Animal Strain: Macaques monkeys (Macaca nemistrina) [vendor was unspecified]

Animal Starting Weight: 5-7 kg

No. of Animals: 4 male animals used throughout study; individually housed at [redacted]

Test Materials: Lyophilized BPD-MA (batch number F93-120-0887) was reconstituted with 14 ml of sterile water for injection to make a final solution of 2 mg/ml.

Route: Intravenous infusion over a 10-minute period

Study Design:

Group Number	Dose Level (mg/kg)	Dose Concentration (mg/ml)
1	0.5	2
2	1.0	2

Methods: On the day of the experiment, the animals were anesthetized, and catheters were introduced into femoral and anticubetal veins for blood sampling and BPD-MA infusion, respectively. BPD-MA at 0.5 or 1.0 mg/kg was infused over 10 minutes at a rate of 2.5 ml/minute. The concentration of BPD-MA in the infused solution was in a range of 0.10-0.28 ml/kg. Blood samples were taken 15 minutes prior to drug infusion (control), and then immediately and at 15, 30, 60, 90, 120, 180, 240, and 360 minutes post infusion. Blood samples were centrifuged and plasma separated from cellular components. Plasma was analyzed for BPD-MA content by fluorescence spectroscopy. The excitation wavelength was 439 nm and emission was at 699 nm. Plasma samples were diluted with PBS containing 1% triton X-100. The values obtained were compared to a standard curved constructed with liposomal BPD-MA in plasma with PBS-1% triton X-100.

Results:

Plasma Levels of BPD-MA: The results are listed in Table 13. The maximum concentration of BPD-MA was detected at the first sampling point after infusion. BPD-MA was completely eliminated by 24 hours after a dose of 0.5 or 1 mg/kg BPD-MA. Rapid clearance takes place during the first hour post intravenous infusion. Complete excretion of BPD-MA occurred earlier in animals infused with the lower dose. When comparing the plasma levels of BPD-MA after an infusion of either 0.5 or 1 mg/kg, the BPD-MA levels in the animals infused with the lower dose were unproportional to the BPD-MA-plasma levels obtained in the animals infused with the higher dose.

Table 13. Plasma levels of BPD-MA in male macaques monkeys intravenously infused with either 0.5 or 1.0 mg/kg.

BPD Concentration in Plasma (µg/mL)		
Time Post i.v.	1 mg/kg Dose: MEAN ± SD	0.5 mg/kg MEAN ± SD
0.5 min	5.621 ± 1.641	
1.0 min		3.105 ± 0.441
4.0 min	4.24	
15.0 min	1.501 ± 0.007	0.445 ± 0.078
30.0 min	1.022 ± 0. 148	0.237 ± 0.083
1.0 hr	0.706 ± 0.050	0.128 ± 0.093
1.5 hr	0.568 ± 0.155	0.090 ± 0.072
2.0 hr	0.440 ± 0.164	0.058 ± 0.535
2.5 hr	0.421	
3.0 hr	0.231 ± 0.024	0.045 ± 0.045
4.0 hr	0.103	
4.75 hr	0.150	0.036
6.0 hr	0.064 ± 0.031	

Average (± SD) plasma levels of BPD-MA in macaques were determined following fast infusion of the drug at 0.5 or 1 mg/kg. Each dose was studied in 3 monkeys.

d. Ocular Pharmacokinetics

i. Title: Ocular distribution of BPD in the rabbit eye [Ref. 89]
Study Identification: PH-94020
Site: [redacted]
Study Dates: March – December 1994
Formulation and Lot No.: Liposomal BPD-MA [CL 318,952] Batch # F93-120-0883 or E93-120-0870
Certificate of Analysis: No [X]
Final Report (X) December 1994
GLP and QA Statements Signed: No (X)
Objective: To define the ocular distribution of liposomal BPD-MA following intravenous administration of the drug to rabbits with either pigmented or nonpigmented eyes.

Dr. Javier Avalos reviewed this study for IND [redacted]/Submission [redacted] review completed May 16, 1995. This review is provided below. Comments by the current Reviewer are in italics. Results from a pilot study evaluating the distribution of drug to ocular tissues following local administration will not be reviewed here since this route of administration is not relevant to the current application.

Laboratory: [redacted]
(Study Number PH-94020)

Animal Strain: New Zealand White rabbits (vendor was unspecified) and pigmented rabbits (strain and vendor were unspecified)

Animal Starting Weight: 1.8 to 3.2 kg

No. of Animals: 16 animals (8 pigmented and 8 non-pigmented); individually housed (24 hour time point only)

Reviewer's Comments - Clarification of number of animals – 4 animals had pigmented eyes and a total of 8 pigmented eyes were evaluated.

Test Materials: Thirty mg of liposomal CL 318,952 (BPD-MA) [batch number-J93-120-0883 or E93-120-0870] were reconstituted with sterile water for injection (15 ml).

Route: Single intravenous administration (6 mg/kg over a 1 minute period)

Methods: Animals were anesthetized with an intramuscular injection of a combination of 50 mg/kg Ketamine, 15 mg/kg Xylazine, and 2.5 mg AC Promazine. Subsequent injections were also given as needed to sustain full anesthesia until sacrifice at the designated time point following BPD-MA administration. Anesthetized animals were given an intravenous dose of 6 mg/kg BPD-MA, over a period of approximately 1 minute. The rabbits were dosed in an ear vein. Blood was taken from the opposite ear for analysis at 30 minutes, 1, 2, and 24 hours after dosing. [***Reviewer's Comments*** - *Tissue samples were collected at the same time points.*] Animals were sacrificed after the last bleeding. Eyes were removed and dissected. A total of 9 tissue compartments were separated out, and the sclera was further subdivided into three segments for comparison with similar segments isolated at or adjacent to the site of local BPD-MA delivery in a corresponding study. The tissue samples were extracted and the fluorescence from each sample was measured using an excitation wavelength of 433 nm and an emission reading at 692 nm. BPD-MA was estimated from a BPD-MA standard curve prepared by serial dilution in 95% ethanol.

Results:

Following administration of 6 mg/kg of BPD-MA, a significant distinction between albino and pigmented animals was not noted and thus, the data were combined for each time point. In the plasma, BPD-MA levels approximated 2 µg/100 µl at 2 minutes after dosing, 500 ng/100 µl at 15 minutes, and in excess of 125 ng/100 µl two hours following injection. Table 10 represents the levels of BPD-MA obtained in various tissues of both strains of rabbits. Maximum levels of BPD-MA (25-30 ng/10 mg of tissue) in choroidal tissue occur at or before 30 minutes following intravenous delivery. A similar pattern occurs in the ciliary body and processes (15-20 ng/10 mg of tissue) to that seen in the choroid. In the retina, the levels of BPD-MA continue to increase up to two hours (20 ng/10 mg of tissue). Tissue contained the lowest levels of BPD-MA 24 hours following injection. Levels of BPD-MA found in the choroid at this time period were 1.2 ng/10 mg of tissue. In the retina, the levels were higher at 3.8 ng/10 mg of tissue for the same time period. CL315,585 plasma concentrations were 5.89 and 2.80 µg/ml for males and females, respectively.

Table 10. Distribution of BPD-MA in ocular tissues of treated NZW and pigmented rabbits following an intravenous administration of 6 mg/kg BPD-MA.

TABLE 1	30 min (n=9)		1 Hour (n=6)		2 Hour (n=9)		24 Hr (n=6)	
	mean	SD	mean	SD	mean	SD	mean	SD
VITREOUS	0.04	0.04	0.01	0.01	0.02	0.02	0.00	0.01
LENS	0.01	0.03	0.01	0.02	0.01	0.03	0.00	0.00
CILIARY B/P	17.39	6.11	16.60	2.38	12.78	3.95	0.61	0.06
IRIS	6.71	2.51	4.66	1.34	4.05	1.36	0.06	0.15
-ANT SEG-	13.64	3.47	13.83	1.92	10.77	2.27	0.72	0.10
CORNEA	0.45	0.34	0.36	0.26	0.51	0.33	0.12	0.07
LIMBAL SCL	1.42	0.98	1.67	0.24	1.95	0.44	0.42	0.09
MID SCLERA	0.90	0.33	1.11	0.35	1.43	0.49	0.21	0.06
POST SCL	1.22	0.61	1.47	0.33	1.97	0.54	0.37	0.48
O NERVE RT	4.02	2.83	2.06	0.79	3.27	2.62	0.00	0.00
RETINA	11.67	2.92	16.14	1.60	17.62	3.79	3.78	0.56
CHOROID	27.22	5.43	24.84	4.73	18.08	4.53	1.17	0.67
AQUEOUS			0.14	0.10	0.07	0.06	0.02	0.03

-The units for the represented values are ng/10 mg of tissue.

Reviewer's Comments -

1. BPD-MA rapidly distributed to the iris, ciliary body, retina, and choroid. In addition, these tissues also demonstrated the greatest accumulation of drug. In most tissues, drug concentration decreased over time. The exception was the retina, in which drug concentrations increased over 2 hours. Minimal drug accumulated in the avascular portions of the eye [e.g. cornea, lens, and vitreous]. This would suggest that the iris, ciliary body, retina, and choroid would be the most susceptible to PDT toxicity.

Ref. 58: Haimovici, R. et. al. [1996] Localization of lipoprotein-delivered benzoporphyrin derivative in the rabbit eye. *Current Eye Res.* 16:83-90. – *Dr. Javier Avalos previously reviewed this study for IND [redacted] Submission [redacted] pp. 33-34; review completed May 16, 1995.[redacted] It is felt that this review is for the same study described here since the tables delineating the ocular distribution are the same. It is indicated, however, in the review that both pigmented and nonpigmented eyes were evaluated.* In the study described in this reference, LDL complexed-BPD-MA was administered at 2 mg/kg to albino rabbits. Eyes were enucleated from 5 minutes to 24 hours later and evaluated with light and fluorescence microscopy or used for porphyrin extraction. At 5 minutes post-injection, fluorescence was brightest in the choroid and retinal pigment epithelium [RPE]. After 20 minutes, choroidal fluorescence had decreased and RPE and photoreceptor fluorescence had increased. By 2 hours, no fluorescence remained in the choroid and photoreceptors. RPE fluorescence had decreased by 2 hours and trace amounts were visible at 24 hours. The authors discussed the potential role of LDL receptors and the uptake of BPD-MA [LDL-complexed and liposomal]. LDL receptors are present on endothelial cell and the expression is increased in proliferating endothelial cells. LDL receptors and phagocytic "scavenger receptors" are also present on RPE cells. Although the Sponsor stated that the findings at 5 minutes were similar with liposomal BPD-MA, it was not indicated whether the similarities persisted over time. Extrapolations to humans should be made cautiously due to differences in the blood supply of the rabbit eye compared with

nonhuman and human primates as well as differences in the formulation used in this study compared to the clinical formulation.

e. Reproductive Pharmacokinetics

i. Title: Placental transfer of BPD-MA [CL 318,592] after a single intravenous dose to pregnant rats [Ref. 345]
Study Identification: A9301
Site: [redacted]
Study Dates: January 22 – March 31, 1993
Formulation and Lot No. - Liposomal BPD-MA - ^{14}C-BPD-MA; Lot No. L921203A
Certificate of Analysis: Yes (X)
Final Report (X): Dec. 13, 1993
GLP Signed: No (X) **QA Statements:** Yes (X)
Objective: "To determine the extent of transfer of CL 318,952 [BPD-MA: Benzoporphyrin derivative of Monoacid] across the placenta to the fetus and to assess its pharmacokinetics in the pregnant rat when given as a single intravenous dose"

Study Design – A single iv dose of ^{14}C-BPD-MA [liposomal] in sterile water [25 mg/kg; 14.4 ml/kg; 2.5 ml/min; 36 µCi/ml] was administered to pregnant Sprague-Dawley rats on Gestation Day 15. At 0.083, 0.5, 1, 2, 4, 8, 12 and 24 hours after dosing, blood [cardiac puncture], amniotic fluid, and fetal and placental tissue samples were collected from anesthetized rats [N generally =2/time point]. Samples were also obtained from 1 rat prior to dosing. Maternal blood was analyzed for radioactivity and regioisomer concentrations. Pharmacokinetic parameters were calculated.

Results – The fetal:maternal plasma and placental:maternal plasma [$AUC_{0\text{-inf}}$] ratios, based on total radioactivity, were approximately 0.007 and 0.25, respectively. The $t_{1/2}$ was comparable for the dam and the fetus. The data are summarized in the table below.

Summary of Pharmacokinetic Parameters of BPD-MA (CL 318,952) Following IV Administration (25 mg/kg) in Pregnant Rats

Tissue	$C_{(max)}$[a] (µg/mL)	C_0[b] (µg/mL)	T_{max} (hr)	AUC(0-24) (µg.hr/mL)	AUC(0-inf) (µg.hr/mL)	CL(T) (mL/min/kg)	$V_{(ss)}$ (L/kg)	$T_{(1/2)}$ (hr)
Total radioactivity in maternal whole blood	70.2[c]	ND	0.083	273	279	1.5	0.5	4.6
Total radioactivity in maternal plasma	92.4[c]	ND	0.083	305	325	1.3	0.5	6.9
Unchanged in maternal plasma (315,555 plus 315,585)	73.1[d]	ND	0.083	215	227	1.8	0.6	7.3
Unchanged in 315,555 only	13.8[d]	13	0.083	33.4[e]	35.5	11.7	2.6	3.4
Unchanged in 315,585 only	59.3[d]	59	0.083	172	174	2.4	0.6	4.1
Total radioactivity in placenta	10.8[c]	ND	0.083	63.5	81.4	ND	ND	11.7
Total radioactivity in fetus	0.1[c]	ND	0.088	2.0	2.4	ND	ND	7.4
Total radioactivity in amniotic sac fluid	0.00[c]	ND	ND	ND	ND	ND	ND	ND

a = Concentration at 5 min, the first sampling time
b = Calculated concentration at zero-time
c = µg equivalents of radioactivity
d = µg /mL concentration determined using HPLC
e = Extrapolated to 24 hrs using plasma concentrations up to 12 hrs
ND = Could not be determined.

Sponsor's Conclusions –

1. Exposure to fetus, when compared to the dam, was small.

2. The fetal:maternal plasma ratio decreased with time suggesting that drug does not accumulate in the fetus.

3. The data indicate that the PK is comparable in pregnant and nonpregnant rats.

Reviewer's Comment – The Reviewer concurs.

II. **Metabolism** – Several studies were conducted, both *in vivo* and *in vitro*, in hepatic preparations and plasma from different species to evaluate the metabolism of BPD-MA. The studies are listed below with a brief synopsis of the methods. The results are summarized.

A. *In Vitro* Metabolism in Liver Slices, S9 Fractions, and Microsomes

a. Title: Species comparison of the in vitro metabolism of benzoporphyrin derivative [BPD-MA; CL 318,952] in liver slices and subcellular fractions [Hepatic S9 and microsomes] from rat, dog, and man [Ref. 359] – Hepatic preparations were incubated with 100 µM of BPD-MA plus oxidative metabolism cofactors [NADPH regenerating system]. Samples were taken at 0, 15, 30, 60, and 120 minutes of incubation and analyzed by HPLC.

b. Title: Metabolism of BPD-MA [verteporfin] by human liver S9 and microsomes [Ref. 285] – Hepatic preparations were incubated ± NADPH generating system with 3 µM of liposomal BPD-MA for 0 30, 60, and 90 minutes. Additional samples were incubated with either UDPGA or PAPS to determine the potential for conjugate formation. Samples were analyzed by HPLC for relative amounts of BPD-MA_C, BPD-MA_D, and BPD-DA.

c. Title: *In vitro* metabolism of BPD-MA by liver slices, from S9 fraction and microsomes from the rat, dog, and human with reference to regioisomers and enantiomers. [Ref. 375] – BPD-MA regioisomers and BPD-DA were quantified by HPLC and enantiomers were quantified by capillary electrophoresis in samples from Study PK-93002 [Ref. 359].

B. *In Vitro* Metabolism in Plasma

a. Species differences in the metabolism of BPD-MA [Ref. 286] - Rat, mouse and human plasma were incubated with aqueous BPD-MA for 15 minutes to 24 hours. Relative amounts of BPD-MA_C BPD-MA_D, and BPD-DA were determined by HPLC analysis.

C. *In Vivo* Metabolism – Plasma, Bile, Liver extracts

a. Species differences in the metabolism of BPD-MA [Ref. 286] – Plasma was collected 15 and 60 minutes from cannulated Sprague-Dawley rats [gender not provided; N=2] following an intravenous injection of 4 mg/kg of radiolabeled liposomal BPD-MA. Plasma was collected 0, 5, 15, 30, 60, 90, 120, 150, and 180 minutes from Yucatan mini swine [gender not provided; N=2] following an intravenous injection of 2 mg/kg of

radiolabeled liposomal BPD-MA. Plasma was collected 15 and 180 minutes from DBA/2 mice [gender not provided; N=2] following an intravenous injection of 4 mg/kg of radiolabeled liposomal or aqueous BPD-MA. Relative and/or quantitative amounts of BPD-MA_C, BPD-MA_D, and/or BPD-DA were determined by HPLC analysis.

- **Liver extracts** were obtained from mice at 15 minutes and 3 hours following an iv injection of radiolabeled BPD-MA at 4 mg/kg.

- **Bile** from pigs was obtained 3 hours post iv injection of radiolabeled BPD-MA at 2 mg/kg. Relative amounts of BPD-MA_C, BPD-MA_D, and BPD-DA were determined by HPLC analysis.

Results and Conclusions - BPD-MA was metabolized to varying degrees in the plasma and liver with the rate and the stereospecificity of the metabolizing enzymes both species and tissue dependent. BPD-MA, and specifically both regioisomers, were metabolized in the cytosolic and microsomal fractions of rats, dogs, and humans. The species rank order for rate of metabolism of BPD=MA as well as the % of peak area of BPD-DA was rat > dog ≥ human. The rank order for rate of metabolism in the hepatic preparation was microsomes > S9 fraction > liver slices. The stereospecificity for metabolism of the regioisomers was greatest in the rat, much less in the dog and, generally, comparable in humans. Stereospecificity was also demonstrated *in vivo*. There did not appear to be any stereospecificity for metabolism of the enantiomers. However, these results were considered preliminary.

