixed in Davidson's solution

Results

- **Mortality** – There were 2 premature decedents, 1 each/group. Death occurred during recovery from anesthesia in the Group 2 animal. Undernutrition, pale mucosae, hollow eyes, and recumbency preceded death in the animal from the control group. Cause of death in this animal was not identified.

- **Clinical Observations** – There were no treatment-related effects.

- **Body Weight, Body Weight Gain, Food Consumption** – There were no treatment-related effects.

- **Ocular Evaluation - Iris Color Evaluation** – There were sporadic differences between the values in the treated vs. untreated eyes [Weeks 9, 13, 52, 70, and 90]. There were no differences between saline and drug treated eyes. The Sponsor suggests that this may reflect dosing-procedure effects.

- **Irritation Grading** – At Week 105, 1 animal in Group 2 exhibited changes in the lens. The relationship to treatment is considered unlikely. Other findings were sporadic and comparable between groups. Grading generally was ≤1.

- **Organ Weights** – There was a statistically significant decrease in relative but not absolute liver weight in the treated compared to controls [14% decrease]. This was considered to be unrelated to treatment.

- **Histopathology** [including electron microscopy and densitometry]– There were no treatment-related effects. The ultrastructural evaluation of the trabecular meshwork did not reveal any differences between the two groups. The Sponsor notes that these findings did not confirm the hypothesis that UF-021 "decreases intraocular pressure by altering the aqueous outflow through the trabecular meshwork". Since this is a pressure dependent system, the effects may become apparent only under conditions of increased intraocular pressure. However, it should be emphasized that decreases in IOP were observed in normotensive rabbits, cats, dogs, and monkeys. The effects of the test article on the ciliary muscle [e.g. uveoscleral outflow] were not assessed. Based on dosimetry, there was comparable local and total transmission for the untreated, NaCl, and UF-021 treated eyes suggesting no changes were induced in the iridic pigmentation.

Reviewer's Comment – Study Design and Data Presentation – The test article concentration in this study [0.12%] is less than that which is present in the clinical formulation [0.15%]. It was administered at the same frequency as will be used in the clinical setting.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. There were no treatment-related effects observed in this study. The transient change in iris color in the drug and saline treated eyes [both groups] compared to the contralateral untreated eyes was not considered to be drug-related. **Reviewer's Comments** – The Reviewer concurs.

iii. Title: 18-month ocular study on iris color changes in the cynomolgus monkey: Unoprostone isopropyl ophthalmic solution versus Latanoprost [Submission dated April 13, 2000]
Study Identification:
Site:
Study Dates: February 19, 1998- September 16, 1999
Formulation and Lot No.: MS-028-Rescula
Comparative/positive control: latanoprost [Xalatan™]
Certificate Analysis: No [X]
Final Report: April 7, 2000
GLP and QA Statements Signed: Yes [X]
Objective: "To evaluate the ocular tolerance of the test articles...Rescula Eye Drops and Latanoprost...with special emphasis on possible iris color changes, when administered topically one to two times daily to the Cynomolgus monkey for at least 18 months."

Study Design:

Test Material/ Group Designation	Dose			Sex	N	Species/Strain
	µl/eye	route	dosing schedule			
Months 1-12						
1- UIOS 0.15%	50 [1 drop]	OD	BID	M/F	5/6*	Cynomolgus monkeys – purpose bred – Best Engineering Co.
2 – Latanoprost 0.005%			SID			
Months 13-18						
1- UIOS 0.15%	100 - [2 drops]		BID			App. 4-10 years F = 2.4-4.9 kg; M=4.7-5.5 kg
2 – Latanoprost 0.005%	50 – [1 drop]		BID			

*One female died on Day 16 and was replaced – apparently replaced from colony since statement on p. 18 indicates that 10 animals were obtained.

Parameter Evaluated	Time Point(s)
Mortality/Morbidity	BID
Clinical observations	BID
Body weight	Weekly
Food consumption	Estimated daily and evaluated weekly
Ophthalmology [blinded evaluation]- indirect ophthalmoscopy [direct when indicated], slit lamp [Baldwin scale for irritation]	Predose, Weeks 12, 26, 39, 52, 65, and 78
Eye lash examination	Predose, Weeks 12, 26, 39, 52, 65, and 78
Iris Examination– iris colorimetry, digital photographs	Predose, Weeks 2, 4, 6, 8, 10, 12, 6, 20, 24, 289, 32, 36, 40, 44, 48, 52, 56, 60, 65, 68, 72, and 78
Necropsy	At sacrifice
Histopathology –eyes with optic nerve* -standard H&E, gross abnormalities	At sacrifice

*fixed in Davidson's solution

Results – Mortality – One female administered UF-021 was found dead on Day 16. The Sponsor states that death was not associated with UF-021 but did not indicate cause of death.

