CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-119

PHARMACOLOGY/TOXICOLOGY REVIEW

N21-119 VISUDYNE

QLT Phototherapeutics, Inc.

Review and evaluation of Pharmacology and Toxicology Data

Division of Analgesics, Anti-inflammatory, and Ophthalmic Drug Products
HFD-550
Reviewer: Susan D. Wilson, D.V.M., Ph.D.

NDA Number: N21-119

Serial Number
Letter Date: August 14, 1999
Type of Submission: Original NDA

Information to Sponsor: Yes(X) No ( )

Completion Date: November 30, 1999

Sponsor or Agent:

QLT PhotoTherapeutics Inc
520 West 6th Avenue
Vancouver, British Columbia
Canada V5Z 4H5
Contact: David Mitchell Phone - (604) 872-7881
Fax - (604) 707-7373

U.S. Representative:
Jonathan Kahan Phone – (202) 637-5600
Fax - (202) 637-5109
Hogan & Hartson
555 Thirteenth St., N.W.
Washington, D.C. 20004-1109

Manufacturer (if different) for drug substance:

Drug name:
1° - VISUDYNE™
2° - verteporfin
3° - BPD-MA
4° - benzoporphyrin derivative monoacid ring A
5° - CL 318,952

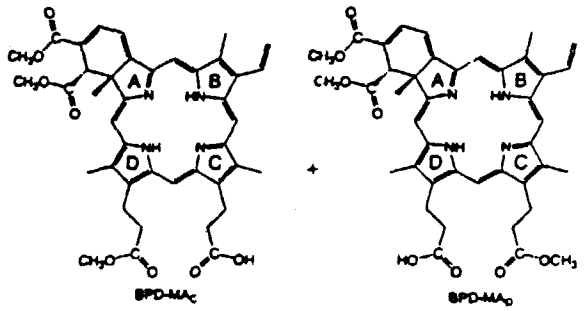
Chemical Name: 1:1 mixture of the following regioisomers

BPD-MA$_C$ - 9-methyl trans-(±)-18-etheneyl-4,4a-dihydro-3,4-bis(methoxycarbonyl)-4a,8,14,19-tetramethyl –23*H*, 25*H*-benzo(b)porphine-9,13-dipropanoate

BPD-MA$_D$ - 13-methyl trans-(±)-18-etheneyl-4,4a-dihydro-3,4-bis(methoxycarbonyl)-4a,8,14,19-tetramethyl –23*H*, 25*H*-benzo(b)porphine-9,13-dipropanoate

CAS Number (if provided by sponsor): Not provided

Structure: $C_{41}H_{42}N_4O_8$



Molecular Weight:718.814

Relevant IND/NDA/DMF:

Drug Class: Photodynamic therapeutic agent

Indication: For the treatment of choroidal neovascularization associated with age-related macular degeneration

Clinical Formulation (and components): 15 mg/vial that is reconstituted with sterile water for injection [USP] to a concentration of 2 mg/ml. This is further diluted with 5% dextrose to yield an appropriate dose

Components/Excipients	Concentration (mg/vial)
Verteporfin – active ingredient	15
Butylated hydroxytoluene – antioxidant	
Ascorbyl palmitate – antioxidant	
Egg phosphatidylglycerol (IV) – solubilizing agent	
Dimyristoyl phosphatidylcholine (IV) –solubilizing agent	
Lactose monohydrate (NF) – lyoprotectant and osmolarity adjustment	

Route of Administration: intravenous infusion

Proposed Clinical Protocol: None provided

Studies Reviewed within this submission:

Report No.	Report Date	Study Title	Test Material Lot
		PHARMACOKINETIC STUDIES	
In Vitro Studies			
PH-97006	Sept. 25, 1997	Uptake and Release Kinetics of the Photosensitizers Liposomal BPD-MA, NC0060, and NC0069 [Ref. 39]	92-902-017
PH-93013	Feb. 28, 1993	Kinetics of Uptake and Release of Liposomal [L-BPD] and Aqueously [A-BPD] Formulated BPD by Tumor and Normal Cells In Vitro [Ref. 36]	Not provided
PH-97009	Dec. 17, 1997	Demonstration of the Transient Existence of Liposomes in the Presence of Plasma [Ref. 46]	Not provided
PH-94024	Feb. 8, 1995	The Distribution of BPD-MA and Liposomal Lipid Among Plasma Proteins [Ref. 47]	Lot # CB2-128, 132, 133, 134, and Powder, QL 1381
PH-151090	Dec. 17, 1990	Tissue Distribution of Radioactivity Following a Single Intravenous Administration of ^{14}C-BPD-MA in the Mouse [Ref.284]	Not provided
In Vivo Studies			
Ocular Pharmacokinetics			
Rabbit Eye			
PH-94020	Dec. 1994	Ocular Distribution of BPD in the Rabbit Eye [Ref. 89]	Batch # J93-120-0883 or E93-120-0870
Systemic Pharmacokinetics			
Mouse			
PH-151090	Dec. 17, 1990	Tissue Distribution of Radioactivity Following a Single Intravenous Administration of ^{14}C-BPD-MA in the Mouse [Ref.284]	Not provided
Rat			
TX-94038	Mar. 4, 1994	Mass Balance and Excretion of Radioactivity Following a Single Intravenous Dose of ^{14}C-CL 318,952 [BPD-MA] in the Rat [Ref. 288]	D93-1200850
A9301	Dec. 13, 1993	Placental Transfer of BPD-MA [CL 318,592] After a Single Intravenous Dose to Pregnant Rats [Ref. 345]	^{14}C-BPD-MA L921203A
PH-011090	Dec. 14, 1990	Excretion and Mass Balance of Radioactivity Following a Single Intravenous Administration of 4.0 mg/kg ^{14}C- BPD-MA in the Rat [Ref. 354]	Not provided
PK-98001	May 7, 1991	An Intravenous Single Dose Pharmacokinetic Study of Verteporfin in Sprague-Dawley Rats Including Assessment of BPD-DA and Enantiomer Ratios [Ref. 361]	Lot No. TC0715
Monkeys			
PK-94001	Oct. 18, 1994	A Single Dose Intravenous Pharmacokinetic Study of Liposomal Benzoporphyrin Derivative Monoacid Ring A [BPD-MA; CL-318,952] in Male Cynomolgus Monkeys [Ref. 334]	F93-120-0880
PH-94023	Dec. 12, 1994	Pharmacokinetic Studies in Pigtail Macaque Monkeys [Macaca nemistrina], Following Fast Infusion of Liposomal BPD-MA [Ref. 363]	F93-120-0887
Pig			
TX-94029	Jan. 30, 1995	Plasma Clearance of Liposomal BPD in Yucatan Micro Swine [Ref. 362]	Not provided