Plasma enzymes were also capable of metabolizing BPD-MA. The greatest enzymatic activity was observed in the rats with low activity in humans and in the mouse. The stereospecificity was apparent in the rat and somewhat in the mouse but not in the human plasma samples. However, the difference in the relative quantities of BPD-MA_C and BPD-MA_D was more apparent following *in vivo* exposure suggesting that the differential elimination depended more on liver clearance and liver enzyme specificity than on plasma enzyme activity. *In vivo,* BPD-MA_C was cleared more rapidly than BPD-MA_D in the rat and pig but not in the mouse.

The major metabolite identified, BPD-DA, was formed quickly in all species in both the *in vivo* and *in vitro* studies. These studies indicated that BPD-DA did not accumulate in the plasma and that the diacid was further metabolized at a fairly rapid rate by hepatic but not plasma enzymes. The regioisomers of the parent decreased with time and BPD-DA increased linearly over time following incubation of BPD-MA with hepatic preparations. In the microsome preparation, the decrease in parent was approximately equal to the increase in BPD-DA [approximately 9% of the starting concentration]. In the S9 fraction, the increase in BPD-DA accounted for only approximately 60% of the loss of parent [approximately 24% of the starting concentration] suggesting metabolism to another compound. The Sponsor stated that no other peaks besides BPD-MA_c, BPD-MA_D, and BPD-DA were observed and that any other metabolite would account for approximately 5-10% of the initial BPD-MA. Microsomal NADPH enzymes did not appear to contribute to BPD-MA metabolism. Conjugation of BPD-MA and BPD-DA did not appear to occur. Liver metabolism appeared to be carried out by esterases.

The following tables delineate the metabolites and rates of formation observed in the various hepatic preparations from the rat, dog, and human [Ref. 359].

In Vitro Metabolism of Regioisomers of BPD-MA (BPD-A1 and BPD A-2) to BPD-DA by Liver Slices, S9 Fraction and Microsomes from the Rat, Dog, and Human

Expressed as the Percentage of the Peak Area on HPLC Chromatogram

Species	Liver Fraction	BPD-DA	BPD-A1	BPD-A2	BPD A1 : A2
Rat	Liver Slices	15.3	19.9	38.2	1 : 1.9
	S9 Fraction	20.5	26.0	44.1	1 : 1.7
	Microsomes	15.7	32.1	46.1	1 : 1.4
Dog	Liver Slices	6.91	31.1	38.1	1 : 1.2
	S9 Fraction	11.8	36.7	44.0	1 : 1.2
	Microsomes	5.25	43.2	47.6	1 : 1.1
Human	Liver Slices	4.16	34.9	41.1	1 : 1.2
	S9 Fraction	5.77	42.3	43.4	1 : 1.0
	Microsomes	7.97	43.3	43.7	1 : 1.0

The Rate of Metabolism of BPD-A1 and BPD-A2 Regioisomers by Liver Slices, S9 Fractions and Microsomes from the Rat, Dog, and Human.

Species	Liver Fraction	BPD-A1 (ng/mg protein/min	BPD-A2 (ng/mg protein/min	BPD A1 + A2 (ng/mg protein/min
Rat	Liver Slices	6.50	5.90	12.4
	S9 Fraction	24.6	3.70	28.3
	Microsomes	134	61.0	195
Dog	Liver Slices	2.90	3.20	6.10
	S9 Fraction	13.1	7.00	20.1
	Microsomes	83.0	65.0	148.0
Human	Liver Slices	1.80	1.90	3.70
	S9 Fraction	10.1	9.80	19.9
	Microsomes	58.0	63.0	121

The following table delineates plasma concentrations of BPD-MA regioisomers and BPD-DA following iv administration of BPD-MA.

TIME	% PEAK AREA											
	RAT				MOUSE				PIG			
	DA	A1	A2	A1:A2	DA	A1	A2	A1:A2	DA	A1	A2	A1:A2
15 min	5.29	31.85	55.77	1:1.80	13.61	31.84	44.22	1:1.42	1.63	35.90	58.68	1:1.66
1 h	3.99	25.76	60.14	1:2.33	-	-	-	-	1.75	27.32	63.12	1:2.31
3 h	-	-	-	-	13.51	46.50	34.98	1:0.54	2.28	23.13	55.24	1:2.39

III. Comparison of the Activity of the Regioisomers, Enantiomers and BPD-DA to BPD-MA

A. Comparison of the activity of the regioisomers and enantiomers - Several studies were conducted to compare the activity of the regioisomers of BPD-MA in various systems. The activity of the 4 enantiomers was also assessed in an *in vitro* study. The table below outlines the studies including results. These studies indicated that the parent and regioisomers demonstrate comparable activity with only minor differences. These differences in the *in vivo* evaluation were attributed to PK and biodistribution differences.

Study Title [Ref.]	Study Design	Results/Conclusions
PH-92013: Comparative studies of the activity of benzoporphyrin derivative monoacid ring A (CL318,952) and its regioisomers CL315,585 and CL315,555) *In Vivo* and *In Vitro* [Ref. 105]	P815 tumor cells incubated with 0-50 ng/ml of parent or regioisomers X 1 hour; irradiated at 690 nm laser light for 30 minutes; viability determined by MTT assay	LD_{50} – 12-20 ng/ml for all 3 test compounds
	DBA/2 mice with M1-tumors were dosed i.v. with 2 mg/kg of parent or regioisomers; 3 hrs later irradiated at 690 nm laser light; observed 20 days	All groups were tumor free for first 8 days After 20 days 7/10, 5/10, and 4.3/10 were tumor free after receiving CL318,952, CL315,555, and CL 315,585, respectively
	DBA/2 mice were dosed i.v. with 2 mg/kg of parent or regioisomers; 3 hrs later irradiated at 690 nm laser light; observed 20 days	Rank order for severity of skin lesions – CL315,555 > CL318,952 > CL315,585
PH-92006: A Comparison Between BPD-MA and its Two Regioisomers with Respect to Dark Toxicity *In Vitro* [Ref. 104]	P815 tumor cells incubated with 0-50 ng/ml of parent or regioisomers X 1 hour; viability determined by MTT assay	LD_{50} – CL318,952 = 12.0 ng/ml - CL315,555 = 23.9 ng/ml - CL315,585 = 13.6 ng/ml
PH-92005: A Comparison Between BPD-MA and Its Two Regioisomers with Respect to Photoactivation *In Vitro* [Ref. 109]	P815 tumor cells incubated with 0-50 ng/ml of parent or regioisomers X 1 hour; irradiated at 690 nm laser light for 30 minutes; viability determined by MTT assay	LD_{50} – CL318,952 = 15.9 ± 1.2 ng/ml - CL315,555 = 18.4 ± 1.4 ng/ml - CL315,585 = 12.8 ± 1.3 ng/ml Max. toxicity for all compounds at 25 ng/ml
91006: Photosensitizing Potency of Two Regioisomers of Benzo-porphyrin Derivative *In Vitro* [Ref. 110]	M1 rhabdomyosarcoma tumor cells incubated with 2-30 ng/ml of regioisomer X 1 hour, irradiated with fluorescent light [300-800 nm]; viability determined by MTT assay	LD_{50} – CL315,555 = 5.8 ng/ml - CL315,585 = 6.4 ng/ml
91004: A Comparative Study of the Effects of Two Regioisomers of BPD-MA Given I.V. to Tumor-Bearing Mice [Ref. 112]	DBA/2 mice with M1-tumors were dosed i.v. with 2 mg/kg of parent or regioisomers; 3 hrs later irradiated at 690 nm laser light; observed 20 days	Day 1 – eschar and edema at exposure site in all groups After 20 days anti-tumor efficacy of regioisomer comparable to parent – 4-5 tumor-free animals in each group
92007: A Preliminary Study Comparing the Skin Photosensitizing Effects of BPDA and Its Two Regioisomers [Ref. 113]	DBA/2 male mice were dosed i.v. with 0 or 2 mg/kg of control, parent or regioisomers, 3 hrs later irradiated at 690 nm laser light, observed 21 days	Days 1-10 – parent and regioisomers resulted in lesions scored moderate to severe, effects development and healing were also comparable CL315,585 generally scored lowered but based on means and S.D. lesions were comparable across groups
92010: A Single Dose Comparative Study of the Anti-Tumor Efficacy of Liposomal BPD-MA and its Regioisomers [Ref. 114]	DBA/2 mice with M1-tumors were dosed i.v. with 2 mg/kg of parent or regioisomers; 3 hrs later irradiated at 690 nm laser light; observed 20 days [3 experiments]	All test articles exhibited antitumor activity with comparable to marginally better efficacy with parent [7 tumor free] vs regioisomers [4.3 and 5 tumor free]

Study Title [Ref.]	Study Design	Results/Conclusions
PH-93031A: Comparison Between BPD-MA and Its 4 Enantiomers with Respect to Photoactivation In Vitro [Ref. 115]	P815 tumor cells incubated with 0-50 ng/ml of regioisomers or enantiomers X 1 hour; irradiated at 690 nm laser light; viability determined by MTT assay	There was considerable variability in the results LD_{50} – CL315,555 AI = 14.9 ng/ml - CL315,585 AII= 8.0 ng/ml - AIEI = 9.19 ng/ml - AIEII = 7.81 ng/ml - AIIEI = 6.44 ng/ml - AIIEII = 3.37 ng/ml

B. Comparison of the Activity of BPD-DA to BPD-MA – The Sponsor provided several literature citations which described the *in vivo* and *in vitro* activity of BPD- DA compared to BPD-MA. These citations included:

a. Ref 100: Richter, A.M., et. al., [1991]. Photosensitising potency of structural analogues of benzoporphyrin derivative [BPD] in a mouse tumour model. *Br. J. Cancer* 63:87-93.

b. Ref. 35: Kessel, D. [1989]. In vitro photosensitization with a benzoporphyrin derivative. *Phochem. Photobiol.* 49[5]:579-582.

c. Ref. 38: Richter, A.M., et. al. [1996]. Photosensitizing potencies of the structural analogues of benzoporphyrin derivative in different biological systems. *Clin. Laser Med. Surg.* 14:335-341.

d. Ref. 102: Richter, A.M., et. al. [1990]. In vitro evaluation of phototoxic properties of four structurally related benzoporphyrin derivatives. *Phochem Photobiol.* 52[3]:495-500.

Results and Conclusions – Since BPD-MA and BPD-DA have the same chromophore, they demonstrate the same absorption spectra, extinction coefficients, and singlet oxygen yields. However, the Sponsor concluded that the BPD-MA was at least five times more potent than BPD-DA in both *in vitro* and *in vivo* test systems. These test systems included tumor cytotoxicity and photosensitization. However, they were comparable in activity in an RBC photohemolysis and VSV cytotoxicity test system. This difference in activity in systems, in which the target is considered to be intracellular, has been attributed to differences in uptake and retention in cells of the 2 test articles. Due to its greater lipophilicity, the uptake and retention of BPD-MA was greater than of BPD-DA. However, in systems in which the target is considered to be the membrane, the activity of BPD-MA and BPD-DA appeared to be comparable. Therefore, the apparent activity of BPD-MA and BPD-D may be dependent on the location of the target for the induction of toxicity.

IV. Drug Interaction Studies

A. In Vitro Studies

a. Title: *In vitro* study of drug interaction in human plasma between methotrexate and BPD-MA [Ref. 290]
Study Identification: PH-97008
Site: [redacted]
Study Dates: September – October 1999

Formulation and Lot No. - Liposomal BPD-MA; Batch # CX08-47, CX08-49, CX08-51
Certificate of Analysis: No (X)
Final Report (X): Jan. 1999
GLP Signed and QA Statements: No (X)
Objective: "To determine whether BPD-MA can displace protein-bound methotrexate in human plasma *in vitro*".

Study Design – Human plasma was incubated with: [1] BPD-MA at 2.86 and 28.6 μg/ml; [2] MTX at 0, 3, 90 and 180 μg/ml; and [3] BPD-MA and MTX. For samples with both MTX and BPD-MA, the MTX was added first and incubated for 30 minutes in order to reach equilibrium of drug between plasma and proteins. The BPD-MA was then added for an additional 30 minute incubation. Following incubation, all samples were centrifuged overnight to obtain a protein free plasma ultrafiltrate. Drug content [MTX, BPD-MA] in the ultrafiltrate was determined by HPLC. The experiment was conducted three times.

Results – Approximately 33% of the total plasma MTX was in the protein free fraction. No BPD-MA was detected in this fraction. The amount of MTX at concentrations of 3-180 μg/ml in the protein free plasma ultrafiltrate did not change in the presence of BPD-MA at 2.86 and 28.6 μg/ml.

Conclusion – Under the conditions of this study, BPD-MA in human plasma did not appear to displace protein-bound MTX.

Summary of Pharmacokinetics/Toxicokinetics –

Uptake and Release Kinetics – Two *in vitro* studies [PH-97006 and PH-93013] indicated that uptake was rapid with a maximum concentration [saturation] observed within 20-30 minutes. The rank order of concentration of drug was tumor cells > stimulated normal spleen cells > unstimulated spleen cells. Release of drug appeared to be faster from normal cells compared to tumor cells. There was greater cytotoxicity observed in cells irradiated following a 60-minute incubation with BPD-MA. However, incubation for more than 30 minutes did not increase the concentration in the cell. This suggested that there was an intracellular redistribution of drug "to more vulnerable sites". Viability of splenocytes, collected from DBA/2 mice 15 minutes, 1, 3, 24, 48, and 72 hours following a 10 mg/kg iv injection of BPD-MA and irradiated, was decreased by approximately 50-60% at all time points.

Plasma Distribution - *In vitro* studies suggested that liposomes of BPD-MA were disrupted in the presence of plasma. Drug predominantly partitions into the plasma with <10% associated with either WBCs or RBCs. At <6 hours, drug was largely [approximately 80-90%] associated with lipoproteins, primarily with HDL and to a lesser extent with VLDL and LDL. Only a small percentage [e.g. approximately 6%] was associated with albumin. However, by 24 hours of incubation, drug was fairly evenly distributed between each of the 3 lipoprotein fractions. Data suggest that BPD-DA has a comparable distribution in plasma with slightly greater association with HDL than that observed for BPD-MA.

Biodistribution – Biodistribution studies were conducted in mice. The Sponsor stated that the biodistribution was comparable between tumor-bearing and normal mice. Clearance of liposomal BPD-MA from the blood and plasma was rapid. Fifteen minutes following administration of liposomal BPD-MA at 4 mg/kg, the highest concentrations, based on radioactivity, were observed in the gall bladder followed by the liver, adrenals, kidney, lung, heart, spleen, small intestine, then fat, salivary glands, pancreas, and tumor. Less than 2 μg/g

tissue were found in other tissues. At 3 hours post drug administration, levels had increased in the gall bladder and small intestine, but were generally decreasing in the other organs. The exception was the skin and pancreas in which tissue concentration at 15 minutes and 3 hours was comparable. By 24 hours, the drug concentration based on radioactivity, was <0.7 µg/g tissue in all tissues but the gall bladder and liver. Levels in the gall bladder and liver were 2-3 µg/g tissue. By 196 hours following drug administration, all tissue levels were <0.4 µg/g tissue. The Sponsor indicated that the elimination half-life between the peak concentration and that at 168 hours was 25 and 29 hours for the skin and liver, respectively. The half-life for the first 24 hours was 5 hours for liver, lung and fat, and 15 hours for skin.

Mass Balance and Excretion – These studies were conducted in the mouse and rat. In the rat, the highest plasma drug levels were observed at 15 minutes. Clearance was more rapid in female than in male rats between 3-8 hours following drug administration. The primary route of excretion was through the bile in both the mouse [approximately 60% of the radioactive dose] and the rat [approximately 90% of the radioactive dose]. In the rat approximately 50% and 80% had been eliminated in the feces by 24 and 48 hours, respectively. Approximately 80% of the excreted drug was unchanged. Urinary excretion accounted for approximately 4% in the mouse and <1% in the rat. Furthermore, in the rat, the drug that was eliminated in the urine was predominantly metabolized. In the rat, approximately 3% was left in the carcass after 168 hours.

Pharmacokinetics – Pharmacokinetic studies were conducted in rats, nonhuman primates, and Yucatan microswine. There was rapid distribution of the drug followed by a slower elimination phase with all analytes exhibiting a bi-exponential decline. In both the rat and cynomolgus monkey, there was a stereospecific disposition of regioisomers. In addition, there was some stereospecific disposition of the enantiomers in the rat [This was not evaluated in the monkey.] The relative exposure to BPD-MA_D was approximately 2-3X BPD-MA_C in both species. There were minimal gender differences in exposure in rats, with females exhibiting slightly higher exposure based on AUC_{0-t}. This differed from the toxicokinetic data presented in the 14-day and 28-day rat studies [92020 and TX-90601]. The reason for this difference is not known, but the Sponsor has been requested to clarify this apparent discrepancy. Only male cynomolgus monkeys were evaluated. The $t_{1/2}$ for BPD-MA_C was approximately 3, 7, and 5 hours in the male monkey, male and female rat, respectively. The $t_{1/2}$ for BPD-MA_D was approximately 5, 7, and 3 hours in the male monkey, male and female rat, respectively. In male macaques, exposure was not dose-proportional at 0.5 and 1.0 mg/kg. No drug was detected by fluorescence spectroscopy at either dose 24 hours following administration. Exposure in anesthetized microswine pigs was 2-3X greater than in conscious pigs. However, rate of drug administration did not appear to significantly affect exposure. Exposure to BPD-DA in rats appeared to represent <10% of the overall exposure to test article.

Ocular Distribution - Studies were conducted in both pigmented and nonpigmented rabbits. No differences were observed in ocular distribution between pigmented and nonpigmented so results were combined. BPD-MA rapidly distributed to the iris, ciliary body, retina, and choroid. In addition, these tissues demonstrated the greatest drug concentrations. Minimal drug accumulated in the avascular portions of the eye [e.g. cornea, lens, and vitreous]. Maximum drug concentration, following an iv injection of 6 mg/kg verteporfin, was observed at 30-60 minutes in the choroid, anterior segment, and the ciliary body and process. By 2 hours, the drug concentration had begun to gradually decrease. Drug concentration in the retina, however, continued to increase up to 2 hours. By 24 hours, drug concentration had significantly decreased with the highest levels still present in the retina and the choroid [3.78 and 1.17 ng/10 mg tissue, respectively].