- **Clinical Observations** – Sporadic emesis, stool changes, rash, and wounds were comparable across both treatment groups.

- **Body Weight, Body Weight Gain, Food Consumption** – These parameters were generally comparable between the two groups.

- **Ophthalmology/Ophthalmoscopy** – Findings were sporadic. The Baldwin irritation scoring was generally 0.

- **Eye Lash Examinations** – Findings were sporadic and comparable across the two treatment groups. There was no increase in lash growth or pigmentation reported for either group.

- **Iridal Color Examination** – Iris color changes were observed in 3/5 males and 1/5 females administered latanoprost at ≥32 weeks. Of these animals, changes in 1 male were not observed until Week 56 after dosing was increased to BID. Iris color change was observed in 1/5 males administered UIOS at ≥32 weeks

- **Necropsy** – Findings were sporadic and/or comparable across treatment groups.

- **Histopathology** - Findings were sporadic and/or comparable across treatment groups.

Reviewer's Comment – Study Design and Data Presentation – These were adequate for the stated objective.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. "There is no conclusive evidence that the finding ...[e.g. iris color change in 1 male]...is definitively induced by UIOS." **Reviewer's Comment** – There is also no conclusive evidence that this finding was not induced by UIOS. The Sponsor did not provide any evidence that this finding was spontaneous. Therefore, it is concluded that the iris color change was induced by UIOS. However, under the conditions of this study, the frequency of iris color change was greater in the group administered latanoprost.

2. **Studies Conducted with Metabolites, Analogues, and Degradation Products**

a. **Rabbits - Irritation Studies**

i. Title: **Comparative eye mucous irritation test with Rescula Ophthalmic Solutions, its analogues, metabolites and degradation products ophthalmic solutions in albino rabbits [Vol. 1.41; pp. 95-157]**
Study Identification:
Site:
Study Dates: October 29-December 17, 1991
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: December 17, 1991
GLP and QA Statements Signed: Yes [X]
Objective: To assess the potential ocular irritation of Rescula ophthalmic solution, metabolites, and degradation products in rabbits.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [redacted] *The review is provided below.*

Groups of 5 male New Zealand white rabbits were treated with a single dose of the products listed in the table below. All products were administered as 0.12% ophthalmic solutions. All treatments were instilled into the left eye as 100 µL aliquots; the right eye remained untreated. The eyes were examined with a slit lamp at 1, 3, 6, and 24 hours after treatment.

Test Substance	Description
Vehicle control*	Vehicle
Rescula ophthalmic solution	Drug product
Related substance A	Synthetic precursor
Related substance B	Secondary product
Related substance H	Decomposition product
Related substance J	Decomposition product
Metabolite M1	Metabolite
Metabolite M2	Metabolite
Degradation product	Rescula after 6 months storage @ 20 C (89.9% Rescula)

[redacted]

Results: All test substances were found to be non-irritating.

B. Acute Systemic Toxicity

1. Oral Administration

a. Mice

i. Title: Acute toxicity tests of UF-021 administered to mice orally and subcutaneously [Vol. 1.11; pp. 1-33]
Study Identification: [redacted]
Site: [redacted]
Study Dates [In-life]: November 12 – December 18, 1987
Formulation and Lot No.: [redacted]
Vehicle: [redacted]
Certificate Analysis: No [X]
Final Report: April 6, 1988
GLP and QA Statements Signed: Yes [X] These studies are translations, therefore, the signatures are not present
Objective: To assess the potential toxicity of UF-021 following a single IV or PO dose in mice.

Results - No mortalities were observed in male and female mice [N=10/sex/group] administered a single dose of 0, 1000, and 2000 mg/kg PO or SC. Test material was still present at the injection site at both sc doses and was associated with subcutaneous induration in 4-9/10 animals per sex per dose; therefore, an LD_{50} was not determined by this route. The LD_{50} was >2000 mg/kg by the oral route. There were no effects on clinical observations, body weight, or necropsy that were considered treatment-related.

2. Intravenous Administration

a. Mice

i. Title: Preliminary toxicity tests of single intravenous administrations of UF-021 using mice [Vol. 1.11; pp. 34-47]
Study Identification:
Site:
Study Dates [In-life]: January 14-21, 1988
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: Not provided
GLP and QA Statements Signed: No [X]
Objective: To determine the LD_{50} of UF-021 following a single iv administration to mice.