Report No.	Report Date	Study Title	Test Material Lot
Metabolism			
In Vitro			
PK-98002	Dec. 10, 1998	Metabolism of BPD-MA [Verteporfin] by Human Liver S9 and Microsomes[Ref. 285]	Not provided
PH-0592	May 1992	Species Differences in the Metabolism of BPD-MA [Ref. 286]	Not provided
PK-93002		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]	H92-902-017
PK-98003		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]	H92-902-017
Comparison of Activity – Drug substance/product, Regioisomers, Enantiomers, BPD-DA			
PH-92006	Nov. 3, 1992	A Comparison Between BPD-MA and its Two Regioisomers with respect to Dark Toxicity In Vitro [Ref. 104]	Not provided
PH-92013	Oct. 28, 1992	Comparative Studies of the Activity of Benzoporphyrin Derivative Monoacid ring A [CL318,952] and its Regioisomers [CL315,585 and CL 315,555] *In Vivo* and *In Vitro* [Ref. 105]	Not provided
PH-92005	Nov. 3, 1992	A Comparison Between BPD-MA and Its Two Regioisomers with Respect to Photoactivation *In Vitro* [Ref. 109]	Not provided
91006	Apr. 11, 1991	Photosensitizing Potency of Two Regioisomers of Benzoporphyrin Derivative *In Vitro* [Ref. 110]	Not provided
91004	Mar. 31, 1991	A Comparative Study of the Effects of Two Regioisomers of BPD-MA Given I.V. to Tumor-Bearing Mice [Ref. 112]	Not provided
92007	Dec. 15, 1992	A Preliminary Study Comparing the Skin Photosensitizing Effects of BPD-A and Its Two Regioisomers [Ref. 113]	Not provided
92010	Dec. 5, 1992	A Single Dose Comparative Study of the Anti-Tumor Efficacy of Liposomal BPD-MA and its Regioisomers [Ref. 114]	Not provided
PH-93031A	Apr. 29, 1994	Comparison Between BPD-NMA and Its 4 Enantiomers with Respect to Photoactivation *In Vitro* [Ref. 115]	QL1794-13-10
Drug-Interactions			
PH-97008	Jan. 1999	In Vitro Study of Drug Interaction in Human Plasma Between Methotrexate and BPD-MA [Ref. 290]	Batch Nos. CX08-47, CX08-49, CX08-51
PHARMACOLOGY STUDIES			
Light Activation			
91002	April 1, 1991	The Photosensitivity Action Spectrum of Liposomal BPD-MA [Ref. 62]	Not provided
Monkeys			
TX-99005	April 21, 1999	Imaging of Experimental Choroidal Neovascularization by Liposomal BPD-MA Angiography [Ref. 61]	Not provided
SAFETY PHARMACOLOGY			
***In Vivo* Studies**			
TX-97007	May 13, 1997	General Pharmacology of L-BPD-MA [Ref. 99]	G93-120-0891

Report No.	Report Date	Study Title	Test Material Lot
Dog			
36624	Apr. 23, 1991	Hemodynamic Effects of Benzoporphyrin Derivative-Monoacid Ring A [BPD-MA] Cryodessicated Powder in Anesthetized Dogs [Ref. 120]	Not provided
PH-93032	Nov. 7, 1994	Cardiovascular Effects of Benzoporphyrin Derivative Monoacid Ring A [BPD-MA], a Photodynamic Therapy Agent, without Photoactivation in the Beagle Dog [Ref. 121]	F93-120-0880
PH-94004	Nov. 11, 1994	Cardiovascular Effects of Benzoporphyrin Derivative Monoacid Ring A [BPD-MA], A Photodynamic Therapy Agent, Without Photoactivation in the Anesthetized Beagle Dog [Ref. 122]	F93-120-0880
Pig			
PH-94001	May 18, 1994	Preliminary Investigations on the Effects of Bolus Injection and Infusion of Liposomally Formulated BPD and Its Constituents [Ref. 123]	J90-120-0883
TX-94025	Dec. 21, 1994	Safety Assessment of Liposomal BPD-Ma Verteporfin to Support Fast Infusion in Humans [Ref. 124]	Not applicable
PH-94021	May 1, 1994	Studies of Complement Interaction with a Liposomal Formulation of BPD [Ref. 125]	Not provided
TX-94007	Nov. 9, 1994	Cardiovascular Effects After a 45 Minute Infusion of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent without Photoactivation in the Conscious Micro Swine [Ref. 126]	F93-120-0883
TX-94009	Nov. 9, 1994	Cardiovascular Effects After an Intravenous Bolus of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent without Photoactivation in the Conscious Micro Swine [Ref. 127]	F93-120-0883
TX-94021	Sept. 21, 1994	Complement Activation in Montreal Pigs [Ref. 128]	F93-120-0883
TX-94010	Nov. 8, 1994	Cardiovascular Effects After an Intravenous Bolus of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent without Photoactivation in the Conscious Micro Swine [Ref. 129]	F93-120-0883
TX-94014	Dec. 20, 1994	Cardiovascular Effects After a 45 Minute Infusion and/or Bolus Injection of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent without Photoactivation in the Anesthetized Micro Swine [Ref. 130]	F93-120-0883
TX-94018	Dec. 29, 1994	Cardiovascular Effects After a Rapid Intravenous Bolus of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent without Photoactivation in the Conscious Micro Swine [Ref. 131]	R1186-92
PH-94022	Nov. 25, 1994	Effect on Complement Level [CH50] of Rapid Bolus IV Injection of BPD-MA Samples from BioResearch [Ref. 132]	Not provided
TOXICOLOGY STUDIES			
Ocular Toxicity Studies			
Nonhuman Primates			
TX-97001	Not provided	Light Only Treatment to the Normal Retina and Choroid in the Cynomolgus Monkey [Ref. 333]	Not provided
TX-96008	Not provided	Photodynamic Therapy Retreatment of Normal Retina and Choroid in the Cynomolgus Monkey [Ref. 95]	Not provided