The Sponsor suggested that one of the mechanisms involved in accumulation of BPD-MA may be the LDL receptor. This receptor is expressed on endothelial cells and is upregulated in neovascular endothelium. Therefore, this would theoretically increase the drug accumulation in CNV compared to other tissues and increase specificity of PDT with BPD-MA. However, as the Sponsor noted, LDL receptors are also expressed on normal endothelium and retinal pigmented epithelium. In addition, scavenger receptors have also been implicated in BPD-MA uptake and these receptors are also present on RPE. It has also been suggested that a third mechanism, diffusion, may be involved in BPD-MA distribution. These mechanisms would further enhance the accumulation of drug in other retinal structures besides CNV. Therefore this suggest that the iris, ciliary body, retina [including RPE and neurosensory retina], and choroid would be the most susceptible to PDT toxicity.

Reproductive Pharmacokinetics – Studies conducted in pregnant rats indicated that based on radioactivity levels [1] exposure to the fetus was small compared to the dam; [2] drug did not accumulate in the fetus; and [3] the pharmacokinetics are comparable in pregnant and nonpregnant rats.

Metabolism – BPD-MA was metabolized to varying degrees in the plasma and liver with the rate and the stereospecificity of the metabolizing enzymes both species and tissue dependent. BPD-MA, and specifically both regioisomers, were metabolized in the cytosolic and microsomal fractions of rats, dogs, and humans. The species rank order for rate of metabolism of BPD-MA as well as the % of peak area of BPD-DA was rat > dog ≥ human. The rank order for rate of metabolism in the hepatic preparation was microsomes > S9 fraction > liver slices. The stereospecificity for metabolism of the regioisomers was greatest in the rat, much less in the dog and, generally, comparable in humans. Stereospecificity was also demonstrated *in vivo*. There did not appear to be any stereospecificity for metabolism of the enantiomers. However, these results were considered preliminary.

Plasma enzymes were also capable of metabolizing BPD-MA. The greatest enzymatic activity was observed in the rats with low activity in humans and in the mouse. The stereospecificity was apparent in the rat and somewhat in the mouse but not in the human plasma samples. However, the difference in the relative quantities of BPD-MA_C and BPD-MA_D was more apparent following *in vivo* exposure suggesting that the differential elimination depended more on liver clearance and liver enzyme specificity than on plasma enzyme activity. *In vivo*, BPD-MA_C was cleared more rapidly than BPD-MA_D in the rat and pig but not in the mouse.

The major metabolite identified, BPD-DA, was formed quickly in all species in both the *in vivo* and *in vitro* studies. These studies indicated that BPD-DA did not accumulate in the plasma and that the diacid was further metabolized at a fairly rapid rate by hepatic but not plasma enzymes. The regioisomers of the parent decreased with time and BPD-DA increased linearly over time following incubation of BPD-MA with hepatic preparations. In the microsome preparation, the decrease in parent was approximately equal to the increase in BPD-DA [approximately 9% of the starting concentration]. In the S9 fraction, the increase in BPD-DA accounted for only approximately 60% of the loss of parent [approximately 24% of the starting concentration] suggesting metabolism to another compound. The Sponsor stated that no other peaks besides BPD-MA_C, BPD-MA_D, and BPD-DA were observed and that any other metabolite would account for approximately 5-10% of the initial BPD-MA. Microsomal NADPH enzymes did not appear to contribute to BPD-MA metabolism. Conjugation of BPD-MA and BPD-DA did not appear to occur. Liver metabolism appeared to be carried out by esterases.

Activity of the Regioisomers and Enantiomers- All regioisomers and enantiomers exhibited activity both in *in vivo* and *in vitro* systems. There were some differences in the activity of the regioisomers, but these differences were considered minimal. The activity of BPD-DA compared to BPD-MA was dependent on the test system in which it was evaluated. In the test systems in which the target is believed to be an intracellular site, e.g tumor cytotoxicity and photosensitization, BPD-MA appeared to be at least five times more potent than BPD-DA in both *in vitro* and *in vivo* test systems. However, in systems in which the target is believed to be the membrane, e.g. RBC photohemolysis and VSV cytotoxicity, the activity of BPD-MA and BPD-DA appeared to be comparable.

Toxicology:

I. Ocular Toxicity

A. Nonhuman Primates

a. Light only treatment to the normal retina and choroid in the cynomologus monkey [Ref. 333]
Study Identification: TX-97001
Site:

Study Dates: Not provided
Formulation and Lot No. – Not applicable
Certificate Analysis: No (X) – Not applicable
Final Report: No (X)
GLP and QA Statements Signed: No (X)
Objective: "To study the effect of exposure to laser light of 689 nm at an irradiance of 600 mW/cm^2 and a fluence of 100 J/cm^2, on the normal primate retina and choroid"

This report was a manuscript. A summary of the data was provided.

b. Title: Photodynamic therapy retreatment of normal retina and choroid in the cynomolgus monkey [Ref. 95]
Study Identification: TX-96008
Site:

Study Dates: Not provided
Formulation and Certificate Analysis: Not provided
Final Report: Not provided
GLP and QA Statements Signed: No (X)
Objective: "To study the effect of repeated photodynamic therapy [PDT] applications on normal primate retina and choroid using an intravenous infusion of liposomal" BPD-MA.

Dr. Javier Avalos has previously reviewed these two studies under IND Submission draft review. This review is provided below with comments by the current Reviewer in italics. Strikethroughs were included by the current Reviewer for clarification.

1. Husain, D. Michaud N., Flotte T., Gragoudas E., Miller J. Light only treatment to the normal retina and choroid in the Cynomolgus monkey. (Study No. TX-97001). [Non GLP Study]

2. Renke M., Miller J., Canakis C., Husain D., Michaud N., Flotte T., Gragoudas E. Photodynamic therapy retreatment of normal retina and choroid in Cynomolgus monkey. (Study No. TX-96008). [Non GLP Study]

Date of Study: Not provided.
Lab: Not provided. [*Laser Research Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA]*
Formulation: Liposomal formulation reconstituted in sterile water and diluted with 5% dextrose; Lot No. not provided

In these two studies, the investigators first evaluated the potential effect of exposure to laser (diode laser; Coherent Medical Laser) light of 689 nm at an irradiance of 600 mW/cm^2 and a fluence of 100 J/cm^2 on normal primate retina and choroid (Study No. TX-97001) and then following repeated photodynamic therapy on normal primate retina and choroid using an intravenous infusion of liposomal benzoporphyrin derivative (BPD or Verteporfin) (Study No. TX-96008). In the first study, the eyes of two cynomolgus monkeys (gender not provided; 1.8 to 4.3 kg) were irradiated with a 4000 µm spot of laser light *centered on the fovea of both eyes* at an irradiance of 600 mW/cm2, a fluence of 100 J/cm2, and a treatment time of 166.66 seconds. *Treatment of the 2 eyes was staggered by 2 weeks.* No photosensitizer dye was administered. ~~An eye was enucleated at 24 hours, 2 weeks, 3 weeks, and 5 weeks after treatment.~~ *The following endpoints were evaluated: [1] fundus photography; [2] fluorescein angiography; and [3] histopathology of the eyes.* Histopathology was "*performed 2 weeks after treatment of the right eye and 24 hours after treatment of the left [in the first monkey]. In the second monkey, enucleation was performed 5 weeks after treatment of the right eye and 3 weeks after treatment of the left eye*".

In Study TX-97001, fundus photography and fluorescein angiography demonstrated no change at any observation period (*pre PDT*, 24 hours *post PDT* then weekly *for up to 5 weeks*). The findings were confirmed by light and electron microscopy. The investigators concluded that no damage was reported in the retina and choroid of normal eyes following laser irradiation with an irradiance of 600 mW/cm^2 and a fluence of 100 J/cm^2. The histological changes observed in the treated eyes at 2, 3, and 5 weeks following treatment were secondary to either fixing or processing of the eye in the postmortem stage. *[**Reviewer's Comment** – There were no control eyes. However, it appears that the Sponsor is basing their conclusion that these findings are associated with postmortem processing on the fact that they are observed in nonirradiated portions of the eye. The qualifications of the individual conducting the histopathological evaluation are not provided.]*

In the second study, cynomolgus monkeys (2/group; gender not given; *1.8-4.3 kg*) were infused with either 6 mg/m^2, 12 mg/m^2, or 18 mg/m^2 (*approximately* 0.47 mg/kg, 0.96 mg/kg, or 1.4 mg/kg, respectively) and then irradiated with the experimental conditions established in the first experiment. *[689 nm, 600 mW/cm^2, 100 J/cm^2, a spot size of 4000 µm, and a treatment time of 166.66 seconds]. These treatment parameters were selected based on previous studies and the parameters used in the Phase III clinical trials. The total infusion volume was 10 ml and was followed by a 3 ml flush of D5W.* Animals were irradiated 20 minutes after the 10 minute infusion. In each animal, irradiation was centered on the fovea in one eye and centered on the optic nerve in the other eye. The treatments of the two eyes were staggered by one week. Each eye received one treatment every other week for a total of three treatments. One animal in each group was followed for 2 to 3 weeks after the third PDT application and then sacrificed. The second animal in each group was followed for 6 to 7 weeks after the last PDT application prior to being sacrificed. Fundus photography and fluorescein angiography were performed *prior to treatment*, at 24 hours and then once weekly. Light and electron microscopy were conducted at 2

or 3 weeks in one animal of each group and 6 or 7 weeks in the second animal following the third treatment.

The Sponsor stated that body weight did not change significantly over the study and that food and water consumption was normal.

Fundus photography revealed deep retinal whitening 24 hours post-PDT #1[foveal treatment] in all dose groups which was dose-dependent with respect to severity. After PDT #2 and 3, no whitening was observed at the low dose, but was observed at the periphery in the mid and high doses in sections of retina previously untreated. This has previously been noted. [Kramer, et.al. [1996] Liposomal benzoporphyrin derivative verteporfin photodynamic therapy. Selective treatment of choroidal neovascularization in monkeys. Ophthalmology, 103:427-738]. Treatment borders tended to become obscure by 1 week post-PDT #3 in both the fovea and optic nerve head [ONH] treatment groups at the low but not the mid and high dose groups. No edema was observed in the ONH treatment group at the low dose. There were 1/2 and 2/2 animals at the mid and high dose, respectively, which showed edema for up to 1 week after PDT #2 and #3. The severe edema in 1-2 eyes at the high dose was associated with marked intra-, sub-, and pre-retinal hemorrhage, and "cotton-wool spots". Optic nerve pallor was observed following resolution of the edema.

A cumulative dose response effect was observed from the evaluation of the fluorescein angiograms and histology. Fluorescein angiography demonstrated early hypofluorescence with late staining 24 hours after treatment. In the mid and high dose groups, persistent altered staining of the treatment sites was present in all eyes at all time points while late staining was only observed at the 24-hour observation period for the low dose group. Fluorescein leakage from and staining of the optic nerve persisted at 2 and 6 weeks after the third treatment in the high dose group but not in the low dose group. Histologically, more severe damage to the outer retina was noted at higher PDT doses. At 6 mg/m^2, foveal and optic nerve treatment led to minimal choriocapillaris damage and mild retinal pigment epithelium and outer photoreceptor damage [*Grade 1*]. At this dose, the optic nerve showed mild atrophy and capillary loss [*Grade 1*]. *Lesions in the fovea treated group had resolved by 3 weeks.* Treatment using the mid and high doses led to choriocapillaris closure and severe retinal pigment epithelium and outer sensory retina damage [*Grade 4 at both doses*]. Severe vascular occlusion and hemorrhage within the optic nerve were also present in the mid and high dose groups.

In conclusion, mild damage to the retina, choroid, and optic nerve was reported in primates following three PDT sessions using 6 mg/m2 (0.47 mg/kg) verteporfin and an irradiance of 600 mW/cm^2 and a fluence of 100 J/cm^2. However, higher doses of verteporfin (12 and 18 mg/m^2) led to significant damage to the normal retina, choroid, and optic nerve. The recommended light dose for the clinical trials is 600 mW/cm^2 with an intensity of 50 J/cm^2 delivered over 83 seconds.

***Reviewer's Comment** – The descriptions provided for fundus photography, angiography, and histopathology for individual animals [Appendix 4] were vague and do not utilize proper terminology. There were several entries [e.g. pp. 22, 24, 29, 33, 34, 37, 38, 39, 43, 45, 47, 49, 50, 51, 52, 53, 64] which suggested that the Sponsor had questions as to the overall severity of lesions in a given animal. This was indicated by use of terminology such as "unable", "?","? suptemp", "? central", "Grade 2, 3, or 4", "? Grade 4", "? +", "? fibrosis", "? patchy", "? amount of ONL pyknosis. It was not clear as to who read the slides or the qualifications of the individual reading the slides. However, it appeared from the terminology used that the individual was not trained in veterinary pathology. It addition, it was not indicated whether the read was blinded or peer reviewed. Since there was only an N of 1, these studies should be considered preliminary. The studies were not conducted in compliance with GLP according to 21 CFR 58. Based on these considerations, these studies are considered inadequate for regulatory purposes.*

c. Title: Pre-clinical BPD-MA pharmacology study for macular degeneration [Ref. 94]
Study Identification: TX-94027
Site:
Study Dates: Not provided
Formulation and Certificate Analysis: Liposomal BPD-MA Batch nos. PC1256, R-1186-102, R-1186-192, and PQ002-94
Final Report (X) Dec. 28, 1994
GLP and QA Statements Signed: No (X)
Objective: [1] "To determine the efficacy and selectivity of PDT using liposomal BPD-MA to treat experimental CNV" in cynomolgus monkeys and [2] "to assess the selectivity of effect, or the ability to close the choriocapillaris with limited damage to the neurosensory retina and deeper choroid" in normal eyes of cynomolgus monkeys.

Dr. Javier Avalos previously reviewed this study under IND Submission review completed May 16, 1995; pp. 12-16. This review is provided below with comments by the current Reviewer in italics. Strikethroughs were included by the current Reviewer for clarification.

Laboratory: *Although it is indicated that the histopathology was conducted under direction, it is not clear as to who read the slides, the qualifications of the individual reading the slides, and whether the read was blinded or peer reviewed.*

Animal Strain: male and female Macaca fascicularis *generally wild monkeys*

Animal Starting Weight: 3-5.5 kg (age 2-8 years, *probably*)

No. of Animals: 25

Test Material: Liposomal BPD-MA supplied
Batch Numbers - PC 1256 (4 vials) 6/8/93
(10 vials) 7/93
R-1186-102 (5 vials) 3/7/94
R-1186-192 (4 vials) 6/1/94
PQ002-94 (3 vials) 10/8/94
The lyophilized powder in vials was reconstituted prior to use with sterile water for injection to a final concentration of 2 mg/ml. The solution and powder forms were stored at 2-8° C until use (up to 2 weeks) and were protected from light at all times.

Route: bolus IV injection

Light Device: Laser light at 692 nm was delivered using an argon/dye laser A slit lamp delivery system and a 200 µm silica optical fiber (modified Laser link, was employed. The treatments were performed using a plano fundus contact lens The spot size at the cornea was set on the slit lamp adapter

and confirmed with a caliper. The laser power at 692 nm was measured at the focal plane with a power meter [redacted]

Treatment Parameters for Each of Experiments:

Number of animals	Dose of Dye (mg/kg)	Time of Irradiance (min)	Light Intensity (mW/cm^2)	Fluence (J/cm^2)
8 normal 10 CNV	1, 0.75, 0.5, 0.375, and 0.25	5, 20, 40, 60, 80, 100, and 120	600	150
1 normal 1 CNV	0.375	-	150, 300, 600, 900, 1200 and 1500	150
2 normal 2 CNV	0.375	-	600	50, 100, 200, 400, and 600
1 normal 3 CNV*	0.375	20-50	600	150

* - These animals were 3 of the 5 animals treated with 0.375 mg/kg BPD in the first set of experiments.

The following table created by the current Reviewer delineates the dosing protocol on an individual animal basis. There were a total of 25 animals evaluated [multiple lesions in each eye]. Histopathology was not conducted on 3 animals [#9719, 8219, and 2122] and, therefore, they are not included in the table.