Results - Mortality was observed in all mice [males; N=5/group] administered a single iv dose of 250 or 500 mg/kg and in 1/5 at 100 mg/kg. LD_{50} was between 100-200 mg/kg. Death was preceded by respiratory suppression, clonic seizures, and prostration. These signs were also transiently observed in animals that survived at 40 and 100 mg/kg, but not at 20 mg/kg. Pulmonary hemorrhage was observed in the premature decedents. Severity was dose-dependent. Necrotic tail changes were observed in surviving animals. According to the Sponsor, there was a dose-dependent decrease in weight gain at ≥40 mg/kg by Day 7.

a. Rats

i. Title: Acute toxicity study of UF-021 administered intravenously in rats [Vol. 1.11; 109-184]
Study Identification:
Site:
Study Dates [In-life]: July 27 – September 16, 1988
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: January 17, 1989
GLP and QA Statements Signed: Yes [X]
Objective: To determine toxicity of UF-021 following a single iv administration of UF-021 to rats.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study . The results are summarized here.

Results – Rats [N=10 sex/group] were administered iv doses ranging from 54-200 mg/kg. The median lethal dose was 93.5 and 125 mg/kg in male and female rats, respectively. Mortality was 100% in males and females at 200 mg/kg and in males at 154 mg/kg. Mortality was 70% in

females at 154 mg/kg. At 200 mg/kg, rats exhibited a decrease in spontaneous movement and diarrhea. Staggering was observed in rats administered 54-118 mg/kg. Depressed respiration and clonic convulsions were observed in rats administered 70-200 mg/kg. Decreased skin temperature was observed in rats administered 118-154 mg/kg. Surviving rats recovered within 1 day of treatment. The necropsy findings in the premature decedents generally included pulmonary congestion, hemorrhage, and foamy fluid in the nose, oral cavity, and trachea.

c. Dogs

i. Title: Acute toxicity study of UF-021 administered intravenously in beagles [Vol. 1.12; pp. 83-178]
Study Identification:
Site:
Study Dates [In-life]: April 25 – August 1, 1989
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: June 11, 1990
GLP and QA Statements Signed: Yes [X]

Objective: To determine toxicity of UF-021 following a single iv administration of UF-021 to beagle dogs.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study The results are summarized here.

Results – There were no premature decedents at doses of 10 and 40 mg/kg. Vomiting, miosis, and increased respiration were observed in dogs [N=2 males/group] administered 10 or 40 mg/kg with resolution within 3-5 hours of treatment. Ataxia, decreased locomotion, hyperemia of the lips and eyes, and facial pruritis was observed in all groups including the excipient control group. Treatment did not induce any toxicologically significant effects in hematology although there was a 50-100% increase in WBC counts characterized by a neutrophilia following treatment. Increases in AST, ALT, and ALP was observed in 1 dog at 10 mg/kg and both dogs at 40 mg/kg with the most pronounced changes observed in ALT [app. 10-20X ↑] on Day 1. Values approached normal by Day 14. The effect did not appear to be dose-dependent. At necropsy, 1 dog administered 40 mg/kg had injection site swelling. Hepatic microgranulomas were observed histopathologically in dogs at 40 mg/kg.

3. Subcutaneous Administration

a. Rats

i. Title: Toxicity test of single subcutaneous administration of UF-021 in rats [Vol. 1.12; pp. 1-82]
Study Identification:
Site:
Study Dates: January 7, 1991 – January 22, 1992

Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: January 22, 1992
GLP and QA Statements Signed: Yes [X]

Objective: To determine the acute toxicity of the UF-021 following a single sc injection in rats.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this ***The results are summarized below.***

Results – There were premature decedents at 1000 mg/kg [1 male] and 2000 mg/kg [5 males and 3 females]. There were no premature decedents at 500 mg/kg. At necropsy pulmonary congestion was generally noted in the premature decedents. Injection site crusting was observed in surviving rats.

C. Repeat Dose Toxicity Studies

1. Oral Administration

a. Mice

i. Title: **MS-016; 4 week dose confirmation study in mice with administration by gavage [Vol. 1.27; pp. 43-208]**
Study Identification:
Site:
Study Dates [In-life]: November 25 – December 24, 1996
Formulation and Lot No.:
Vehicle:
Certificate Analysis: Yes [X]; Appendix 1
Final Report: April 26, 1999
GLP and QA Statements Signed: Yes [X]
Objective: "To confirm the acceptability of doses of MS-016...identified for a 2 year study in mice after oral administration by gavage for 4 weeks."