Report No.	Report Date	Study Title	Test Material Lot
TX-94027	Dec. 28, 1994	Pre-Clinical BPD-MA Pharmacology Study for Macular Degeneration [Ref. 94]	PC1256 MGH Pharmacy R-1186-102 R-1186-192 PQ002-94
Dogs			
TX-93003	Aug. 4, 1994	A Single Dose Intravenous Retinal Toxicity Study in Dogs of Liposomal Benzoporphyrin Derivative Monoacid A [BPD-MA; CL-318,952] [Ref. 330]	F93-120-0880
Systemic Toxicity Studies			
Acute Studies			
Rat			
90060	May 23, 1991	A Range-Finding Study of Benzoporphyrin Derivative Monoacid (a Photodynamic Anticancer Agent) Given I.V. to Male Rats [Ref. 316]	H90-120-0123
90225	May 22, 1991	A single dose study of benzoporphyrin derivative monoacid (an anticancer agent) given intravenously to rats [Ref. 310]	H90-120-0123
90061	May 23, 1991	A single dose study of benzoporphyrin derivative monacid (a photodynamic anticancer agent) given IV to rats [Ref. 311]	H90-120-0123
Dog			
90063/ 90235	May 30, 1991	A single dose toxicity study of benzoporphyrin derivative monoacid (a photodynamic anticancer agent) given IV to beagle dogs [Ref. 309]	H90-120-0123
Repeat Dose Studies			
Rats			
90237/ 90241	May 24, 1991	An intermittent, multiple dose study of benzoporphyrin derivative monoacid (a photodynamic anticancer agent) given IV to rats [Ref. 313]	H90-120-0123
92020/ 92052	June 17, 1993	A two-week intravenous toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rats [Ref. 312]	J90-120-0175 J90-120-0182
TX-96010	Nov. 17, 1998	A 28-Day Intravenous Injection Toxicity Study [With a 28-Day Recovery] of Benzoporphyrin Derivative Monoacid [BPD-MA] in the Albino Rat [Ref. 317]	PQ009-96
Dogs			
93031	Aug. 13, 1993	An Escalating Dose Intravenous Toxicity Study of CL 318,952 [Benzoporphyrin Derivative Monoacid Ring A, A Photodynamic Therapy Agent] in Dogs [Ref. 318]	J90-120-0182
90064	May 24, 1991	An intermittent, multiple dose study of benzoporphyrin derivative monoacid (a photodynamic anti-cancer agent) given IV to beagle dogs [Ref. 314]	H90-120-0123
TX-93004	April 11, 1994	A Two-Week Intravenous Toxicity Study of CL 318,952 [Benzoporphyrin Derivative Monoacid Ring A], A Photodynamic Therapeutic Agent, in Dogs [Ref. 318]	K92-120-0794 L92-120-0809 D93-120-0850 E93-120-0870 E93-120-0876 D93-120-0864 E93-120-0877
In Vitro			
TX-94015	June 24, 1994	Direct Hepatotoxicity of BPD Verteporfin [BPD-MA in Human Liver Slices Cultured *In Vitro* [Ref. 336]	Not provided