Dose of BPD-MA [mg/kg]	Animal No.	Irradiance [mW/cm^2]	Fluence [J/cm^2]	Time to irradiance after injection [min.]	No. of Lesions Evaluated
Dose and Time to Irradiance After Injection Varied					
Normal eyes – evaluated histopathologically 24 hours post PDT					
1	9781	600	150	50-120	Total of 7 treated areas; 1 area at each time point
	9775[a]	300			Total of 7 treated areas; 1 area at each time point
0.75	9615[b]	600		5-60	Total of 4 treated areas; 1 area at each time point
0.5	9615[b]			5-60	Total of 4 treated areas; 1 area at each time point
0.375	1669			10-100	Total of 8 treated areas; 1 area at each time point
	9815				Total of 9 treated areas; 1 area at each time point
	1234				Total of 8 treated areas; 1 area at each time point
0.25	9588			10-60	Total of 9 treated areas; 1-2 areas at each time point, [both eyes were used]
Eyes with laser induced CNV - evaluated histopathologically 24 hours post PDT					
1	9797[a]	600	150	5-120	Total of 7 lesions treated; 1 area at each time point
0.5	9727		100	10-80	Total of 6 lesions treated; 1 area at each time point
0.375	9520		150	10-100	Total of 6 lesions treated; 1 area at each time point
	9747				Total of 6 lesions treated; 1 area at each time point
	9773			20-60	Total of 4 lesions treated; 1 area at each time point
0.25	9704			10-80	Total of 7 lesions treated; 1 area at each time point
	9808				Total 7 lesions treated; 1 area at each time point

Dose of BPD-MA [mg/kg]	Animal No.	Irradiance [mW/cm^2]	Fluence [J/cm^2]	Time to irradiance after injection [min.]	No. of Lesions Evaluated
Variation of Irradiance[c]					
Normal eyes – evaluated histopathologically 24 hours post PDT					
0.375	**324**	300	150	16 or 51	Total of 10 lesions treated; 1-2 areas treated at each irradiance [generally 1 timepoint/eye, both eyes were used]
		600		27 or 37	
		900		27 or 38	
		1200		21 or 16	
		1500		15	
		1800		56	
Eyes with laser induced CNV - evaluated histopathologically 24 hours post PDT					
0.375	**178**	150	150	15 or 62	Total of 9 lesions treated; 1-3 areas treated at each irradiance [generally 1 timepoint/eye, both eyes were used]
		300		20 or 36	
		600		34	
		900		42, 51, and 54	
		1200		52	
Variation of Fluence[d]					
Normal eyes – evaluated histopathologically 24 hours post PDT					
0.375	**549**	600	50	20	Total of 5 lesions treated; 1 area treated at each fluence
			100	65	
			200	32	
			400	40	
			600	43	
Eyes with laser induced CNV - evaluated histopathologically 24 hours post PDT					
0.375	**1319**	600	50	45	Total of 5 lesions treated; 1 area treated at each fluence
			100	64	
			200	37	
			400	20	
			600	47	
	336-18		50	20	Total of 5 lesions treated; 1 area treated at each fluence
			100	24	
			200	31	
			400	39	
			600	53	
Evaluation of Long Term Effects of PDT					
Eyes with laser induced CNV					
0.375	**9773**	600	150	Approximately 20-60	Total of 5 lesions treated; 1 area at each time point
	423-87				Total of 6 lesions treated; 1 area at each time point
	41				Total of 8 lesions treated; 4 lesions per eye; 2 areas at each time point

[a] *It was not clear when these animals were sacrificed. Apparently, it was days after PDT, but it was not readily apparent how many days.*

[b] *This animal apparently underwent 2 treatment protocols with different doses.*

[c] *Since time to irradiance after BPD-MA administration is known to significantly alter efficacy and safety, the same irradiance administered at different times after drug administration should be considered a different light dose. Consequently, evaluation of the effect of changes of irradiance on efficacy and safety were, at best, difficult to assess.*

[d] *Since time to irradiance after BPD-MA administration is known to significantly alter efficacy and safety, the same fluence administered at different times after drug administration should be considered a different light dose. Consequently, evaluation of the effect of changes of fluence on efficacy and safety were at best, difficult to assess.*

[e] *Long term effects of PDT on normal eyes were not evaluated in this study.*

CNV Model: Animals were anesthetized for all procedures. Choroidal neovascularization was induced by placing 9 laser burns in each macula using an argon/dye laser. The laser parameters were 50 µm spot size, 0.1 second duration, and powers ranging from 350-450 mW. The monkeys were followed weekly, for 2-3 weeks, by fundus photography and fluorescein angiography (FA), to detect choroidal neovascularization. Approximately 40-50% of the laser lesions developed neovascularization detectable by angiography.

Methods: Liposomal BPD was injected intravenously through a catheter as a bolus, followed by a saline flush. Laser light was delivered to the retinas of the animals after the dye injection at a specified time. Light was focused on the retina using an contact lens, producing a 1250 um spot, which was centered on the area to be treated. The areas of CNV were identified using a pre-treatment angiogram. Areas of normal/choroid were selected in a configuration which facilitated identification during histologic preparation; treatment spots were applied in the posterior pole approximately 1500 um from the optic disc in a peripapillary distribution. After each PDT treatment, fundus photographs were performed immediately. Fundus photographs and FA were performed the day after PDT, prior to sacrifice, to determine efficacy of closure of experimental CNV. The effects were confirmed using light and electron microscopy on bisected, trimmed, fixed, and embedded *eyes*. Four animals were followed for 4-7 weeks following PDT with weekly fundus photography and angiography.

Results:

Mortality: No apparent compound related deaths were reported.

Clinical Observations: No treatment-related effects were noted.

Efficacy of Treatment: Effective closure of CNV was demonstrated by fluorescein angiography as early hypofluorescence corresponding to the treated area. CNV closure was demonstrated at all doses (1, 0.5, 0.375, and 0.25 mg/kg) examined. Irradiation of all lesions in 2 animals between 5-100 minutes after being treated with 1 mg/kg dye induced CNV closure. CNV closure was found in 4 of the 4 membranes that were treated 10-50 minutes after a dye injection of 0.5 mg/kg while 2 CNV lesions were found open when irradiated 50 minutes after dye infusion. In the 36 membranes (5 monkeys) treated with a dose of 0.375 mg/kg BPD-MA, 21 of the 25 CNV irradiated within 50 minutes of dye injection demonstrated closure. Only 4 of 11 CNV irradiated beyond 50 minutes of dye infusion demonstrated angiographic closure. Closure was induced in 2 of 2 lesions irradiated in the first 20 minutes of a dye dose of 0.25 mg/kg. Only 2 of 4 CNV irradiated between 20 and 40 minutes after dye injection showed closure. No effect was observed in CNV irradiated 40 minutes after the 0.25 mg/kg bolus injection. The angiographic closures were confirmed with light and electron microscopy.

Eight CNV in one monkey were treated using variable irradiances. The dose was 0.375 mg/kg and the fluence was 150 J/cm2 in all treatments. All membranes were closed at 150, 300, 600, and 900 mW/cm2. The time of FA measurement was not reported. The lesion treated with 1200 mW/cm2 showed some hemorrhage in the choroid and open vessels in the CNV. However, this lesion was treated at 52 minutes of dye injection.

Ten membranes in two monkeys with CNV were treated with variable fluences. The dye dose was 0.375 mg/kg and irradiance was 600 mW/cm2 in all cases. At 50, 100, and 200 J/cm2, 3 of 6 lesions treated in each group were open. At 400 and 600 J/cm2, all CNV (4) were closed, and in one of each of the CNV treated with 400 or 600 J/cm2, an overlying serous retinal detachment was evident following treatment.

Twenty lesions were treated in 3 monkeys with a dye dose of 0.375 mg/kg, fluence of 150 J/cm2, and irradiance of 600 mW/cm2. The animals were irradiated 20-50 minutes post dye injection and underwent angiography at 24 hours, 2 weeks, and 4 weeks after dye infusion. At 24 hours after treatment, 14 of the 20 CNV were closed by angiography. At 2 weeks after treatment, 15 of the 20 CNV were angiographically closed. At 4 weeks after treatment, 13 of the membranes were closed angiographically. Histopathology was done on 11 lesions in two monkeys. Eight lesions showed the presence of a few identifiable vessels in the membrane; angiographically half of these lesions were classified as open. Three lesions showed one identifiable vessel in the area of CNV; angiographically all 3 of these lesions were closed. *The most common lesion reported in the monkeys with surgically induced CNV, apart from CNV or choriocapillaris closure, was damage to the ONL. The degree of damage and/or pyknosis was not always clearly indicated. However, it appeared that damage was dependent on the dose of BPD-MA and inversely related to the interval between drug administration and irradiation. Other findings reported at a dose of 1 mg/kg included INL and photoreceptor damage. Serous detachment was reported at 1 mg/kg and irradiation at 5 minutes as well as at 0.375 mg/kg with fluences of 400-600 mW/cm^2. Other findings did not clearly demonstrate a relationship to drug dose, irradiation dose or timing and included [1] damage/congestion and/or closure of medium or large choroid vessels; [2] minimal retinal damage; and [3] possible break in Bruch's membrane. In the animals evaluated at 4 weeks post irradiation, although it would appear that the lesions are resolving, RPE damage and macrophage infiltration persisted regardless of timing of irradiation and choriocapillaris closure was no longer observed. It is not clear which lesions were secondary to the laser-induction of CNV.*

Fundus photography, fluorescein angiography, and light microscopy were used to assess the retinas of dye only controls with no irradiation. No adverse effects were observed in the retina in these animals.

Selectivity of the Treatment: Selectivity of treatment was investigated by performing PDT on normal retina and choroid using the same parameters as in the CNV. A total of 56 lesions were placed in normal retina/choroid of 9 monkeys, with varying dye doses, at a fluence of 150 J/cm2 and irradiance of 600 mW/cm^2. Irradiation of lesions within 50 minutes of dye injection caused a dose dependent increase in retinal/choroidal damage. At a dose of 1 mg/kg of dye, 5 of 6 lesions had choriocapillaris closure, RPE damage, photoreceptor damage, outer nuclear layer pyknosis greater than 50% or medium/large choroidal vessel damage or retinal vessel damage (a grade of 5 by the author's grading). The sixth lesion was not histologically found. At a dose of 0.75 mg/kg of dye, 4 of 4 lesions showed a Grade 5 damage. At a dye dose of 0.5 mg/kg, 1 of 4 lesions had a Grade 5 damage and 3 of 4 lesions demonstrated Grade 4 damage (no medium/large choroidal or retinal vessel damage). At a dye dose of 0.375 mg/kg, 3 of 15 lesions had a Grade 4 damage, 2 of 15 lesions had a Grade 3 damage (pyknosis in outer nuclear layer was less than 50%), 6 of 15 lesions had a Grade of 2 damage (minimal, <20%, pyknosis in ONL), and 3 of 15 lesions had a Grade 1 damage (RPE damage with or without choriocapillaris closure and occasional pyknosis in the ONL). At a dose of 0.25 mg/kg, 6 of 7 lesions demonstrated a Grade 1 damage while 1 lesion had a Grade 5 damage (irradiated within 5 minutes).

The damage caused by varying the irradiance (300-1800 mW/cm^2) at a constant dye dose (0.375 mg/kg) and fluence (150 J/cm^2) resulted in Grade 1-5 damage. At 300 mW/cm^2, 1 lesion had Grade 1 damage while a second lesion showed a Grade 4 damage. Lesions irradiated at 1200 and 1500 mW/cm^2 demonstrated Grade 5 damage while 1 lesion irradiated at 1800 mW/cm^2 had Grade 3 damage.

The damage observed in normal retina/choroid treated with variable fluence (50-600 J/cm^2, constant dye dose (0.375 mg/kg) and irradiance (600 mW/cm^2) were Grade 1 and 2. These results are from one animal with 15 lesions.

At doses of 0.375-1 mg/kg [600 mW/cm^2;150 J/cm^2] and generally at all time points evaluated for each dose, there was some degree of damage to the RPE, the ONL, and the outer and inner segments of the photoreceptors as well as closure or damage to the choriocapillaris. The severity tended to be dependent on dose and time to irradiation. Damage to the medium and large choroidal vessels [platelets, congestion, occlusion] and varying degrees of INL damages tended to be observed at doses of 0.5, 0.75, and 1.0 mg/kg. At a dose of 0.375 mg/kg, the lesions tended to become more severe as the irradiance, but not the fluence, increased.

In the animals evaluated at 7 weeks post irradiation, both those with normal choroid or laser-induced CNV, RPE damage and macrophage infiltration persisted regardless of timing of irradiation and vessel closure was no longer observed.

No effect or *minimal residual damage* to normal retina/choroid treated with 0.375 mg/kg dye, irradiance of 600 mW/cm^2, and fluence of 150 J/cm^2 were observed in eight lesions of one monkey at 7 weeks post PDT. *The residual damage included minimal RPE damage and the presence of large macrophages.*

The following table provides a description of the grading system.

Grade	Damaged Retinal / Choroidal Layers
1	RPE only or RPE + slight photoreceptor changes + occasional pyknosis in the ONL; with or without choriocapillaris (c-c) closure.
2	choriocapillaris (c-c) closure + RPE damage + photoreceptor damage + minimal (< 20%) pyknosis in outer nuclear layer(ONL)
3	c-c closure +RPE damage + photoreceptor damage + ONL pyknosis < 50%
4	c-c closure + RPE damage + photoreceptor damage + ONL pyknosis > 50% .
5	c-c closure + RPE damage + photoreceptor damage + ONL pyknosis > 50% or medium / large choroidal vessel damage or retinal vessel damage.

TABLE 2. EFFECT OF PDT WITH VARIOUS DRUG DOSES AND TIMES OF LIGHT DELIVERY[a] AFTER DRUG ADMINISTRATION ON CHOROIDAL MEMBRANES

Drug Dose (mg/kg)	No. of Animals	Type[b] of Membrane	No. of Lesions Treated	Time (minutes) of Light Delivery After Drug Administration[c]									
				5	10	20	30	40	50	60	80	100	120
1.0	2	CNV	13	C-2		C-2		C-2		C-2	C-1	C-2	C-2
	2	NORMAL	14	GR5-2		GR5-2		GR5-1 NF-1		GR4-2	GR4-2	GR4-1 NF-1	GR4-1 NF-1
0.75	1	NORMAL	4	GR5-1		GR5-1		GR5-1		GR5-1			
0.5	1	CNV	6		C-1	C-1		C-1	C-1	O-1	O-1		
	1	NORMAL	4	GR5-1		GR4-1		GR4-1		GR4-1			
0.375	5	CNV	34		C-2	C-6 O-1	C-3 O7-1	C-6 O-1	C-3 O-1	C-2 C7-3 O-1	O-2	O-2	
	3	NORMAL	24		GR2-2 GR1-1	GR3-1 GR2-2	GR4-1 GR2-1 GR1-1	GR3-1 GR2-1 GR1-1	GR4-2 GR2-1	GR4-1 GR2-1 NF-1	GR4-1 GR2-1 NF-1	GR4-1 GR2-1 GR1-1	
0.25	2	CNV	14		C-2	C-1 O-1	O-2	C-1 O7-1	O-2	C7-1 O-1	C7-1 O-1		
	1	NORMAL	9	GR5-1	GR5-1 GR1-1	GR1-2		GR1-1 NF-1	NF-1	GR1-1			

[a] Light dose - 150 J/cm²; 600 mW/cm²
[b] CNV - choroidal neovascularization; NORMAL - normal retina/choroid
[c] C-closed angiographically; O - open; NF - not found; GR - histology grade- number of lesions

The following tables summarize the results.

TABLE 4. EFFECT OF PDT[a] USING VARIOUS FLUENCES ON CNV AND NORMAL CHOROID

Type[b] of Membrane	No. of Animals	No. of Lesions Treated	Fluence (J/cm²)[c]				
			50	100	200	400	600
CNV	2	10	C at 20 min[d] O at 45 min	C at 23 min O at 64 min	C at 31 min O at 37 min	C at 20 & 39 min SRD[e] at 39 min	C at 47 & 53 min SRD[e] at 53 min
NORMAL	2[f]	15	GR1 at 20 min	GR1 at 32 min	GR1 at 40 min	GR2 at 50 min	GR1 at 60 min

[a] Drug dose - 0.375 mg/kg; light irradiance - 600 mW/cm²
[b] CNV - choroidal neovascularization; NORMAL - normal retina/choroid
[c] C - closed angiographically; O - open; NE - no effect; GR - histology grade
[d] Time (minutes) of light delivery after drug administration
[e] Serous retinal detachment
[f] No histopathology for one animal

TABLE 3. EFFECT OF PDT[a] USING VARIOUS LIGHT IRRADIANCES ON CNV AND NORMAL CHOROID

Type[b] of Membrane	No. of Animals	No. of Lesions Treated	Irradiance (mW/cm²)[c]						
			150	300	600	900	1200	1500	1800
CNV	1	8	C at 15 & 62 min[d]	C at 20 & 36 min	C at 34 min	C at 42 & 51 min	O at 52 min		
NORMAL	1	10		GR1 at 16 min GR4 at 51 min	GR3 at 27 min GR5 at 37 min	GR3 at 36 min GR5 at 27 min	GR5 at 21 & 46 min	GR5 at 15 min	GR3 at 56 min

[a] Drug dose - 0.375 mg/kg; light dose - 150 J/cm²
[b] CNV - choroidal neovascularization; NORMAL - normal retina/choroid
[c] C - closed angiographically; O - open; GR - histology grade
[d] Time (minutes) of light delivery after drug administration

TABLE 5. LONG-TERM EFFECTS OF PDT ON CNV AND NORMAL CHOROID

Type[b] of Membrane	No. of Animals	No. of Lesions Treated	Time of Assessment After PDT[c]			
			24 hours	2 weeks	4 weeks	7 weeks
CNV	3	20	C-14 O-5 O?-1	C-15 O-5	C-13 O-7	NT
NORMAL	1	8	C-8	ND	ND	All lesions: minimal damage, normal retina

[a] Drug dose - 0.375 mg/kg; light dose - 150 J/cm² and 600 mW/cm²
[b] CNV - choroidal neovascularization; NORMAL - normal retina/choroid
[c] C - closed angiographically; O - open; ND - not done

Conclusion: The combined data regarding the effectiveness and selectivity of the treatment led to the conclusion that optimal PDT parameters for CNV were a fluence of 150 J/cm^2, irradiance of 600 mW/cm^2, and a dye dose of 0.375 mg/kg with light irradiation performed 20-50 minutes after dye injection.

***Reviewer's Comment** – The descriptions listed in the pathology for individual animals [Appendix 4] were inconsistent, vague and did not utilize proper terminology. The grading system was weighted primarily towards changes in the ONL [e.g. pyknosis] and damage/closure of the medium and large choroidal vessels. The grading system did not indicate the severity of changes observed in the INL, RPE, and photoreceptors nor was the severity always indicated in the individual animal data. It was not clear as to who read the slides or the qualifications of the individual reading the slides. However, it appeared from the terminology used that the individual was not trained in veterinary pathology. In addition, it was not clear as to whether the read was blinded or peer reviewed. The N was small generally ranging from 1-2 animals, although there were multiple lesions per eye. In general, there was only 1 lesion evaluated under a given test article and light dose regimen. This was essentially an N of 1, which is inadequate for a pivotal study. The study was not conducted in compliance with GLP. Based on these considerations, this study is considered inadequate for regulatory purposes.*

***Additional Conclusions** – As noted above, there were a number of concerns that makes interpretation of the study difficult. Taking into account the various caveats, the parameters that appeared to exhibit the greatest impact on treatment outcome were dose of BPD-MA and timing of irradiation following drug administration.*

- The Sponsor considered the damage to normal retina/choroid following a dose of 1 mg/kg unacceptable.