Test Material/ Group Designation*	Dose				Sex	N	Species/Strain
	mg/kg	ml/kg	route	# of doses			
1- Vehicle Control							CD-1 [Crl:CD®(ICR) BR] -
2- Distilled water	0		gavage	SID			M – 18-20 g; F – 15-18 g at arrival
3- MS-016	1.2						App. 8 weeks at study start
4- MS-016	3.6						Individually housed
5- MS-016	12						

*There were 30 M/F included in a satellite group for TK analysis, which was subsequently not performed.

Endpoints – Mortality, clinical signs, body weight, food consumption, water consumption, hematology, serum chemistry, necropsy, organ weights and limited histopathology [abnormal tissue, eye, heart, kidney, liver, lung, ovary, spleen, and testis]. Histopathology was not conducted on all premature decedents.

Results

Mortality – Twelve animals died during the pretrial period. The cause of death was not indicated. The table below indicates the number of premature decedents during the study conduct.

	Group 1	Group 2	Group 3	Group 4	Group 5
Males	1	0	3	3	1
Females	1	1	2	2	5

There were 12/30 male and 18/30 female premature decedents in the satellite group. The clinical signs, necropsy and/or histopathological findings in the premature decedents were generally consistent with gavage error and included [1] abnormal respiration, hypothermia, decreased activity, hunched posture, pale body, trembling, and/or yellow staining of the fur; [2] esophageal rupture and abnormal contents in the thorax; and [3] purulent pleuritis, purulent esophageal inflammation, purulent pericarditis, and splenic red pulp depletion. There is concern with the validity of the study due to the high number of premature decedents in the pretrial period since no cause of death was identified. In addition, there were only 50% of the high dose females available for evaluation at terminal sacrifice.

There was approximately a 40% decrease in ovarian weight in the group mean in the high dose compared to the Group 2 controls and 3 of the Group 5 females had ovarian cysts. The relationship to treatment is not known.

There were no treatment-related effects on body weight, body weight gain, food consumption, hematology, and serum chemistry. The other findings occurred sporadically and/or were distributed evenly across treatment groups. [Note: Individual animal histopathology data were not provided.]

There were several deficiencies in this study. Since this study is not pivotal, these deficiencies were less of a concern.

The Sponsor based the doses used in the 2-year bioassay in mice on this study. This study was not conducted.

b. Rats

i. Title: Preliminary subacute toxicity tests with 14 days of oral UF-021 administration to rats [Vol. 1.27; pp. 1-42]
Study Identification:
Site:
Study Dates [In-life]: December 25, 1987 – January 8, 1988
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: June 1, 1988
GLP and QA Statements Signed: No [X]
Objective: "To establish doses for subacute toxicity tests with 90 days of daily oral administration of UF-021 to rats

Study Design

Test Material/ Group Designation	Dose				Sex	N	Species/Strain
	mg/kg	ml/kg	route	# of doses			
1- Vehicle Control							Sprague Dawley [Crj:CD(SD) –
2- UF-021	50						Age – app. 6-week-old
3- UF-021	150						M – 187-209 g; F – 140-166 g
4-UF-021	450						Group housed [5]

Endpoints – General condition, body weight, hematology, serum chemistry, necropsy, and organ weights.

Results – There were no premature decedents. Transient, intermittent salivation was observed in males and females at doses ≥150 mg/kg within about 1-3 minutes after dosing. Signs generally resolved within 40 minutes. According to the Sponsor, this sign was first observed on Days 3-4 and increased in frequency with increased duration of dosing.

There was a statistically significant increase of 22% in absolute and relative liver weight in males at 450 mg/kg. There were no serum chemistry correlates and the relationship to treatment is not known.

There were no effects considered to be treatment-related on body weight, hematology, serum chemistry, and necropsy.

The Sponsor set the NOAEL at 50 mg/kg based on a 6% decrease in Hb at 150 and 450 mg/kg. Since there was no change in other RBC indices, this change is of questionable relationship to treatment. Therefore, a more appropriate NOAEL for this study would be 450 mg/kg.

Although only summary data were presented and no histopathology was conducted, both the study design and data presentation were considered adequate for a preliminary study.

ii. Title: MS-016 [DR 40002 Rescula]: 4 week dose confirmation study in rats with administration by gavage [Vol. 1.28]
Study Identification:
Site:
Study Dates [In-life]: November 14 – December 13, 1996
Formulation and Lot No.:
Vehicle:
Certificate Analysis: Yes [X] Appendix 1
Final Report: October 7, 1999
GLP and QA Statements Signed: Yes [X]
Objective: "To confirm the acceptability of doses of MS-016...for a 2 year study in rats after oral administration by gavage for 4 weeks."