Report No.	Report Date	Study Title	Test Material Lot
		REPRODUCTIVE TOXICOLOGY	
Rats			
TX-96009	Dec. 22, 1998	An Intravenous Fertility Study of Benzoporphyrin Derivative Monoacid (BPD-MA) in the Rat [Ref. 340]	TC0715
3151.11	Feb. 14, 1994	An Intravenous Range-Finding Developmental Toxicity Study of CL 318,952 [Benzoporphyrin Derivative Monoacid, A Photosensitizer for Photodynamic Therapy] in Rats [Ref. 344]	H92-902-017
TX-93002	Sept. 30, 1994	An Intravenous Developmental Toxicity Study of CL 318,952 (Benzoporphyrin Derivative Monoacid, A Photosensitizer for Photodynamic Therapy) in Rats [Ref. 341]	E93-120-0877
TX-98003	Mar. 29, 1999	An Intravenous Pre and Postnatal Study of Benzoporphyrin Derivative Monoacide [BPD-MA] in the Rat [Ref. 347]	TC0175
Rabbits			
3151.3	Mar. 29, 1994	An Intravenous Range-Finding Developmental Toxicity Study of CL 318,952 [Benzoporphyrin Derivative Monoacid, A Photosensitizer for Photodynamic Therapy] in Rabbits [Ref. 345]	H92-902-017
TX-93001	Sept. 1, 1994	An Intravenous Developmental Toxicity Study of CL 318,952 (Benzoporphyrin Derivative Monoacid, A Photosensitizer for Photodynamic Therapy) in Rabbits [Ref. 342]	E93-120-0877
		GENOTOXICITY	
90077	May 10, 1991	Ames/Salmonella-E. coli platelincorporation Assay on Benzoporphyrin Derivative [BPD-MA; CL 315,555/315,585] With and Without Metabolic Activation and With and Without Light Irradiation [Ref. 348]	PC1094
90078	May 9, 1991	Test for Induction of Unscheduled DNA synthesis in Rat Primary Hepatocyte Cultures by Benzoporphyrin Derivative Monoacid [BPD-MA; CL 315,555/315,585] With and Without Light Irradiation [Ref. 349]	PC1094
90080	May 9, 1991	Test for Induction of Gene Mutation at the HGPRT Locus in cultured Chine Hamster Ovary [CHO] cells by Benzoporphyrin Derivative Monoacid [BPD-MA; CL 315,555/315,585] With and Without Metabolic Activation and With and Without Light Irradiation [Ref. 350]	PC1094
90081	May 15, 1991	Test for Induction of Chromosome Aberration in cultured Chine Hamster Ovary [CHO] cells by Benzoporphyrin Derivative Monoacid [BPD-MA; CL 315,555/315,585] With and Without Metabolic Activation and With and Without Light Irradiation [Ref. 351]	PC1094
90079	May 31, 1991	Micronucleus Test on Benzoporphyrin Derivative, Monoacid [BPD-MA; CL 315,555/315,585] Administered Intravenously to Male Mice With and Without Light Irradiation [Ref. 352]	H90-120-123
98008	April 13, 1999	Test for Chemical Induction of Gene Mutation at the HGPRT Locus in cultured Chine Hamster Ovary [CHO] cells by Benzoporphyrin Derivative Monoacid [BPD-MA; CL 315,555/315,585] With and Without Metabolic Activation With a Confirmatory Assay [Ref. 353]	TC0992