B. Dog

a. <u>A single dose intravenous retinal toxicity study in dogs of liposomal benzoporphyrin derivative monoacid A [BPD-MA; CL-318,952] [Ref. 330]</u>
Study Identification: 930106
Site: [redacted]
Study Dates [in-life]: Feb. 15 – March 3 [pilot study] and May 2-16 [main study], 1994

Formulation and Lot No. – liposomal BPD-MA- F93-120-0880 [both studies]; test article prepared on each day of dosing

Vehicle – 10% D-lactose monohydrate

Certificate Analysis: Yes (X)

Final Report: Aug. 4 [pilot study] and Aug. 22 [main study], 1994

GLP and QA Statements Signed: Yes (X)

Objective: "To determine the effects of Liposomal Benzoporphyrin Derivative Monoacid Ring A [BPD-MA; C-318,952] after intravenous infusion on the retina of dogs following exposure to sunlight at different postdose intervals"

Dr. Javier Avalos has previously reviewed this study for IND [redacted] Submission [redacted] draft review. This review is provided below with comments by the current Reviewer in italics.

A pilot study was conducted in which beagle dogs [N=1/sex/group] were administered 20 mg/kg and exposed to sunlight 24, 48, 72, and 96 hours later. Animals were maintained under subdued lighting until exposure to sunlight. The most severe phototoxicity occurred in animals exposed to sunlight 24 hours post-dosing. The female in this group was euthanized prematurely in moribund treatment-related condition, apparently secondary to phototoxicity. With the exception of bilateral conjunctivitis in this premature decedent, ocular angiography, direct and indirect ophthalmologic examinations, and ocular histopathology revealed no evidence of retinal or ocular tissue toxicity in any of the treated animals. The male dog exposed to sunlight at 24 hours exhibited well-defined erythema and alopecia of the forelimbs that persisted until study termination [Day 14]. Animals exposed to sunlight at ≥48 hours post drug administration, exhibited phototoxicity for 3-4 days including slight to moderate erythema of the shaved forelimb and perinostril area. Animals were normal for the remainder of the study period. Red urine was also observed in 4 dogs 24-48 hours following BPD-MA administration.

Animal Strain: Beagle dog [redacted]

Animal Starting Weight: 8.7 to 12.68 kg

No. of Animals: 4/sex; individually housed

Test Materials: Liposomal BPD-MA [redacted]

Batch Numbers - F93-120-0880

The lyophilized powder in vials was reconstituted prior to use with sterile water for injection to a final concentration of 2 mg/ml. The solution and powder forms were stored at 2-8° C until use and were protected from light at all times.

Route: bolus IV injection, 7 ml/min

Study Design and Dose Levels:

Group	Treatment	Dose of BPD-MA (mg/kg)	Concentration of BPD-MA (mg/ml)	Dose Volume (ml/kg)
1	D-Lactose	0	0	5.0
2	BPD-MA	10	2	5.0

Methods: Liposomal BPD-MA was intravenously infused in cephalic vein of the forelimb of male and female Beagle dogs and followed with an exposure to sunlight (actual) *for 6 hours.*

Animals were housed with subdued fluorescent lighting (19.3 + 2.9 or 31.2 + 2.5 mW/cm^2; 12 hr dark cycle) 24 hours post-infusion. At approximately 24 hours postdosing, animals were exposed to ca. 1200 J/cm^2 by placing dogs into outside runs in the sunlight. After sunlight exposure, dogs were kept under normal lighting conditions (187 + 11.1 or 201.1 + 6.5 mW/cm^2) until sacrifice on study day 15. Direct and indirect ophthalmoscopic and fluorescein angiography evaluations were conducted approximately 10 days prior to dose administration and on day 13. Clinical observations were made twice daily to detect any adverse findings, as well as daily detailed observations. On day 15, all dogs were euthanized and eyes were collected, fixed, trimmed and embedded. The eyes underwent histopathological examination [*5 sections*].

Results:

Mortality: No mortalities were reported.

Clinical Observations: *During exposure to sunlight, the control males developed slight redness to the ears and stomach. Signs in the treated males suggested increased photosensitivity and included; [1] swelling and redness around the eyes; [2] rubbing the eyes on ground; and [3] whimpering or hiding in the shade. All 3 females exhibited clinical signs of photosensitivity during the 6-hour sunlight exposure period. No signs associated with light exposure were observed in the female controls at any time point.* Signs of phototoxicity were evident in 2 of 3 females in Group 2. Well-defined erythema of the skin around the eyes and on the face, pinna (ears), and shaved forelimb appeared on the animals on day 3 and persisted through out the study. The erythema progressed to scabbing *and/or open wounds* in these animals. ***[Reviewer's Comment** – The table describing the signs observed in the females indicates that there was a sore on the scrotum. The Sponsor has been asked to clarify this discrepancy.]*

Body Weights: Body weight loss occurred in the two animals with the phototoxic response. The weight loss experienced was approximately 10%. *There was also a decrease in overall food consumption observed in these animals.*

Ophthalmologic evaluation: [*Robert Munger, DVM, DACVO conducted the direct and indirect ophthalmoscopic exams.*] Biomicroscopic and indirect ophthalmoscopic examinations were performed on all animals. One animal (a female animal from Group 2) exhibited thickening of the upper lids of both eyes with scabbed ulcerations. The globes and conjunctiva were within normal limits. Bilateral temporal muscle atrophy was also noted. No other ocular effects were noted from the intravenous infusion of the test article in any other animal. *Conducted prior to dosing and on Day 13.*

Ocular Angiography: No abnormal findings were reported after reviewing the fluorescein angiograms obtained after sunlight exposure. *Conducted prior to dosing and on Day 13.*

Anatomic Pathology: No treatment-related findings were reported. No evidence of retinal or other ocular tissue toxicity *was observed* as a result of treatment with BPD-MA *based on histopathology.*

***Reviewer's Comment** – The design of the study [e.g. evaluation of direct and indirect ophthalmoscopy on Day 13 and histopathology on Day 15 post dosing] does not address acute or early changes that may have resolved. The conclusion is that either no lesion developed under these experimental conditions or that any lesion that developed resolved.*

C. Literature Reviewed

a. Rabbits

1. Ref. 55: Schmidt-Erfurth, U., et. al. [1994]. Vascular targeting in photodynamic occlusion of subretinal vessels. *Ophthalmology*: **101**; pp. 1953-1961

BPD-MA, dissolved in DMSO, was complexed with human LDL [0.8 mg/ml BPD + 2 mg human LDL/mg BPD-MA]. **[Reviewer's Comment** – This is not the same formulation as used in the clinical trials.] Anesthetized albino NZW and pigmented Dutch belted rabbits [N = 4/strain; gender not indicated] were administered 2 mg/kg of BPD-MA/LDL intravenously. The right eye was irradiated [10, 50, and 100 J/cm^2] immediately after or 3 hours following dosing [N=2/strain/time point]. The left eye either was not irradiated [N=2] or was irradiated prior to drug administration [N=2]. Two rabbits were included as naïve controls. The following endpoints were evaluated: [1] fundus photography 1 hour post treatment; [2] fluorescein angiography following fundus photography; and [3] histopathology – light and electron microscopy 2 hours post irradiation. **Results** – The extent of damage to the retina [including choroidal vessels, RPE , ONL] and the degree of vessel closure and thrombosis were a function of both light dose and time interval between drug administration and irradiation. Irradiation alone did not induce any histopathological lesions. There were no differences in the pathology between pigmented and non-pigmented eyes. The design of this study did not allow for evaluation of lesions that developed at >2 hours post irradiation. The results are summarized in the table below. [**Note:** The exact time of irradiation at the early time point was not provided. In the text, it was stated that treatment occurred immediately after administration of LDL-complexed BPD-MA. The table indicates that this was within 30 minutes.] Electron microscopy revealed denuded endothelial cells with choroidal vessels packed with platelets.

Table 1. Ophthalmoscopic Findings

Treatment within 30 mins of BPD–LDL Application	Treatment 3 hrs after BPD–LDL Application
10 J/cm^2	
Immediate discoloration	No ophthalmoscopic change during PDT
Loss in retinal transparency	Late discoloration (faint)
Early serous detachment (localized)	No serous detachment seen ophthalmoscopically Occlusion of choriocapillaris only
Occlusion of choriocapillaris and some large choroidal vessels	
RPE completely disrupted	RPE disrupted, but partially attached
Damage throughout entire retina	Photoreceptors intact with swollen inner segments, inner retina unchanged
50 J/cm^2	
Immediate discoloration	No ophthalmoscopic change during PDT
Loss in retinal transparency	Late discoloration (slight)
Early serous detachment (expanding)	Late minimal serous detachment
Occlusion of choriocapillaris and most large choroidal vessels	Occlusion of choriocapillaris and some large choroidal vessels
RPE completely disrupted	RPE disrupted, but partially attached
Damage throughout entire retina	Photoreceptors and focally outer nuclear layer altered
100 J/cm^2	
Immediate discoloration	No ophthalmoscopic change during PDT
Loss in retinal transparency	Late discoloration (intensive)
Early serous detachment (expanding)	Late moderate serous detachment
Full-thickness choroidal occlusion	Occlusion of choriocapillaris and some large choroidal vessels
RPE completely disrupted	RPE disrupted
Damage throughout entire retina	Damage throughout entire retina

BPD-LDL = benzoporphyrin derivative–low-density lipoprotein; PDT = photodynamic therapy; RPE = retinal pigment epithelium.

Conclusion: "Endothelial cells and in particular proliferating endothelium equally demonstrate an increased uptake of LDL. Thus, LDL or liposomes should be useful as carriers to

Site:

Study Dates: study initiated Sept. 20, 1990
Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; approximately on analysis of dosing solutions; Stock solution may have been used over the "course of a few days"
Vehicle – liposomal solution/BPD-MA carrier
Certificate Analysis: Yes (X)
Final Report: May 23, 1991
GLP and QA Statements Signed: No (X)
Objective: "To identify the potential toxicity in rats given a single intravenous dose of BPD-MA followed by exposure to activating light".

Dr. Will Coulter previously reviewed this study by for IND Submission final review; pp. 8-9. Comments by the current Reviewer are in italics below.

The NOAEL for this study was [1] 20 mg/kg without light activation; [2] 0.2 mg/kg + 100 J/cm2; and [3] 0.5 mg/kg + 50 J/cm² based on clinical observations [e.g. local effects]. Gross and histopathology evaluations were not conducted.

b. Title: A single dose study of benzoporphyrin derivative monoacid (a photodynamic anticancer agent) given iv to rats [Ref. 311]
Study Identification: 90061
Site:

Study Dates: study initiated Nov. 7, 1990
Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; solutions were used within 24-48 following reconstitution; HPLC analysis was conducted on frozen samples taken on the day of dosing; approximately
Vehicle – liposomal solution/BPD-MA carrier
Certificate Analysis: Yes (X)
Final Report: May 23, 1991
GLP and QA Statements Signed: Yes (X)
Objective: "To identify potential toxicity in rats given a single intravenous dose of BPD-MA followed by exposure to activating light".

Dr. Will Coulter previously reviewed this study for IND Submission final review; pp. 9-10. Additional comments by the current Reviewer [in italics] are provided below.

1. *Histopathological lesions extended into the muscle [inflammation and/or necrosis] at 2 mg/kg +50 and 100 J/cm² and 10 mg/kg + 25, 5, and 100 J/cm². Lesions were dose dependent both as a function of BPD-MA and irradiation dose.*
2. *There were sporadic statistically significant changes in several organ weights [relative and absolute]. A dose-dependent effect was not observed. These changes were not considered to be treatment-related.*
3. *There was an increased incidence of interstitial pneumonia and mononuclear infiltration. Interstitial pneumonia was generally grade 2 (slight) and was observed in 0/5 vs. 4/5 and 2/5 animals at 0 vs. 10 mg/kg without or with irradiation, respectively. Mononuclear*

infiltration, generally grade 2, was observed in 1/5 vs. 4/5 animals at 0 vs. 10 mg/kg without or with irradiation. This was observed in females only. The significance of this finding is not known.

4. *A saline control [e.g. negative control] should be included in toxicology studies. However, lack of a negative control was not considered to significantly compromise this study. Use of a vehicle control in this study does provide useful data with respect to the toxicological profile of the excipients in the final formulation.*
5. *The NOAEL was [1] 0.5 mg/kg +100 J/cm² and [2] 10 mg/kg without irradiation.*

c. Title: A single dose study of benzoporphyrin derivative monoacid (an anticancer agent) given intravenously to rats [Ref. 310]
Study Identification: 90225
Site: [redacted]
Study Dates: study initiated Nov. 28, 1990
Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; dosing solutions were prepared the day of dosing and based on the study design, the solution would be used in ≥8 hours following preparation; HPLC analysis was conducted on frozen samples taken on the day of dosing; approximately [redacted]
Vehicle – liposomal solution/BPD-MA carrier
Certificate Analysis: Yes (X) [redacted]
Final Report: May 22, 1991
GLP and QA Statements Signed: Yes (X)
Objective: "To determine the single dose toxicity of BPD-MA when administered intravenously to rats".

Dr. Will Coulter previously reviewed this study for IND [redacted] Submission [redacted] final review; pp. 10-11. [redacted] Additional comments by the current Reviewer [in italics] are provided below.

1. *The statistically significant decreases in mean food consumption in the low and high dose groups in males and the high dose in females correlated with decreases of approximately 25-50% in body weight gain over the same period. However, there was not a statistically significant change in mean body weight with differences between control and high dose weights ≤5%. The relationship to treatment is not known.*
2. *Clarification of organ weight data – Animal #4 [male/Group 1 control] was inadvertently not recorded. [Protocol amendment].*
3. *Absolute and relative thymus weights were decreased in all treated females [approximately 15-24%] reaching statistical significance in the low and high dose groups. The significance of this finding is not known.*
4. *A saline control [e.g. negative control] should be included in toxicology studies. However, lack of a negative control was not considered to significantly compromise this study. Use of a vehicle control in this study does provide useful data with respect to the toxicological profile of the excipients in the final formulation.*
5. *The NOAEL for this study was 100 mg/kg.*

B. Dog
a. Title: A single dose toxicity study of benzoporphyrin derivative monoacid (a photodynamic anticancer agent) given IV to beagle dogs [Ref. 309]
Study Identification: 90063/90235

Site:

Study Dates: Dosing initiated on Nov. 21 [high-dose] and Dec. 27 [low dose], 1990
Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; dosing solutions were prepared either the day of before or the day of dosing. HPLC analysis was conducted on frozen samples taken on the day of dosing; approximately

Vehicle – liposomal solution/BPD-MA carrier
Certificate Analysis: Yes (X)
Final Report: May 30, 1991
GLP and QA Statements Signed: Yes (X)
Objective: "To identify potential toxicity in [beagle] dogs given a single intravenous does of BPD-MA followed by exposure to activating light".

Dr. Will Coulter previously reviewed this study for IND Submission final review; pp. 11-12. Additional comments by the current Reviewer [in italics] are provided below.

Reviewer's Comments

1. *The Sponsor amended the summary report to correct a typographical error. The irradiation was 687-713 nm rather than 683-713 nm as stated in the original summary and in the appended review.*
2. *Results for Study 90235 – The NOAEL was 0.1 mg/kg + 50 J/cm^2. A single dog at this dose exhibited slight erythema on Day 6. Local reactions [erythema, edema, and microscopic damage to skin, sc., and muscle in the high dose group] but no systemic toxicity was observed at doses of 0.5 - 1 mg/kg + 50 J/cm^2. Local lesions at 1 mg/kg progressed to ulcerations.*
3. *Study 90235 Toxicokinetics - Plasma concentrations of the regioisomers CL 315,555 and CL 345,585 were measured by HPLC at 0.5, 1, 3, 5, 8, 12, 24, 48, 72, 120, and 168 hours post dosing in the animals [N=2] dosed at 1 mg/kg + 50 J/cm^2. **Results***

Pharmacokinetic Parameters For Beagle Dogs Given A Single IV Dose of 1 mg/kg BPD-MA (CL 315,555/ CL 315,585)

Dog #	C_{max} (ug/mL)	AUC_{0-last} (ug•hr/ml)	Total Clearance (mL/min/kg)	V_{ss} (L/kg)	Half Life (hrs)

4. *Study 90063*
   - *RBC indices were decreased in all irradiated males by approximately10-25% compared to both the concurrent and predose control values and in females at 20 mg/kg ± irradiation.*
   - *There was considerable variability in white blood cell counts. However, there tended to be an increase in absolute neutrophil counts [approximately 15-200%] in all treatment groups [± irradiation] compared to concurrent controls with the exception of females administered 0.5 mg/kg + 100 J/cm^2. The increase was also apparent when values were compared to baseline measurements, was observed on both Days 1 and 14, and tended to be greater in males than females. This increase in PMNs was associated with a decrease in absolute lymphocyte counts [approximately 40-80%] in males at ≥2 mg/kg and females at ≥10 mg/kg [irradiated only]. The combination of neutrophilia/lymphopenia would be consistent with a stress leukogram. The neutrophilia may also reflect an inflammatory response secondary to local tissue damage in those animals that were irradiated. Due to differences in the irradiation dose it was not possible to determine any dose-response relationship.*
   - *An increase in AST 3 hours post irradiation of approximately 25-100% was observed in most irradiated groups. Increases in AST were associated with increases in CK. In the absence of a concurrent increase in ALT, this increase suggests skeletal muscle injury rather than hepatic toxicity.*
5. *Due to the small N [e.g. 1/sex/group] results must be interpreted cautiously.*
6. *A saline control [e.g. negative control] should be included in toxicology studies. However, lack of a negative control was not considered to significantly compromise this study since predose measurement of both serum chemistry and hematology were obtained. Use of a vehicle control in this study does provide useful data with respect to the toxicological profile of the excipients in the final formulation.*

III. Repeat Dose Toxicity Studies

A. Rat

a. Title: <u>An intermittent, multiple dose study of benzoporphyrin derivative monoacid (a photodynamic anticancer agent) given IV to rats [Ref. 313]</u>
Study Identification: 90237/90241
Site:
Study Dates: Dosing initiated Dec. 7, 1990
Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; dosing solutions were prepared on the day of dosing; HPLC analysis was conducted on frozen samples taken on the day of dosing; approximately
Vehicle – liposomal solution/BPD-MA carrier
Certificate Analysis: Yes (X)
Final Report: May 24, 1991
GLP and QA Statements Signed: Yes (X)
Objective: "To identify potential toxicity in rats given multiple intravenous doses of BPD-MA followed by exposure to activating light"

Dr. Will Coulter previously reviewed this study for IND Submission final review; pp. 17-18 for the toxicology portion [90237] and pp. 6-8 for the toxicokinetic portion [90241]. Additional comments by the current Reviewer [in italics] are provided below.