Study Design

Test Material/ Group Designation*	Dose				Sex	N	Species/Strain
	mg/kg	ml/kg	route	# of doses			
1- Vehicle Control							Sprague-Dawley [Crl:CD BR] - [redacted] M – 73-81 g; F – 51-57 g at arrival App. 6-7 weeks at study start Individually housed
2- Distilled water	0		gavage	SID			
3- MS-016	1.2						
4- MS-016	3.6						
5- MS-016	12						

*Satellite Animals for TK analysis - 10 animals/sex were administered 12 mg/kg for 1 day.

Endpoints – Mortality, clinical signs, body weight, food and water consumption, ophthalmology, toxicokinetics [Days 1 (satellite animals) and 28/29 (main study animals)], hematology, serum chemistry, urinalysis, necropsy, organ weights, and histopathology [eye, GI tract, heart, kidney, liver, lung, ovary, spleen, submandibular lymph node, and/or testis in the VH placebo control and the high dose animals].

Results - There were no premature decedents.

Low and high dose males and females were bled 5 times for TK analysis on the day prior to sample collection for hematology and serum chemistry. Although this confounds the interpretation of hematology data, it would account for the statistically significant decrease [15-25%] in RBC indices when compared to the distilled water control at the low and high doses.

There were no treatment-related effects on clinical observations [according to the Sponsor], body weight, food and water consumption, serum chemistry, urinalysis, ophthalmoscopy, and organ weights. Necropsy and histopathology findings occurred sporadically, were comparable across treatment groups, and/or did not demonstrate a dose response. [Note: Comparison of the histopathology from Group 5 to Group 1 [saline control] would have been more appropriate since the VH will be administered in the clinical setting. However, lesions were generally very mild to mild.]

Toxicokinetics – The metabolite M1 was measured. The TK data are provided in the table below.

PARAMETER	DAY 1		DAY 28/29			
	12 mg/kg/day		12 mg/kg/day		1.2 mg/kg/day	
	Male	Female	Male	Female	Male	Female
T_{max} [hr.]*	0.25	0.25	0.25	0.25	0.25	0.25
C_{max} [ng/ml]	29.8	58.6	48.7	73.9	7.4	4.8
AUC_{0-8hr} [ng•hr/ml]**	68.6	74.8	98.6	83.2	ND#	ND

*First time point evaluated

**Values were obtained through 8 hours on Day 28/29

#ND = not determined

Higher values were observed on Days 28/29 compared to Day 1 suggesting that there was either accumulation or an induction of UF-021 metabolism. Exposure in females tended to be higher at the high dose than in males on both Days 28/29 and 1. The Sponsor suggests that the data "may be of limited reliability" due to interanimal variability [standard deviations of 30-50%]. There is further concern with data reliability since there were detectable levels of M1 prior to

dosing, predominantly in females. [Note: There were discrepancies in the presentation of the TK data: [i] Appendix 23 Tables 2 and 3 – It appears that the labels for the Group 5 and Group 3 animals were reversed; and [ii] Appendix 24 – The graphs indicate the data were for a 13 TK study. The Sponsor will be asked to clarify these discrepancies.]

2. Subcutaneous Administration

a. Rats

i. Title: Toxicity test in rats through subcutaneous administration of UF-021 for 3 months, followed by successive withdrawal thereof for 1 month [Vol. 1.29]
Study Identification:
Site:
Study Dates [In-life]: July 25 – December 1, 1989
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: November 14, 1990
GLP and QA Statements Signed: Yes [X]
Objective: To determine the toxicity of UF-021 in rats when administered for 3 months by the subcutaneous routes.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study ***The results are summarized below.***

Results – Reversible injection site crusting was observed in the majority of rats [N=12/sex/group] at 5 and 50 mg/kg, but not 0.5 mg/kg, of UF-021. Histopathology findings at the injection site in the VH and treated groups were reversible and included hemorrhage, edema, focal necrosis, crust, granulation, ulcer and/or healed ulcer.

There was a statistically significant, reversible decrease in RBC indices [<10%] in male rats at the end of the treatment period that occurred concomitantly with a 25% increase in percent reticulocytes, an increase in bone marrow [2 males and 5 females] extramedullary splenic [1 female] hematopoiesis, and a 12% increase in platelets compared to the VH control at 50mg/kg. A statistically significant [<5%] decrease in Hb was also observed in females at 50 mg/kg and in males at 5 mg/kg. Male and female rats at 50 mg/kg exhibited approximately a 15% decrease in total protein and albumin. These changes were considered by the Sponsor to be secondary to the injection site lesions. There was a 15-20% decrease in phospholipids in both males and females at 50 mg/kg/day. The relationship of this finding to treatment is not known.