Report No.	Report Date	Study Title	Test Material Lot
		SPECIAL TOXICOLOGY	
PHOTOTOXICITY			
Mice			
90223	May 23, 1991	A Single Dose Range-Finding Study of Benzoporphyrin Derivative Monoacid [a Photodynamic Anticancer Agent] Give I.V. to Male Mice, Followed by Exposure to Light from a Solar Simulator [Ref. 325]	H90-120-0123
90058	May 24, 1991	A Single Dose Range-Finding Study of Benzoporphyrin Derivative Monoacid [a Photodynamic Anticancer Agent] Give I.V. to Male Mice, Followed by Exposure to Light from a Solar Simulator [Ref. 328]	H90-120-0123
90059	May 23, 1991	A Single Dose Phototoxicity Study of Benzoporphyrin Derivative Monoacid [a Photodynamic Anticancer Agent] Give I.V. to Mice, Followed by Exposure to Light from a Solar Simulator [Ref. 326]	H90-120-0123
Pigs			
PH-93002	June 4, 1993	Development of Skin Photosensitivity Following IV Administration of BPD-MA to Normal Pigs [Ref. 323]	R1186-101
EVALUATION OF IMMUNE EFFECTS			
PH-95011R	Mar. 6, 1996	Assessment of Anti-BPD Immunity in Mice Treated with Liposomal BPD Verteporfin [137]	L90-120-0216 E93-120-0878
PH-95010	Sept. 28, 1995	Immunogenicity of BPD-MA – Studies in Rabbits [Ref. 138]	E93-120-0878
TX-99001	Jan. 9, 1999	Antigenicity Study of Liposomal BPD-MA [verteporfin] in Guinea Pigs [Ref. 140]	TC0715
PH-94026	-	Effect of BPD-MA [Verteporfin] on a Murine Model of Delayed Type Hypersensitivity [Ref. 253]	Lot # N-011-1 Lot # N-011-13 H92-902-017
PH-96001	Jan. 13, 1997	The Effect of BPD-MA Activated by UVA Light on Contact Hypersensitivity to 2,4, Dinitrofluorobenzene [DNFB] in Mice [Ref. 254]	E93-120-0878 H92-902-017
PH-97005	Aug. 1, 1997	The Effect of BPD-MA Activated by Broad Spectrum Light 24 Hours Post Injection on Contact Hypersensitivity to 2,4, Dinitrofluorobenzene [DNFB] in Mice [Ref. 255]	H92-902-017
TX-94019	May 1, 1995	Studies on the Effect of BPD-MA on Primary and Secondary Immune Responses to Soluble Protein Antigens [Ref. 266]	H92-902-017
TX-94037	Sept. 28, 1994	Effects of BPD on Parameters of the Immune Response in Naïve Mice in the Presence or Absence of Light Applied Transdermally [Ref. 267]	H92-902-017
In Vitro Compatibility			
90057	Mar. 6, 1991	In Vitro Human Blood Compatibility of CL-315,555/315,585 Benzoporphyrin Derivative Monoacid; [A Photodynamic Anti-Cancer Agent] [Ref. 337]	H90-120-0123
Stability,			
91067	May 24, 1991	Homogeneity, Stability and Dosing System Compatibility of CL 315, 555/315,585 [BPD-MA] Formulation Reconstituted with Sterile Water for Injection USP [Ref. 338]	H90-120-0123
92053	Mar. 18, 1994	Homogeneity, Stability and Dosing System Compatibility of CL 318,592 [Benzoporphyrin Derivative Monoacid] in 5% Dextrose Injection USP [Ref. 339]	J90-120-0175

Literature Reviewed:

Pharmacology

1. **Ref. 36:** Waterfield, E. M., et. al. [1994]. Wavelength-dependent effects of benzoporphyrin derivative monoacid ring A *in vivo* and *in vitro*. *Photochem. Photobiol.* **60[4]**:383-387.
2. **Ref. 11:** Fernandez, J.M., et. al. [1997]. Singlet oxygen generation by photodynamic agents. *J. Photochem. Photobiol. B. Biology* **37**:131-140.
3. **Ref. 19:** Hadjur, C., et. al. [1997]. EPR and spectrophotometric studies of free radical [O_2•, •OH, BOD-MA•] and singlet oxygen [$^1O_2$] generated by irradiation of benzoporphyrin derivative monoacid ring A. *Photochem. Photobiol.* **65[5]**:818-827.
4. **Ref 21:** Aveline, B., et. al., [1994]. Photophysical and photosensitizing properties of benzoporphyrin derivative monoacid ring A [BPD-MA]. *Photochem. Photobiol.* **59[3]**: 328-335.
5. **Ref. 118:** Gillies, r., et. al. [1996]. Spectral characterization of the benzoporphyrin derivative monoacid ring-A photoproduct formed in fetal calf solution during irradiation with 694 nm continuous-wave radiation. *J. Photochem. Photobiol.* **33**:87-90.
6. **Ref 119: Jain, A.K., et. al. [1994]. The potential of benzoporphyrin derivative [BPD] as a photosensitizer of blood-borne targets. *Oncol.* 13:33-42.**
7. **Ref. 35:** Kessel, D. [1989]. *In vitro* photosensitization with a benzoporphyrin derivative. *Photchem. Photobiol.* **49[5]**:579-582.
8. **Ref. 58:** Haimovici, R., et. al. [1996]. Localization of lipoprotein-delivered benzoporphyrin derivative in the rabbit eye. *Current Eye Res.* **16**:83-90.
9. **Ref. 77:** Husain, D., et. al., [1996]. Intravenous infusion of liposomal benzoporphyrin derivative for photodynamic therapy of experimental choroidal neovascularization. *Arch. Ophthalmol.* **114**:978-985.

Pharmacokinetics

1. **Ref. 37:** Richter, A.M., et. al. [1994]. Kinetics of cellular uptake and retention of the benzoporphyrin derivative [BPD]; relevance to photodynamic therapy. *SPIE* **2325**:189-197.
2. **Ref. 52:** Alison, B.A., et. al. [1990]. The plasma distribution of benzoporphyrin derivative and the effects of plasma lipoproteins on its biodistribution. *Photochem. Photobiol.* **52[3]**:501-507.
3. **Ref. 48:** Richter, A.M., et. al. [1993]. Liposomal delivery of a photosensitizer, benzoporphyrin derivative monoacid ring A [BPD], to tumor tissue in a mouse tumor model. *Phochem. Photobiol.* **57[6]**:1000-1006.
4. **Ref. 359:** Richter, A.M., et. al. [1990]. Biodistribution of tritiated benzoporphyrin derivative [^{3}H-BPD-MA], a new potent photosensitizer, in normal and tumor-bearing mice. *J. Photchem. Photobiol. B* **5**:231-244.
5. **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.
6. **Ref. 35:** Kessel, D. [1989]. *In vitro* photosensitization with a benzoporphyrin derivative. *Photchem. Photobiol.* **49[5]**:579-582.
7. **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.
8. Ref. 102: Richter, A.M., et. al. [1990]. *In vitro* evaluation of phototoxic properties of four structurally related benzoporphyrin derivatives. *Photochem. Photobiol.* **52[3]**:495-500.

Systemic Toxicity

1. **Ref. 315:** Kobayashi, T., et. al. [1983]. Lysis of Erythrocytes by Phosphatidylcholine Containing Polyunsaturated Fatty Acid. *J. Biochem.* **93:**675-680.
2. **Ref. 320:** Discussion related to the need to conduct a 28-day repeated dose toxicity study in nonrodents with verteporfin™ [Liposomal benzoporphyrin derivative monoacid: BPD-MA]