1. *Histopathology of irradiated skin and spleen was conducted in all animals.*
2. *Toxicokinetic study – Samples were collected predose [day 9 only], immediately after dosing, and 0.5, 1, 3, 5, 8, 12, 24, 48, and 72 hours after dosing. Each rat was bled 2-3X/72 hours.*
3. *It would appear that the Sponsor has incorrectly stated [p.14] that a Group5 female died on Day 3. All females survived until sacrifice but Male #42 was sacrificed on Day 3.*
4. *Clinical observations - The NOAEL for skin lesions was 0.5 mg/kg. There were two liposomal control females which developed lesions that were similar to lesions developing in the some of the animals administered 1 mg/kg.*
5. *The 50% decrease in weight gain on Days 3-6 in the high dose females when compared to the sham injected was due to weight loss in 2 animals and low weight gain [e.g. 2 gm] in 1 animal.*
6. *There were no treatment-related ophthalmoscopic changes reported by the Sponsor.*
7. *There were no treatment-related effects on urinalysis parameters.*
8. *Clarification of hematology*
   - *Hb was variably affected in Groups 2 and 3 [increased, decreased, unchanged at different time points in males and females].*
   - *Neutrophils [%] were increased on **both** Days 9 and 13 with a larger increase observed on Day 13 in both males and females. Absolute number was also increased.*
   - *Although percent lymphocytes decreased [16-19%] in Group 4 and 5 animals compared to sham injected, the absolute numbers of lymphocytes increased by approximately 1.5-2X.*
   - *Changes in RBC morphology were observed in Groups 2, 4 and 5, generally characterized by an increase in the incidence of polychromasia and/or an increase in the severity and/or incidence of anisocytosis and poikilocytosis.*
9. *Organ weights*
   - *There was a 15-27% decrease in absolute and relative thymus weights in the high dose irradiated males and females compared to saline controls. There was also an increase in these organs compared to the vehicle controls. It was postulated that this decrease was secondary to stress [e.g. development of skin lesions].*
   - *There was approximately a 30% increase in absolute and relative adrenal weights in the high dose males.*
   - *There was approximately a 40% decrease in absolute and relative pituitary weights in high dose females compared to the saline control. The decrease was dose-responsive. VH control was decreased [approximately 30%] compared to the saline control. The significance of this finding is not known.*
10. *Clarification of Histopathology*
    - ***The incidence of lesions [e.g. scabbing in females] was comparable in the saline controls and the low dose males and females. Lesions were observed primarily in Groups 4 and 5. In addition, several lesions, including skeletal muscle necrosis, granulation tissue, and epidermal regeneration, were observed in females administered liposomal solution without BPD-MA.***
    - ***The incidence of mixed, multifocal inflammatory cells in the liver was as follows: [1] Sham Control - 3/10 and 5/10 males and females; [2] VH Control – 6/10 and 4/10 males and females; and [3] 2 mg/kg – 5/9 and 7/10 males and females, respectively. The severity in all animals was minimal.***
    - ***The incidence of spleen congestion was as follows: [1] Sham Control - 3/10 and 5/10 males and females; [2] VH Control – 6/10 and 7/10 males and females; and [3] 2 mg/kg – 5/9 and 6/10 males and females, respectively. The severity was not graded.***

11. There were no apparent differences in pharmacokinetics between males and females. PK parameters were comparable on Days 0 and 9. The AUC for CL 315,555 was 25% less than that for CL315,585. This was attributed to a 3X higher plasma clearance for CL 315,555.

b. Title: A two-week intravenous toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rats [Ref. 312]
Study Identification: 92020/92052
Site:
Study Dates [in-life]: April 29 – May 15, 1992
Formulation and Lot No. – BPD-MA – Batch # J90-120-0175 for both studies and J90-120-0182 for Study 92052; solutions were used on the day of preparation: HPLC analysis was conducted on frozen samples taken on April 29 and May 6, 1992; 97-

Vehicle Controls – liposomal solution/BPD-MA carrier
- D5W
Certificate Analysis: Yes (X)
Final Report: June 17, 1993
GLP and QA Statements Signed: Yes (X)
Objective: "To identify the antemortem and target organ toxicity of formulated CL 318,952, when administered intravenously to rats for 2 weeks [and] to identify potential effects of a liposomal solution without CL 318,952"

Dr. Javier Avalos previously reviewed this study for IND Submission final review; pp. 21-24 [92020] and pp. 24-27 [92052]. This review is provided below with comments by the current Reviewer in italics.

A two-week intravenous toxicity study of CL 318,952 in rats (GLP study)

Laboratory: (Study Number 92020)

Test Materials: Liposomal (CL 318,952 / BPD-MA) solution or liposomal solution diluted with 5% dextrose. Control article was 5% dextrose for injection or liposomal solution without CL 318,952.

Animal Strain: Crl:CD BR albino rats

Animal Starting Weight: *F* = 164 to 212 g; *M = 205-276 g;* (7 weeks of age)

No. of Animals: 5 or 9/sex/group; individually housed

Route: Intravenous

Duration: Once a day for 15-16 days

Study Design:

Group Number	Dose Level (mg/kg/day)	Dose Concentration (mg/ml)
1	0[a]	0
2	0[b]	0
3	2[c]	0.16
4	10[c]	0.8
5	25[d]	2.0
6	2[c]	0.16
7	10[c]	0.8
8	25[d]	2.0

a - 5% dextrose for injection, USP
b - Liposomal solution without CL318,952
c - Liposomal solution diluted with 5% dextrose as the carrier
d - Liposomal solution (undiluted) as the carrier.

High dose was based on solubility and volume factors.

Methods: Animals were given daily intravenous doses of CL 318,952 (BPD-MA) for 15-16 days. Additional groups received 5% dextrose for injection, USP, or the liposomal solution in a similar manner. The dose was infused at a rate of 2.5 ml/min, over a period of approximately 1.5 minutes. The rats were dosed in the tail vein using an appropriately sized syringe and butterfly infusion set. The rats of Groups 1-5 were assigned to the toxicology aspect of this study and were observed daily. *Body weight and food consumption were measured on predose Day -1 and Days 2, 6, 9, and 13. Body weight was also determined on predose days –7 and –2. Ophthalmoscopy was performed on predose Day –8 and Day 13.* Blood was taken for hematology [*hematocrit, hemoglobin, RBC count, reticulocytes, MCV, MCH, MCHC, platelet count, WBC count and differential*] and serum chemistry [*Na, K, Cl, Ca, P, BUN, creat., total bili., gluc., chol., tri., SAP, AST, ALT, TP, alb., glob., alb:glob ratio*] evaluations on day 15 and urine was collected for urinalysis evaluations [*pan collected - color, appearance, pH, protein, gluc., ket., urobil., bili., RBCs/Hb, SpG, sediment analysis*] on day 14. In addition, these animals were sampled at 21-23 hours post-infusion. Animals were necropsied on day 15 or 16 *and organ weights were obtained. Histopathology was conducted on the D5W controls, liposomal VH controls, and the high dose animals. In addition, gross lesions were evaluated microscopically.* The rats of Groups 6-8 were assigned to the toxicokinetic aspect of the study and were similarly dosed for 1 day. These animals were bled immediately after dosing and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dosing. Both Groups 5 and 8 were administered with the maximum feasible dose as limited by the solubility of BPD-MA (2 mg/ml) and maximum dose volume of 12.5 ml/kg. Animals were sacrificed after the last bleeding. For these animals, antemortem observations were limited to mortality and moribundity checks and body weight and food consumption measurements. These data were not evaluated.

Results:

Mortality: All animals survived until scheduled necropsy.

Clinical Observations: No treatment-related differences between the control groups or test groups were noted. Occurrences were sporadic and common to the species.

Body Weights *and Food Consumption*: No statistically significant differences in mean total body weight change or total food consumption data were observed between the dextrose treated animals and BPD-treated animals.

Ophthalmoscopy [*Evaluation conducted by James M. Clinton, VMD, Diplomate ACVO*]: No ocular findings attributable to the administration of CL 318,952 were reported in this study. The focal retinopathy found in a male animal treated with 2 mg/kg/day on day 13 of the study was reported to occur spontaneously in rats.

Clinical Pathology: All comparisons were made to the group receiving 5% dextrose (Group 1). The group of animals receiving a liposomal solution without CL 318,952 were not considered an appropriate control since the Sponsor believes "that the physicochemical characteristics of the liposomal solution may change as a function of the absence or presence of CL318,952".

Hematology – No compound-related effects on hematological parameters in groups given 2 or 10 mg/kg/day were reported. Statistically significant reductions (5-26%) in measured blood cell parameters (hematocrit, hemoglobin, and red blood cell count) were reported in the groups given 25 mg/kg/day of formulated CL 318,952. Additional findings in these groups consisted of statistically significant increases in mean reticulocyte count (*percent:* approximately 6-10 fold) and mean cell volume (13-19%). *Although there was no change in Hct, Hb, and RBC count at 10 mg/kg, there was an increase of 2X in percent reticulocytes at this dose group for both males and females compared to the dextrose control.* A slight decrease was observed in mean cell hemoglobin concentration [*4% in females only*]. A slight increase was seen in the mean cell hemoglobin [*14-19%*] and platelet count [*20% in males only*]. Additionally, red cell morphology findings of the presence of macrocytes, spherocytes, and polychromasia were noted in these groups. Similar effects were seen in the group that received the liposomal solution without BPD. Statistically significant increases (20 [*should be 45%*] and 120%) in mean white blood cell count were observed in both the male and female groups, respectively, given 25 mg/kg/day. *The Sponsor suggesteds that the WBC count in the control females was low, but did not provide historical control data to support this statement. The increase in white count, which was observed in the treated animals compared to control animals, was due primarily to an increase in absolute lymphocyte and neutrophil counts.*

Serum Chemistry - A statistically significant increase (7%) in calcium was reported in all female animals receiving BPD *and liposomal solution only*. The female animals treated with 10 mg/kg/day also had statistically significantly increases in total protein (9%) and globulin (13%). The female group treated with 25 mg/kg/day had statistically significant increases in alanine aminotransferase (27%), total protein (8%), and globulin (15%) [*Total protein levels and globulins were increased 9% and 13%, respectively, at 10 mg/kg*]. A statistically significant decrease was observed in potassium (20%) and *an increase in* total bilirubin (2-5 fold) in male and female animals treated with 25 mg/kg/day [*A 2X increase in bilirubin in males and females was also observed at 10 mg/kg.*] The male animals treated with 25 mg/kg/day *and with the liposomal solution* also showed a statistically significant increase in creatinine *and urea nitrogen* (20%).

Urinalysis - No compound-related effects on urinalysis parameters were reported.

Gross Pathology: A green discoloration (due to test material) was observed in the internal organs (parotid gland, stomach, thymus, testes, large intestines, and small intestines) and skin surfaces including the tail. This discoloration over the entire body surface was reported in 7 of 10 animals receiving 25 mg/kg/day and in 3 of 10 animals receiving 10 mg/kg/day. Only 1 of 10 animals and 2 of 10 animals treated with 25 mg/kg/day had the green discoloration in the parotid gland and thymus, respectively. The majority of the reported discolorations were for animals treated

with 10 and 25 mg/kg/day. However, the stomach, and small and large intestines were discolored in one animal receiving 2 mg/kg/day.

Organ Weights: The mean absolute and relative weights of the spleen were statistically significantly increased in the group receiving 25 mg/kg/day of CL 318,592 in liposomal formulation when compared to the dextrose treated animals. The male animals had an increase of 160% in absolute and relative spleen weights, respectively, and the female animals had an increase of 111% and 119% in absolute and relative spleen weights, respectively. Other significant weight differences observed were sporadic and not dose related. The liposomal control animals were also reported to have similar increases in relative and absolute spleen weights.

Histopathology: Microscopic findings in the group receiving the liposomal control solution and the group receiving 25 mg/kg/day of formulated CL 318,952 were compared to the dextrose treated animals. Moderately increased extramedullary hematopoiesis in the spleen, slight to moderate erythroid hyperplasia of the bone marrow, and minimal extramedullary hematopoiesis in the liver were observed in the majority of animals in the liposomal solution group and the 25 mg/kg/day group. These findings were accompanied by decreased red blood cell count and hematocrit; increased mean cell volume, spherocytes, and peripheral reticulocyte count. No compound-related findings were reported in the 2 or 10 mg/kg/day groups. *The table below outlines these findings [Sponsor provided].*

In females, incidence and/or severity of plasma cell hyperplasia was slightly increased in the cervical and mesenteric lymph nodes.

	Males					Females				
CL 318,952:										
Dose (mg/kg/day)=	0	0	2	10	25	0	0	2	10	25
Vehicle=	D	L	DL	DL	L	D	L	DL	DL	L
Number of Animals=	5	5	5	5	5	5	5	5	5	5
Liver										
Extramedullary hematopoiesis	0	4	0	1	4	0	3	0	0	2
severity	0	1.0	0	1.0	1.0	0	1.0	0	0	1.0
Spleen										
extramedullary hematopoiesis	5	5	5	5	5	5	5	5	5	5
severity	1.0	3.4	1.0	1.0	3.2	1.0	3.0	1.0	1.0	3.0
Bone marrow										
erythroid hyperplasia	0	4	0	0	5	0	5	0	0	5
severity	0	2.7	0	0	2.8	0	2.8	0	0	2.8

D = Dextrose 5% in water (D_5W)
L = Liposomal solution
DL = D5W + Liposome
Severity score code: 1 = minimal; 2 = slight; 3 = moderate; 4 = severe.

Toxicokinetic Results: "Following administration of 2, 10, or 25 mg/kg of CL 318,952 (BPD-MA), day 0 peak plasma concentrations of CL315,555 (a regioisomer of BPD-MA) averaged 9.9, 45 or 122 µg/ml, respectively, and exhibited dose proportionality. AUC_{0-24} values increased in a greater than proportional manner, reflecting a dose dependent decrease in CL_T. The apparent elimination t½ was quite variable, ranging from 6.8 to 11.7 hours. Also, no consistent differences between mean male and female parameters of CL315,555 were reported and thus, they were

combined. For CL315,585, day 0 C_{max} values averaged 14, 67, or 172 µg/ml, respectively, after administration of 2, 10, or 25 mg/kg of CL 318,952. C_{max} were dose proportional and independent of sex. However, the AUC_{0-24} values were different with respect to gender, but dose proportional. Males exhibited AUC values approximately 45% greater than females. CL_T was inversely related to dose. The apparent elimination t½ was estimated to be 3.5 to 4.6 hours.

Systemic exposure to CL315,585 was approximately 4 times higher than CL315,555, as seen by AUC comparison. The ratio of CL315,555 AUC to CL315,585 AUC remained approximately 0.2 for all doses, indicating that the changes in CL_T of CL315,555 and CL315,855 were concomitant, and not stereo selective. On day 15, CL315,555 plasma concentrations, approximately 21-23 hours post dose, were 0.088, 0.24, and 45 µg/ml, respectively, for the doses of 2, 10, and 25 mg/kg of CL 318,952. For CL315,585, the plasma concentrations at 21-23 hours post infusion were 0.12, 0.71, and 3.4 µg/ml, respectively, for doses of 2, 10, and 25 mg/kg of BPD-MA". *The table below outlines these findings [Sponsor provided.]*

Toxicokinetic Parameters of CL 315,555 and CL 315,585, Following an IV Dose of CL 318,952 in Rats

DOSE (mg/kg)		C_{max} (µg/mL)	AUC_{0-24} (µg•hr/mL)	AUC_{0-inf} (µg•hr/mL)	CL_T (mL/min/kg)	$V_{ss/obs}$ (L/kg)	$T_{1/2}$ (hr)
		CL 315,555					
2.0							
	Male	9.31	2.51	2.55	6.54	1.44	5.21
	Female	10.5	N/C	N/C	N/C	N/C	N/C
	Combined	9.91	N/C	N/C	N/C	N/C	N/C
10							
	Male	49.5	19.4	21.3	3.92	1.64	9.30
	Female	41.2	14.5	17.3	4.83	3.13	14.1
	Combined	45.4	17.0	19.3	4.37	2.38	11.7
25							
	Male	125	55.9	58.4	3.57	0.924	7.32
	Female	119	41.4	42.6	4.88	1.13	6.32
	Combined	122	48.7	50.5	4.23	1.03	6.82
		CL 315,585					
2.0							
	Male	14.1	10.7	10.8	1.54	0.431	4.37
	Female	14.0	7.43	7.44	2.24	0.335	2.76
	Combined	14.1	N/A	N/A	N/A	N/A	N/A
10							
	Male	70.8	76.9	79.0	1.05	0.323	5.26
	Female	63.9	54.1	54.5	1.53	0.316	3.78
	Combined	67.4	N/A	N/A	N/A	N/A	N/A
25							
	Male	174	245	257	0.810	0.320	6.00
	Female	170	166	166	1.25	0.262	3.10
	Combined	172	N/A	N/A	N/A	N/A	N/A

N/C = Not Calculated; Insufficient Data for PK analysis
N/A = Not Applicable, due to male vs. female differences
Combined = Male and Female values combined, n=6

A two-week intravenous toxicity study of CL318,952 (BPD) using a maximum dose in rats (GLP Study)

Laboratory: [redacted] (Study Number 92052)

QLT Phototherapeutics, Inc.

Test Materials: Liposomal (CL 318,952 / BPD-MA) solution or liposomal solution diluted with 5% dextrose. Control article was ~~5% dextrose for injection or~~ liposomal solution without CL 318,952.

Study Design:

Group Number	Dose Level (mg/kg/day)[a]	Dose Concentration (mg/ml)
1	0	0
2	50	2.0
3	50	2.0

[a] Administered as two divided doses of 25 mg/kg every 8 hours.