There was approximately a 15-20% increase in relative spleen weights in both males and females that persisted through the recovery period in males. There was a reversible 85% increase in ovary weight at 50 mg/kg that was associated with an increase in the number of corpora lutea.

The NOAEL, based on findings other than injection site changes, was 5 mg/kg. [Note: The toxicological significance of the <5% decrease in Hb was unclear.] MTD was not reached, but adequate multiples of the human exposure were attained. The deficiencies noted by the previous reviewer were corrected in the NDA submission.

ii. Title: Toxicity test of UF-021 after repeated subcutaneous administration for 12 months and withdrawal of the drug administration for 3 months in rats [Vol. 1.32-33]
Study Identification:
Site:
Study Dates [In-life]: November 29, 1990 – March 6, 1991
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X] but concentration analyses are included
Final Report: April 6, 1992
GLP and QA Statements Signed: Yes [X]
Objective: To determine the toxicity of UF-021 in rats when administered for 12 months by the subcutaneous routes.

Dr. Terry Peters reviewed this study [Review completed April 15, 1997; pp. 1-3]. The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, also reviewed this study [Review completed October 8, 1997; pp. 15-19].

Animals: Sprague-Dawley rats (Crj: CD (SD), SPF, aged 6 weeks at study initiation.

Dose groups: 14 animals/sex/group for the interim sacrifice at 1 year; 8 animals/sex/group for the terminal sacrifice at 3 months after the final dosing.

Doses administered: 0.2, 2, and 20 mg/kg/d for 12 months given subcutaneously.

Results: The sponsor reports no effect of drug administration on clinical condition, body weight and feed consumption, urinalyses, or ophthalmoscopic examination. Decreased hemoglobin concentration and hematocrit (<10% for each parameter) were noted in the high dose females. The sponsor attributes these changes due to the ulceration of the injection sites. Lowered albumin (~10% difference from controls) and A/G ratio were seen in all mid and high dose males.

Gross necropsy: Scabs, alopecia and subcutaneous hemorrhages were noted at injection sites in mid and high dose animals. Induration of the injection site was noted in many high dose animals for the duration of the study.

Organ weights: The relative liver weights and the relative uterine weights were higher in the mid and high dose females, when compared to vehicle controls at the end of the 1 year dosing period. No significant differences from controls were found in absolute organ weights. No significant differences were noted at the end of the recovery period.

Histologic examination: Histology was performed on all treated animals. They state that 'relevant regions of 1 or more animals of the untreated or vehicle control group were subjected to histopathological examination.' At the 1 year sacrifice, scabs, ulcers, necrosis of subjacent muscle fibers, inflammation and fibrosis of the injection sites were noted in all animals with the severity of the lesions increasing with increasing dose. Increased hematopoiesis was seen in the females only. Sacrifices after the recovery period showed scars at injection sites.

Mammary Gland Findings in Sprague-Dawley Females at 1 Year Sacrifice

Dose/ Finding	Untreated	Vehicle Control	0.2 mg/kg	2 mg/kg	20 mg/kg
Mammary carcinoma	1	1	2	1	4
Fibroadenoma	1	1	1	1	1
Alveolar dilatation	4	7	6	7	10

Mammary Gland Findings in Sprague-Dawley Females after Recovery Period

Dose/ Finding	Vehicle Control n=8	20 mg/kg n=7
Mammary carcinoma	2	4
Fibroadenoma	1	0
Alveolar dilatation	0	5

Therefore, the total incidence of mammary carcinoma was 1/14 (7%), 3/22 (14%), 2/14 (14%), 1/14 (7%), and 8/21 (38%) for the untreated, vehicle control, 0.2 mg/kg, 2 mg/kg, and 20 mg/kg groups respectively. The sponsor contends that 'some physical property of UF-021, which is an unsaturated fatty acid, may have been a causative factor in the formation of these tumors.' With the rapidity of tumor formation at 1 year (decreased time-to-tumor), the lack of other toxicologically significant findings, and increased mammary carcinoma incidence at 20 mg/kg, this compound should be considered to have produced the findings of a potential animal carcinogen, and possibly acts as an initiator in Sprague-Dawley rats.

The sponsor assigned a toxicological NOEL of 0.2 mg/kg under the conditions of the present study. As no other toxicologically significant findings than mammary carcinoma were found in any of the parameters measured, no NOEL is assigned to this study.

b. Dogs

i. Title: Toxicity study of repeated subcutaneous administration of UF-021 for 3 months in beagles. [Vol. 1.31]
Study Identification:
Site:
Study Dates [In-life]: December 1, 1989 – March 24, 1990
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: January 8, 1992
GLP and QA Statements Signed: Yes [X]
Objective: To determine the toxicity of UF-021 in dogs when administered for 3 months by the subcutaneous route.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [Review completed October 8, 1997; pp. 11- 14]. The results are summarized below.