Animal Strain: Crl:CD BR albino rats [redacted]

Animal Starting Weight: *F* = 159 to *202 g; M = 238* - 273 g (7 weeks of age)

No. of Animals: 5 or 9/sex/group; individually housed

Route: Intravenous

Duration: 15 days

Methods: Animals were given daily intravenous doses of CL 318,952 (BPD-MA) for 15-16 days. Additional groups received ~~5% dextrose for injection, USP, or~~ the liposomal solution *without BPD-MA* in a similar manner. The dose was infused at a rate of 2.5 ml/min, over a period of approximately 1.5 minutes. The rats were dosed in the tail vein using an appropriately sized syringe and butterfly infusion set. All animals were maintained below 20-foot candles. The rats of Groups 1 and 2 were assigned to the toxicology aspect of this study and were observed daily. *Ophthalmic examination [focal illumination, indirect ophthalmoscopy] was conducted predose Day –8 and Day 13.* Blood was taken for hematology [*hematocrit, hemoglobin, RBC count, reticulocytes, MCV, MCH, MCHC, platelet count, WBC count and differential*] and serum chemistry [*Na, K, Cl, Ca, P, BUN, creat., total bili., gluc., chol., tri., SAP, AST, ALT, TP, alb., glob., alb:glob ratio*] evaluations on day 15 and urine was collected for urinalysis evaluations [*pan collected - color, appearance, pH, protein, gluc., ket., urobil., bili., RBCs/Hb, SpG, sediment analysis*] on day 14. In addition, these animals were sampled at 21-23 hours post-infusion. Animals were necropsied on day 15 or 16. *Histopathology was conducted on all toxicology groups.* The rats of Groups 3 were assigned to the toxicokinetic aspect of the study and were similarly dosed for 1 day. These animals were bled immediately after the first dosing and at 2, 4, and 8 hours after dosing; and immediately after the second dose and 2, 4, 8, and 16 hours after dosing. Animals were sacrificed after the last bleeding. For these animals, antemortem observations were limited to mortality and moribundity checks and body weight and food consumption measurements. These data were not evaluated.

Results:

Since the Sponsor considered the results from the liposomal solution as inappropriate for use as a control, comparisons were made to the dextrose control animals from Study 92020. Since they were dosed at the same time and were the same age, it is felt that this was appropriate.

Mortality: Three females given the liposomal solution and three females given 50 mg/kg/day were found dead on day 15. These animals died shortly after being bled. All other animals survived until scheduled necropsy on day 15.

Clinical Observations: *All treated animals exhibited a green-yellow color of the ventral fur, tail, ears, and feet by Day 7.* No *other* treatment-related differences between the control groups or test groups were noted. Occurrences were sporadic and common to the species.

Body Weights *and Food Consumption*: No statistically significant differences in mean total body weight change or total food consumption data were observed between the dextrose treated animals and BPD-treated animals. *There was a 30-50% decrease in body weight gain in the treated males Days 6-9 and 9-13, in treated females and liposomal control females Days 6-9, and in the liposomal control males Days 9-13 compared to the dextrose control animals. This did not correlate with a change in food consumption.*

Ophthalmoscopy [*Evaluation conducted by James M. Clinton, VMD, Diplomate ACVO*]: Focal retinopathy was reported on Day 13 of the study in a male animal treated with 50 mg/kg/day. Although this event is alleged to occur spontaneously in rats, no findings were observed in control animals nor was historical data submitted. Thus, this finding may be related to the administration of BPD-MA. No other ocular findings attributable to the administration of CL 318,952 were reported in this study.

Clinical Pathology: All comparisons were made to the group receiving 5% dextrose (Group 1-*Study 92020*). The group of animals receiving a liposomal solution without CL 318,952 were not considered an appropriate control since it was "believed that the physicochemical characteristics of the liposomal solution may change as a function of the absence or presence of CL318,952".

Hematology - Statistically significant reductions (10-20% *in males and up to approximately 35% in females*) in mean red blood cell parameters (hematocrit, hemoglobin, and red blood cell count) were reported in the group given 50 mg/kg/day of formulated CL 318,952. *Reticulocytes [percent] increased in males and females by approximately 8X and normoblasts also tended to be increased for both genders.* Additional findings in this group consisted of increases in mean cell volume (21-21.9%). Additionally, red cell morphology findings of the presence of macrocytes, spherocytes, and polychromasia were noted in these groups. Similar effects were seen in the group that received the liposomal solution without BPD. *WBC counts were increased in males and females administered either drug substance or liposomal solution [45-60% and 115-130%, respectively] compared to the dextrose control. The Sponsor suggested that the WBC count in the control females was low, but did not provide historical control data to support this statement. The increase in white count when compared to the dextrose controls was due primarily to an increase in absolute neutrophil counts in males and females and absolute lymphocyte counts in females.*

Serum Chemistry - A decrease was observed in potassium (15-23%) and albumin (15-14%) in animals treated with 50 mg/kg/day. The animals treated with 50 mg/kg/day had an increase in BUN (8-12%), total bilirubin (4-7 fold), globulin (14%), and alanine aminotransferase (15-37%). *The difference in mean creatinine values between control and treated animals was <10%.*

Urinalysis - No compound-related effects on urinalysis parameters were reported.

Gross Pathology: A green discoloration was observed in the internal organs (pancreas, parotid gland, stomach, thymus, testes, large intestines, and small intestines, *and peripheral nerve*) and skin surfaces including the tail. This discoloration over the entire body surface was reported in all animals receiving test material.

Organ Weights: The mean absolute and relative weights of the spleen were increased (3 fold) in the treated animals when compared to the dextrose treated animals. Other significant weight differences observed were sporadic and not dose related. The liposomal control animals were also reported to have similar increases in relative and absolute spleen weights.

Histopathology: Microscopic findings in the group receiving the liposomal control solution and the group receiving 50 mg/kg/day of formulated CL 318,952 were compared to the dextrose treated animals. Moderately increased extramedullary hematopoiesis in the spleen, slight erythroid hyperplasia of the bone marrow, and minimal extramedullary hematopoiesis in the liver were observed in the majority of animals [*3-5 animals/sex/group*] in the liposomal solution group and the 50 mg/kg/day group". These findings were accompanied by decreased red blood cell count and hematocrit; increased mean cell volume, spherocytes, and peripheral reticulocyte count. These observations were not reported for animals treated with the 5% dextrose solution of Study No. 92020. *There was slight to moderate injection site perivenous inflammation and hemorrhage in both males and females in the animals administered the liposomal solution ± BPD-MA compared to the dextrose controls. BID administration in this portion of the study may have contributed to the difference when compared to dextrose controls [SID administration].*

Toxicokinetic Results: "Following administration of 50 mg/kg of CL 318,952, day 0 peak plasma concentrations of CL315,555 and CL315,585 averaged 105 and 154 µg/ml, respectively". Males exhibited $AUC_{0\text{-}24}$ values of CL315,585 which were 25% higher than females (577 vs. 460 µg*hr/ml), while no real differences between genders were observed for the CL315,555 isomer. CL_T for CL315,585 was 2.2 ml/min/kg and for CL315,555 the total systemic clearance was 0.67 ml/min/kg for males and 0.89 ml/min/kg in females. The apparent elimination t½ was quite 4.5 hr for CL315,585, and 6.7 in males and 3.3 hours in females for CL315,555.

Systemic exposure to CL315,585 was approximately 3 times higher than CL315,555, as seen by AUC comparison. On day 15, plasma concentrations of CL315,555 and CL315,585 were 0.41 and 5.89 µg/ml in males, respectively. In female rats, the plasma concentrations of CL315,555 and CL315,585 were 0.62 and 2.80 µg/ml, respectively.

The current reviewer evaluated the following study.

c. **A 28-day intravenous injection toxicity study [with a 28-day recovery] of benzoporphyrin derivative monoacid [BPD-MA] in the albino rat [Ref. 317]**

Study Identification: TX-96010
Site: [redacted]
Study Dates [in-life]: March 11– May 7, 1997
Formulation and Lot No. – liposomal BPD-MA- PQ009-96; purity range [redacted] dosing solutions were prepared at least weekly and stored at 4° C; HPLC analysis was conducted on frozen samples taken at each preparation; [redacted]
Vehicle – 5% Dextrose
Certificate Analysis: Yes (X) [redacted]
Final Report: Nov. 17, 1998
GLP and QA Statements Signed: Yes (X)
Objective: "To investigate the potential toxicity of liposomal BPD-MA during daily intravenous injection to the rat for a minimum of 28 consecutive days and following a 28 day recovery period"

Study Design

Test Material/ Group Designation*	Dose**				N***		Species/Strain
	mg/kg	ml/kg	Route	# days dosed	M	F	
Group 1 –5% dextrose	0	12.5	iv	28	15	15	Sprague-Dawley CD – Experimental
Group 2 - BPD-MA	2						
Group 3 - BPD-MA	10						M = 13 wk, F = 9 wk start of study.
Group 4 - BPD-MA	25						M = 409-483 g, F = 193=224 g start of study

*Light level was reduced to <20 foot-candles [app. –0.1 mW/cm^2]

** "The dose levels were selected according to the anticipated clinical dose level, maximum solubility [2 mg/ml], and maximum volume to be administered". There were some sporadic errors in dosing [p. 14] however these were not felt to significantly compromise the study

***10 animals/sex were sacrificed after 28 days of dosing; 5 animals/sex were sacrificed after a 28 day recovery period. The Sponsor indicates that 6 animals were replaced on Day 1, 4 on Day 6, and 3 on Day 7. The Sponsor was requested to clarify [1] which animals [e.g. number and group] were replaced, and [2] if those replaced on Days 6 and 7 received only 22 and 21 doses, respectively.

Parameters Evaluated	Time Point(s)
Clinical examination Mortality/moribundity Physical examination	 BID Weekly, day of sacrifice
Body Weight	Day –1, then weekly until sacrifice
Food Consumption	Weekly until sacrifice
Ophthalmology [indirect ophthalmoscopy, slip lamp examination] – included modifications of lighting, conducted by a board certified veterinary ophthalmologist	Weeks 4 and 8
Reproductive Assessment Estrous Cycle Determination – vaginal lavage Sperm Analysis – Epididymal sperm counts, motility, and morphology	 Daily during Weeks 3-4 At necropsy
Hematology [abdominal aorta, fasting] – RBC count and morphology, MCV, MCH, MCHC, RDW, Hct, Hb, platelet count and MPV, reticulocyte count, WBC and differential Coagulation parameters – APTT, PT	At sacrifice
Serum Chemistry [abdominal aorta, fasting] – A/G ratio, ALT, alb., SAP, AST, BUN, Ca, Cl, chol., creat., glob., gluc., P, K, Na, total bili., TP, tri.	At sacrifice
Urinalysis [16-hour collection, metabolic cage] – appearance, bili., blood, color, gluc., ket, nitrite, pH, prot., SpG, urobil., vol., sediment exam	Weeks 4 and 8
Gross Pathology	At sacrifice
Organ Weights – adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroids, uterus	At sacrifice
Histopathology - adrenals, aorta, bone and bone marrow [sternum], brain [cerebrum, cerebellum, midbrain, and medulla oblongata], cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, injection site*, jejunum, kidneys*, liver*, lungs, lymph nodes [mandibular, mesenteric], macroscopic lesions, mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, salivary gland [mandibular], sciatic nerve seminal vesicles, skeletal muscle, skin, spinal cord [cervical], spleen*, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, urinary bladder, uterus	At sacrifice
Toxicokinetics [orbital sinus N=3/sex/group/time point] – calculated based on trapezoidal rule, HPLC with UV detector	Days 1 and 28 – pre-dose, 1 min, 1, 2, 4, 8, 12, and 24 hours post dose

**These tissues only were examined in the low and mid dose groups, all tissues examined for vehicle and high dose groups

Results –

Mortality

- Four females in the toxicokinetic group died [2 each at 10 and 25 mg/kg/day] post-bleeding on Day 28. The Sponsor suggested that reduced oxygen carrying capacity 2° to blood loss and anemia was contributory.
- One female at 10 mg/kg/day died Day 24 after dosing. Cause of death was not determined.

Body Weight, Weight Gain, and Food Consumption

- Males in all treatment groups lost weight during Week 1 of dosing [range of –5.6 to -11.6 g]. Control rats gained weight. Body weight gain was also decreased by 24% in males at 25 mg/kg/day Days 7-28 compared to controls with the decrease primarily due to reduced weight gain Days 21-28. The weight loss and decreased body weight gain correlated with decreases in food consumption of 7-10%.
- Total body weight gain was decreased by 22% in the high dose females compared to control females. The decrease was primarily due to reduced weight gain Days 14-28. The decrease in body weight gain did not correlate with changes in food consumption. Females at 25 mg/kg in the recovery group lost weight and exhibited decreased food consumption [12%] for Days 43-50 only. A relationship to treatment for the change that was observed during the recovery period is questionable.

Ophthalmoscopic Exam [P. Blouin, DVM, Ph.D., ACVO]– All findings were considered incidental and not treatment-related.

Hematology

- **RBC Indices** - There were a number of perturbations in RBC indices in males at ≥10 mg/kg and in all treatment groups of females [≥2 mg/kg]. These changes were indicative of a regenerative anemia [e.g. decreases in RBC counts, Hb, Hct and increases in MCV, MCH, RDW, and reticulocyte counts]. The findings in males and females are outlined in the following tables.

WEEK 5 - MALES

	RBC $X10^6$	Hb G/DL	Hct %	MCV UM^3	MCH PG	MCHC G/DL	RDW %	RETIC %
GROUP 1 - VEHICLE CONTROL	7.89 .458	14.8 .49	43.8 1.81	54.4 1.98	18.8 .69	34.5 .93	12.6 .69	1.0 .40
GROUP 2 - LOW DOSE 2 MG/KG/DAY	7.44 .238	13.9 .87	48.5 2.39	54.4 2.28	18.7 .98	34.4 .70	12.4 .49	1.2 .75
GROUP 3 - INTERMEDIATE DOSE 10 MG/KG/DAY	6.86 B .652	14.2 1.23	39.3 B 3.38	57.4 1.83	20.7 B .60	36.1 B .46	14.0 B .93	7.5 4.67
GROUP 4 - HIGH DOSE 25 MG/KG/DAY	4.96 B .408	12.2 B .92	35.6 B 2.43	70.7 B 3.86	24.6 B 1.27	34.9 .48	15.2 B .98	35.9 E 18.00

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: B - P<.01 (DUNNETT'S) E - P<.001 (DUNN'S)

WEEK 5 - FEMALES

	RBC $\times 10^6$	Hb G/DL	Ht %	MCV UM^3	MCH PG	MCHC G/DL	RDW %	RETIC %
GROUP 1 - VEHICLE CONTROL	7.32 .249	14.2 .47	40.4 1.35	55.3 1.61	19.3 .52	35.0 .52	11.2 .51	1.5 .74
GROUP 2 - LOW DOSE 2 MG/KG/DAY	6.03 B .533	12.0 B 1.14	33.8 B 2.89	56.0 .85	19.8 .36	35.5 .79	11.6 .49	1.2 .80
GROUP 3 - INTERMEDIATE DOSE 10 MG/KG/DAY	5.52 B .590	11.8 B 1.19	32.4 B 3.40	58.7 B 1.70	21.4 B .68	36.4 B .51	13.6 B .67	8.2 3.15
GROUP 4 - HIGH DOSE 25 MG/KG/DAY	4.36 B .544	11.2 B 1.31	31.7 B 3.41	72.8 B 3.05	25.7 B .89	35.2 .58	14.3 B 1.53	22.0 E 7.22

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: B - P< .01 (DUNNETT'S)
E - P< .001 (DUNN'S)

There was also a 6-14% increase in platelet counts in the animals dosed at 25 mg/kg/day. There were no changes in coagulation parameters [e.g. PT and APTT].

Altered RBC morphology was also observed with an increase in the incidence and severity of polychromasia, anisocytosis, nucleated red blood cells, spherocytes, and crenation. The table below delineates these changes.

RBC Morphology	Dose [mg/kg]							
Incidence	0		2		10		25	
Severity	M	F	M	F	M	F	M	F
Polychromasia	10/10 1	10/10 1	10/10 1	8/8 1	10/10 2	9/9 2	10/10 3	2/8 2.5
Anisocytosis	4/10 1	2/10 1	6/10 1	3/8 1	10/10 2.3	9/9 1.6	10/10 1.8	8/8 2.5
Crenation	2/10 1	4/10 1	8/10 1	3/10 1	1/10 1	3/9 1	0 -	8/8 2
nRBC	2/10	0	1/10	0	4/10	0	6/10	1/8
Spherocytes	0	0	0	0	1/10	0	9/10	4/8

- **WBC Indices** – Elevated WBC counts were characterized by a neutrophilia, lymphocytosis, monocytosis, and eosinophilia in both males and females. These changes were most pronounced in the high dose animals but similar trends were observed at the mid dose. The findings are outlined in the tables below.

The following table represents results in the males.

WEEK 5 - MALES

	WBC $\times 10^3$	WBC DIFFERENTIAL COUNT (ABSOLUTE) NEUT SEG	NEUT NSEG	LYMPH	MONO	EOSIN
GROUP 1 - VEHICLE CONTROL	7.2 1.62	1239.8 420.80	0.0 0.00	5705.9 1646.91	188.0 120.46	96.3 105.02
GROUP 2 - LOW DOSE 2 MG/KG/DAY	6.9 2.45	1167.2 437.53	0.0 0.00	5378.2 2159.40	199.2 209.26	105.4 107.40
GROUP 3 - INTERMEDIATE DOSE 10 MG/KG/DAY	10.1 3.18	2068.4 882.06	0.0 0.00	7637.6 2589.79	248.6 240.27	135.4 169.21
GROUP 4 - HIGH DOSE 25 MG/KG/DAY	22.5 B 5.24	6259.6 E 2551.30	34.3 108.47	16357.2 B 3050.18	597.4 B 379.36	211.5 209.44

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: B - P <.01 (DUNNETT'S)
SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: E - P <.001 (DUNN'S)

The following table represents results in the females.