Results – Injection site swelling was observed in all dogs [N=3/sex/group] in the 5 mg/kg group throughout the study and in 1-3 dogs in the 0.5 mg/kg group during Weeks 12-14. Females at 5 mg/kg exhibited a statistically significant decrease [<10%] in body weight generally during Weeks 4-13.

Statistically significant increases in WBC counts [approximately 50-100%] were noted in males throughout the study at 5 mg/kg and in females at Weeks 5 and 9 in the 5 mg/kg group. This increase was characterized by a neutrophilia. RBC indices in the treated groups were comparable to the VH control and were decreased by ≤10% compared to the saline control. Albumin was decreased by approximately 10-20% in males and females in the high dose.

The primary findings on necropsy and histopathology were associated with injection site reactions and included induration, reddish discoloration of the hypodermis, abscess, cell infiltration, edema, hemorrhage, and necrosis of the hypodermis. Some of these findings occurred with greater frequency in the 5 mg/kg group, but no well-defined dose relationship was present.

There were no treatment-related effects on ophthalmology and urinalysis. The relationship of the statistically significant increases in relative kidney and lung weight in females at 5 mg/kg to treatment was unclear. There were histopathological correlates with the increased organ weights.

ii. Title: Toxicity study of repeated-dose subcutaneous administration of UF-021 for 1 year in beagle dogs followed by 1 month recovery study [Vol. 1.34]
Study Identification:
Site:
Study Dates [In-life]: July 5, 1990 – August 5, 1991
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: January 17, 1992
GLP and QA Statements Signed: Yes [X]
Objective: To determine the toxicity of UF-021 in beagles when administered for 12 months by the subcutaneous route.

Dr. Terry Peters reviewed this study [Review completed April 15, 1997; pp. 3-4]. This review is provided below. The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, also reviewed this study [Review completed October 8, 1997; pp. 15-19].

Animals: 3 beagles/sex/dose aged 7 months at study initiation for the main study; 2 beagles/sex/vehicle control and 5 mg/kg/d given a 1 month recovery period after 1 year of dosing.

Doses administered: saline control, vehicle control, 0.05, 0.5, 5 mg/kg UF-021/day administered subcutaneously.

Parameters evaluated: clinical condition, body weight, feed consumption, ophthalmologic examination, urinalysis, clinical chemistries, gross necropsy, organ weight, and histopathology.

Results: Clinical condition: At 5 mg/kg/d, males and females showed pain and induration at the injection sites. Mucoid, bloody feces, miosis, and swelling between the toes were also noted in these animals. The sponsor discounts these findings as compound-related as 'these were conditions observed in beagle dogs generally'. Additionally, conjunctival discharge, swelling or crusting of

the lips, opacity of the cornea were sporadically noted in the females. The low dose animals did not show these signs. The sponsor also discounted these findings as drug effects as they were only found in the females and 'the relationship to the test substance was not clear'.

Significant increases in total white blood cell counts were seen in the high dose animals at the ≥3 month timepoints. Although the increases were up to 21000/cc, the lymphocyte:neutrophil ratios were inverted, indicating a possible bacterial infection of unidentified origin. By the 1 year timepoint, treated animals were comparable to controls. No dose relationship is evident.

Sporadic differences from controls were noted in albumin, A/G ratios, and platelet numbers. None of these differences have toxicologic significance.

At gross necropsy, there was a pale, greenish gelatinous substance (possibly breakdown products from dorsal muscles or residual drug product, but not identified by the laboratory) was found in the hypodermis and dorsal muscles of all high dose animals. Thickening of the hypodermis was found in these animals as well. All other gross findings were sporadic in nature and not dose-related.

Histopathology revealed hemorrhage, necrosis, inflammatory cell infiltrates and fibrosis at the administration sites of the high dose males. At the lower doses, necrosis and cellular infiltration were noted in 1 male/dose. One vehicle control had hemorrhage, perivascular cell infiltration and degeneration of the epidermis at the administration sites. No abnormalities were noted in the recovery males.

In the high dose females, hemorrhage, necrosis, inflammatory cell infiltrates and fibrosis were noted at the administration sites. Similar findings to the males were noted in the controls and lower dose groups. No abnormalities were noted after the recovery period that were related to drug administration.

On the basis of the findings in this study, the sponsor concluded that the toxicity was mainly a function of becoming inflamed, and that the inflammation was cured after dosing was terminated. They suggest that the NOEL for this study with UF-021 is 0.05 mg/kg/d in beagle dogs. It is more likely that administration of the test compound caused damage to the subcutis and/or panniculus. On the basis of the clinical signs, the NOEL for this study is 0.5 mg/kg/d in beagle dogs.