	WBC $\times 10^3$	WBC DIFFERENTIAL COUNT (ABSOLUTE) NEUT SEG	NEUT NSEG	LYMPH	MONO	EOSIN	BASO
GROUP 1 - VEHICLE CONTROL	4.5 1.86	811.7 583.29	0.0 0.00	3523.6 1590.13	94.1 77.37	46.0 50.57	4.6 14.55
GROUP 2 - LOW DOSE 2 MG/KG/DAY	4.6 1.71	748.6 455.55	0.0 0.00	3685.9 1480.85	75.8 57.25	39.8 39.23	0.0 0.00
GROUP 3 - INTERMEDIATE DOSE 10 MG/KG/DAY	7.7 4.55	1885.6 1683.43	0.0 0.00	5558.1 2794.12	123.6 111.07	177.2 259.75	0.0 0.00
GROUP 4 - HIGH DOSE 25 MG/KG/DAY	18.5 E 10.85	4560.30 4650.86	0.0 0.00	13317.4E 6032.11	462.5 666.90	122.4 188.47	0.0 0.00

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: D - P< .01 E - P< .001 (DUNN'S)

There was a dose-dependent decrease in the myeloid:erythroid ratio at ≥10 mg/kg/day in both males and females ranging from approximately 50-75% compared to control values. This change was a function of [1] erythroid hyperplasia; [2] a decrease in the number of band and mature neutrophils; and [3] a decrease in the number of lymphocytes.

Recovery Phase – RBC indices were slightly elevated [generally <15%] following the recovery period. With the exception of absolute neutrophil counts [segmented and nonsegmented] in the high dose females, the WBC indices were comparable across dose groups. The Sponsor indicates that the M:E ratios in the majority of the recovery animals were comparable to control values. However, 2 mid dose females, and 1 and 3 high dose male and females, respectively, had slight to marked increases in the M:E ratio. The myeloid:erythroid ratio was increased in high dose males [34%] and females [134%] compared to the control rats. The significance of this finding is not known.

Serum Chemistry – Bilirubin – There was a statistically significant increase in bilirubin in males at 10 and 25 mg/kg/day [0.41 ± 0.081 and 0.98 ± 0.18 mg/dl, respectively] and

in females at 25 mg/kg/day [0.69 ± 0.238 mg/dl] compared to control males and females [0.15 ± 0.029 and 0.19 ± 0.039 mg/dl, respectively].

The other changes observed were considered not to be related to treatment.

Recovery Phase – Bilirubin – In the high dose males, bilirubin was still increased by approximately 60%. Bilirubin concentrations were comparable across all groups of females.

Urinalysis – In both high dose males and females, there was a 2X increase in urine volume compared to the control animals. There was a correlation between the increase in volume and a decrease in SpG. There was also an increase in urobilinogen in high dose males [7.5 µmol/L] and females [10 µmol/L] compared to control males [5µmol/L] and females [3.2 µmol/L]. In addition, 3/15 and 0/15 high dose and control females, respectively, exhibited +1 bilirubinuria.

Recovery Phase – With the exception of an increase in urine volume in all treated males compared to controls [2.9, 11.1, 11.8, and 16.5 ml at 0, 2, 10, and 25 mg/kg/day, respectively], values were comparable across treatment groups.

Organ Weights –Spleen weights – There was a statistically significant increase in absolute and relative spleen weights following the 28-day exposure to BPD-MA in mid and high dose males and in high dose females when compared to controls. Weights were still elevated [statistically significant] in high dose males [45%] and females [10%] compared to control values following the recovery period. The table below outlines these changes.

Dose [mg/kg]	Spleen Weight [mg] ± S.D.							
	Treatment Period				Recovery Period			
	Male		Female		Male		Female	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
0	.83 ± .09	.18 ± .02	.51 ± .11	.23 ± .04	.80 ± .04	.16 ± .01	.57 ± .04	.25 ± .01
10	1.04 ± .14	.23 ± .03	.59 ± .11	.27 ± .05	.87 ± .17	.18 ± .03	.53 ± .08	.23 ± .03
25	2.28 ± .26	.52 ± .05	1.36 ± .21	.64 ± .09	1.17 ± .17	.24 ± .03	.62 ± .04	.27 ± .02

The relationship of other changes to treatment, including [1] approximately a 20% increase in absolute and relative thyroid weights in high dose males; [2] a decrease in absolute and relative prostate weights of approximately 10-15% in all treated males after the recovery period; and [3] approximately a 20-30% decrease in absolute and relative thymus weights in all treated males and high dose females compared to controls, is not known. The absolute and relative thymus weights in mid dose females were increased by approximately 15% compared to controls.

Necropsy – The primary finding following 28 days of treatment of BPD-MA was splenic enlargement which occurred in 0/10, 3/10 and 10/10 males at 0, 10, and 25 mg/kg and in 0/10 and 8/10 females at 0 and 25 mg/kg. Following the recovery period, 2/5 high dose males still exhibited splenic enlargement. The relationship of the following findings to treatment are not known: [1] discolored digesta in 1/10 and 2/10 males and females, respectively, at 25 mg/kg; [2] small thymus in 1/10 males at 10 and 25 mg/kg each; and [3] dark areas on the ovaries of 3/5 females in high dose recovery group.

Histopathology- Lesions occurred primarily in the spleen, liver, kidneys, and at the injection site. The incidence and the average severity of the lesions are delineated in the table below. Generally, lesions were graded slight to mild.

Organ/Lesion [N=10] Incidence [Severity]	Dose [mg/kg]							
	0		2		10		25	
	M	F	M	F	M	F	M	F
Injection Site								
Fibrosis	1	4	6	2	3	3	2	4
Perivasculitis	1 [1]	5 [1.2]	7 [1.3]	8 [1.2]	4 [1.2]	9 [1.2]	6 [1.5]	9 [1.7]
Vasculitis	-	-	1 [2]	3 [1]	1 [1]	3 [1.3]	2 [1]	3 [1.3]
Thrombosis	-	1 [1]	-	4 [1.2]	-	2 [1]	1 [2]	5 [1.2]
Kidney								
Tubular pigment accumulation	-	-	-	-	-	-	6 [1]	8 [1]
Liver								
Extramedullary hematopoiesis	-	-	-	-	2 [1]	-	9 [1]	10 [1]
Sinusoidal cell pigment accumulation	-	-	-	-	5 [1]	4 [1]	6 [1]	9 [1]
Lymph node, mandibular								
Congestion and/or erythrophagocytosis	-	-	5	6	3	3	-	-
Spleen								
Extramedullary hematopoiesis	-	-	-	-	6 [1]	5 [1.2]	10 [2]	10 [2.2
Pigment accumulation	-	-	-	-	-	-	-	1 [1]
Lung								
Granuloma	5	4	-	2	-	1	7	8

Perivasculitis, vasculitis, and thrombosis at the injection site; and renal, splenic, and hepatic pigment accumulation were observed at recovery. However, the incidence and severity were generally decreased compared to animals sacrificed Day 28.

Reproductive Parameters

- **Estrous Cycle** – There were no treatment-related effects.

- **Sperm Analysis** – There were no treatment-related effects. There was a single male at the high dose [1/10] that had an increase in % abnormal sperm morphology [app. 4X the average for the control animals].

Toxicokinetics – T_{max} was observed at the first sampling time point [app. 1 minute]. There was a greater than dose-proportional increase in exposure based on $AUC_{0.02\text{-}24\ hr}$ in the mid dose on Day 28 and in the high dose on Days 1 and 28. This is indicative of a decrease in total body clearance. There tended to be an approximately 20-40% greater exposure in males than females although the C_{max} in females was approximately 30% greater compared to males. Values on Day 1 and 28 were comparable indicating that drug did not accumulate with repeated dosing. The table below outlines the C_{max} and $AUC_{0.02\text{-}24\ hr}$ at both time points in males and females.

Group	Dose Level (mg/kg/day)	Sex	Dose Factor[1]	C_{max}		$AUC_{0\text{-}t_{24}}$	
				Day 1 ([2])	Day 28 ([2])	Day 1([2])	Day 28 ([2])
2	2	M F	1 1	14.340 (1) 18.630 (1)	12.323 (1) 10.730 (1)	23.20 (1) 18.23 (1)	21.13 (1) 14.57 (1)
3	10	M F	5 5	71.733 (5.0) 91.267 (4.9)	118.600 (9.6) 74.997 (7.0)	147.61 (6.4) 107.56 (5.9)	168.97 (8.0) 106.37 (7.3)
4	25	M F	12.5 12.5	200.260 (14.0) 254.920 (13.7)	273.070 (22.2) 224.470 (20.9)	441.13 (19.0) 371.80 (20.4)	496.70 (23.5) 376.43 (25.8)

[1] Groups 3 and 4 dose level divided by Group 2 dose level.
[2] Values obtained by dividing the calculated pharmacokinetic parameter by the corresponding Group 2 value.

Reviewer's Comments – Study Design and Data Presentation

1. On p. 12, the Sponsor stated that animals were replaced – 8 on Day 1, 4 on Day 6, and 3 on Day 7 – due to difficulty in dosing the animals. Although one can determine which animals in each group were replaced based on numbering, it was not clear when they were replaced. The Sponsor was asked to clarify this and to indicate how many doses the replaced animals received.
2. In Appendix 19, the SOP compliance statement for formulation analysis was not signed.
3. Based on changes in the M:E ratio, no histopathological lesions in the bone marrow were anticipated. However, there were no changes described in the marrow evaluation.

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

1. A greater than dose proportional increase in exposure in high dose animals on Days 1 and 28 and in mid dose animals on Day 28 suggested a decrease in systemic clearance of BPD-MA. **Reviewer's Comment** - The Reviewer concurs. In addition, a slightly greater exposure based on AUC was observed in males and a slightly higher C_{max} was observed in females. The AUC values were comparable on Days 1 and 28 indicating that drug did not accumulate following repeated exposure.
2. At doses of ≥2 mg/kg, there were "transitory losses in body weight and mild dose-related anemia" characterized by alterations in RBC indices, erythroid hyperplasia, splenic enlargement, extramedullary hematopoiesis, increased total bilirubin, increases in white blood count, and pigment accumulation in the renal cortical epithelium, hepatic cells and sinusoids. These changes indicate a regenerative anemia. Based on these findings, the NOAEL for this study would be <2 mg/kg. **Reviewer's Comment** – In general, the Reviewer concurs. The anemia is considered to be secondary to hemolysis. In addition, the increase in urine volume and urinary urobilinogen would be consistent with hemolysis.

Additional Comments –1. The values for exposure based on AUC to BPD-MA on Day 1 in the 28-day study are approximately 50-80% greater than the sum of the two regioisomers in the 14-day rat study at comparable doses. The Sponsor was asked to discuss the potential source[s] of this difference.

B. Dog

a. Title: <u>An intermittent, multiple dose study of benzoporphyrin derivative monoacid (a photodynamic anti-cancer agent) given IV to beagle dogs [Ref. 314]</u>
Study Identification: 90064

QLT Phototherapeutics, Inc.

Site:

Study Dates: Dosing initiated Jan. 11, 1991

Formulation and Lot No. – BPD-MA – Batch # H90-120-0123; dosing solutions were prepared on the day of dosing; HPLC analysis was conducted on frozen samples taken on first and last day of dose preparation;

Vehicle – liposomal solution/BPD-MA carrier and a saline control

Certificate Analysis: Yes (X)

Final Report: May 24, 1991

GLP and QA Statements Signed: Yes (X)

Objective: "To identify potential toxicity in dogs given multiple intravenous doses of BPD-MA followed by exposure to activating light"

Dr. Will Coulter previously reviewed this study for IND Submission final review; pp. 18-20 [toxicology] and pp. 7-8 [toxicokinetics]. Additional comments by the current Reviewer [in italics] are provided below.

1. *Ophthalmic examinations were conducted predose and after the 3rd dose.*
2. *The organs listed in the review are those which were weighed. Histopathology was conducted on light application site, injection site, skin, skeletal muscle, mammary gland, salivary glands, thyroid and parathyroid, tonsils, tongue, heart, aorta, trachea, lungs, esophagus, stomach, small and large intestine, pancreas, liver, gall bladder, spleen, lymph nodes, thymus, bone marrow, bone [rib], sternum, adrenals, kidneys, ureters, urinary bladder, urethras, prostate, testes, epididymides, ovaries, uterus, vagina, pituitary gland, brain, spinal cord, peripheral nerves, and eyes in the saline controls, liposomal controls, and high dose dogs.*
3. *Clinical observations – Transient erythema was observed in 1/8 liposomal control dogs. [Table I (summary) indicates 2 animals; Appendix II (individual animal) indicates 1 animal]. No lesions at the irradiated site were observed at 0.1 mg/kg + 50 J/cm^2.*
4. *Body Weight – Females in groups 2, 4 and 5 lost weight between Days 8 and 12 [approximately 70, 200 and 100 grams, respectively] while the dogs in the other 2 groups gained weight over the same time period. The weight loss was accompanied by a reduction in food consumption. In males, however, the saline and liposomal control, and Group 2 dogs lost weight [approximately 20-50 gm] compared to Group 4 and 5 dogs which gained weight. Food consumption was comparable across all groups. Due to the magnitude of the weight loss, it is of questionable toxicological significance.*
5. *There were no treatment-related effects on urinalysis or ophthalmologic changes.*
6. *Clarification of organ weight changes – With the exception of thymus weights in females, these changes were considered unrelated to treatment due to a lack of a dose-dependent response. The relationship of the decrease in thymus weights in females to drug treatment is not clear. The magnitude of the changes in brain weight is considered to be secondary to interanimal variability.*

Organ		Liposome		0.1 mg/kg		0.2 mg/kg		0.7 mg/kg	
		Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Thymus	M	↑27%	↑24%	↓29%	↓31%	↓48	↓48	↑17%	↑15%
	F	↓10%	↓5%	↓33%	↓32%	↓30%	↓15%	↓27%	↓20%
Adrenals	M	-	-	-	-	↑49%	↑46%	-	-
Brain	F	-	-	-	-	↑4%	↑18%	↑8%	↑15%
Heart	F	-	-	-	-	↑4%	↑18%	-	-

b. Title: An escalating dose intravenous toxicity study of CL 318,952 [Benzoporphyrin derivative monoacid ring A, A photodynamic therapy agent] in dogs [Ref. 318]
Study Identification: 93031
Site:
Study Dates [in-life]: March 29 – April 30, 1993
Formulation and Lot No. –liposomal BPD-MA- Batch # J90-120-0182; dosing solutions were prepared on each day of dosing; HPLC analysis was conducted on frozen samples taken on March 29, April 5, 12, and 26, 1993;
Vehicle – 10% lactose in sterile water
Test and control solutions were prepared on each day of dosing
Certificate Analysis: Yes (X)
Final Report: Aug. 13, 1993
GLP and QA Statements Signed: Yes (X)
Objective: "To establish the tolerability and overt toxicity of formulated CL 318,952 when given intravenously to dogs"

Dr. Oluwadare M. Adeyemo previously reviewed this study for IND Submission final review; pp. 15-16. Additional comments by the current Reviewer [in italics] are provided below.

1. Clarification on study design

- *Clinical pathology sampling times were predose, Days 3, 10, 17, and 31 [e.g. approximately 24 hours post 3rd dose at each dose level]*
- *Coagulation parameters were assessed predose and Day 31*
- *Urinalysis was performed on samples collected predose, and Days 15 and 29*
- *Hemolysis was evaluated on Days 0, 1, 7, 14, 16 and 28 with several samples collected on each day – due to discoloration of serum it was not always possible to assess this parameter*
- *No organ weights were obtained*
- *Tissue samples were collected and preserved but were not evaluated*

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2. Toxicokinetic Results – The table below delineates the PK data.

BPD-MA Dose (mg/kg)	Cmax (µg/mL)	AUC(0-24) (µg*hr/mL)	Ratio AUCo-inf/ Dose	Dose Normalied CL(t) (mL/min/kg)	Dose Normalied Vss (L/kg)	T½ (hr)
			CL 315,555			
0.2	1.3 ±0.5	nd	nd	nd	nd	nd
1	4.2 ±0.8	3.0 ±0.3	3.0	2.76 ±0.26	0.26 ±0.03	1.6 ±0.1
5	17.1 ±2.3	17.2 ±1.9	3.3	2.45 ±0.25	0.28 ±0.04	1.7 ±0.2
10	26.0 ±6.1	34.3 ±5.9	3.2	2.48 ±0.40	0.34 ±0.07	1.8 ±0.2
			CL 315,585			
0.2	1.5 ±0.5	1.4 ±0.3	6.8	1.28 ±0.27	0.22 ±0.08	2.7 ±0.4
1	5.0 ±0.8	5.3 ±0.5	5.4	1.57 ±0.16	0.32 ±0.04	3.2 ±0.2
5	24.1 ±3.0	34.2 ±2.5	7.2	1.17 ±0.09	0.36 ±0.07	5.2 ±0.3
10	37.8 ±7.6	75.1 ±12.8	7.5	1.10 ±0.17	0.38 ±0.06	5.1 ±0.4

CL(t) and Vss values corrected for contribution (~50%) of each regioisomer
Note = Since the dosing solution concentration and rate of dosing were kept constant across dose groups, the duration of dosing increased as dose increased; (average duration of dosing was 12 seconds, 0.9 min., 4.3 min and 8.9 min)

c. A two-week intravenous toxicity study of CL 318,952 [Benzoporphyrin derivative monoacid ring A], A photodynamic therapeutic agent, in dogs [Ref. 319]

Study Identification: TX-93004

Site: [redacted]

Study Dates [in-life]: June 19 – July 16, 1993

Formulation and Lot No. – liposomal BPD-MA- Batch #K92-120-0794; L92-120-0809; D93-120-0850; E93-120-0870; E93-120-0876; D93-120-0864; E93-120-0877; dosing solutions were prepared on the day of dosing or within 24-72 hours of dosing; HPLC analysis was conducted on frozen samples taken on June 28 and July 2, 1993; [redacted]

Vehicle – 10% lactose

Certificate Analysis: Yes (X) [redacted]

Final Report: April 11, 1994

GLP and QA Statements Signed: Yes (X)

Objective: "To evaluate the potential antemortem and target organ toxicity of CL 318,952 in dogs dosed intravenously for 2 weeks"

Study Design