3. Intravenous Administration

a. Dogs

i. Title: Subacute toxicity study of intravenous administration of UF-021 for 13 weeks in beagles [Vol. 1.30]
Study Identification:
Site:
Study Dates [In-life]: August 28 – November 29, 1989
Formulation and Lot No.:
Vehicle:
Certificate Analysis: No [X]
Final Report: February 5, 1992
GLP and QA Statements Signed: Yes [X]
Objective: To determine the toxicity of UF-021 following subacute intravenous administration [13 weeks] to beagle dogs.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [Review completed October 8, 1997; pp. 9-11]. The results are summarized below.

Results – A number of clinical signs were observed in male and female beagle dogs [N = 3/sex/group], which received daily intravenous injections of excipient vehicle, 0.2, 2.0 or 20 mg/kg of UF-021. Signs included lacrimation; reddening of the lips, ears, and eyes; face scratching; restlessness; head-shaking; hyperemia of the mucous membranes; and miosis. The dogs in the saline control group did not exhibit these effects. Injection site reactions, including edema, necessitated discontinuation of treatment in the 20 mg/kg group. Injection site reactions were observed at necropsy. The histopathogical findings in the 20 mg/kg group included [1] lung granuloma, thickening of alveolar wall, and yellowish granules in granulomas; [2] increased kidney vacuoles in the distal tubular epithelium; [3] degeneration of spermatogenic cells and decrease of mitosis; [4] degeneration of epididymal epithelium, prostate atrophy and degeneration of epithelium; and [5] thymic atrophy. Injection site pathology included hemorrhage, edema, and necrosis.

There were no treatment-related effects on ophthalmology, hearing, ECG, urinalysis, clinical pathology, and organ weights. The significant increases in ALT, AST, and SAP as well as the hepatic granulomas observed in the single dose iv at doses of 10 and 40 mg/kg were not observed in this study.

D. Acute Toxicity of UF-025 [M1] and Other Metabolites, Etc.

a. Mice

i. Title: Comparative toxicity test of single intravenous administration with Rescula, its related compounds, metabolites, and decomposed products in mouse [Vol. 1.11; 48-108]
Study Identification:

Site:
Study Dates: November 20 – April 10, 1992 [Note: The test dates indicated on p. 52 extended beyond the apparent report date. The Sponsor will be asked to clarify this discrepancy]
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: Jan. 10, 1992
GLP and QA Statements Signed: Yes [X]

Objective: To determine the acute toxicity of Rescula and various related compounds [e.g. metabolites, synthetic precursor, by-product, degradation products, deteriorated product] following a single iv injection in mice.

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [Review completed October 8, 1997; p. 32]. The results are summarized below.

Results - Prostration, depressed respiration, reduced motor activity, piloerection, and clonic convulsions were observed in mice [N=5 males/group] administered 70 mg/kg of Rescula, Substance A, B, H, and J, or the deteriorated product with recovery generally within 5 to 30 minutes. Depressed respiration, prostration, and reduce spontaneous motor activity but not convulsions was observed in mice that received 70 mg/kg of M1. Deaths occurred in 1 Rescula treated mouse on Day 2, 1 Substance B mouse 4 hours after treatment, and 2 Substance H mice within 30 minutes after dosing. Injection site swelling, which resolved within approximately 1-2 weeks, was observed in all groups except in the M4 group. There were no treatment-related effects on body weight or necropsy in the surviving animals. Premature decedents generally had a red-brown discoloration of the lungs.

b. Rats

i. Title: Toxicity test of UF-025 single intravenous administration in rats [Vol. 1.11; pp. 185-285]
Study Identification:
Site:
Study Dates: February 13, 1991 – January 22, 1992
Formulation and Lot No.:
Certificate Analysis: No [X]
Final Report: January 22, 1992
GLP and QA Statements Signed: Yes [X]

Objective: To determine the acute toxicity of the metabolite M1 following a single IV injection in rats [N=6/sex/group].

The previous Pharmacology/Toxicology Reviewer, Dr. Andrea Weir, reviewed this study [Review completed October 8, 1997; p. 32]. The results are summarized below.

Results: Pallor, bradypnea, tonic convulsions generally preceded death in rats [N=6/sex/group] administered UF-025 [M1] at 118, 154, and 200 mg/kg. Death was observed in 1 male at 91 mg/kg, 3 males and 2 females at 118 mg/kg 5/sex at 154 mg/kg and all animals at 200