Ocular Toxicity

1. **Ref. 55:** Schmidt-Erfurth, U., et. al. [1994]. Vascular Targeting in Photodynamic Occlusion of Subretinal Vessels. *Ophthalmology*: **101:**1953-1961.
2. **Ref. 73:** Lin, S.C., et. al. [1994]. The photodynamic occlusion of choroidal vessels using benzoporphyrin derivative. *Current Eye Research* **13(7):**513-522.
3. **Ref. 57:** Schmidt-Erfurth, U., et. al. [1995]. In Vivo Uptake of Liposomal Benzoporpyhyrin Derivative and Photothrombosis in Experimental Corneal Neovascularization. *Lasers in Surgery and Medicine.* **17:**178-188

Reproductive Toxicology

1. **Ref. 343:** Expert Report on Intravenous Developmental Toxicity of CL 318,952 [Benzoporphyrin Derivative Monoacid, A Photosensitizer for Photodynamic Therapy] in Rat and Rabbits; TX-94026; Prepared by Mildred S. Christian, Ph.D., ATS; Dec. 9, 1994.

Special Toxicology

Immunotoxicology

1. **Ref. 256:** Simkin, G.L., et. al. [1997]. Inhibition of contact hypersensitivity with different analogs of benzoporphyrin derivative. *Immunopharmacol.* **37:**221-230.

Studies not reviewed within this submission – The following studies were not reviewed because [1] they evaluated efficacy for applications other than for proposed indication of CNV associated with age related macular degeneration; [2] the drug was administered by a route other than that for the proposed indication; and/or [3] the studies utilized light activation at wavelengths other than those intended for use in the proposed indication

Report No.	Report Date	Study Title
		ASSAY VALIDATION
AM-97002	July 23, 1997	Development of an Assay for Demonstrating the Transfer of Liposomal BPD-MA to Plasma Components Using Fluorescence Quenching [Ref. 45]
A9035		Benzoporphyrin derivative monoacid [BPD-MA; CL 318,952) – a photodynamic anti-cancer agent]. The Determination of Two Regioisomers [CL 315,555 and CL 315,585] of BPD-MA [CL 318,952] in rat plasma by reverse phase High Performance Liquid Chromatography [Ref. 355]
A9149		Validation of Method LC91; HPLC Analysis of CL 315,555 and CL 315,585 in Rat Plasma [Ref. 356]
A9145		Validation of Method LC91; HPLC Analysis of CL 315,555 and CL 315,585 in Dog Plasma [Ref. 357]
		ACTIVATION – BLUE LIGHT
PH-93003		Action Spectrum of BPD-MA In Vitro Using Blue Light [Ref. 143]
PH-94004		Action Spectrum of BPD-MA In Vivo Using Blue Light [Ref. 144]

Report No.	Report Date	Study Title
		PHARMACOLOGY STUDIES - *IN VIVO* AND *IN VITRO* TUMOR MODELS
91008	May 31, 1992	A Dose-Ranging Study of Light at 690 nm and 630 nm Applied to Tumour-Bearing Mice Treated with Liposomal BPD-MA [Ref. 64]
PH 91010	Oct. 1, 1991	A Dose-Ranging Study of Liposomal BPD-MA Given I.V. to Tumour-Bearing Mice Followed by Exposure to 690 NM Laser Light II [Ref. 65]
PH 92001	Mar. 27, 1992	A Comparison of Light Emitted by Diode Laser and Argon Pumped Dye Laser with Respect to Activation of a Photosensitizing Drug [Ref. 66]
PH-91015	Jan. 24, 1992	A Comparison of LED and Continuous Wave Laser Light with Respect to Activation of a Photosensitizing Drug [Ref. 67]
PH-91014	Feb. 20, 1992	A Preliminary Dose-Ranging Study of LED Light with Respect to Activation of Liposomal BPD-MA Given I.V. to Tumour-Bearing Male Mice [Ref. 68]
PH-92005	Nov. 3, 1992	A Comparison Between BPD-MA and Its Two Regioisomers with Respect to Photoactivation *In Vitro* [Ref. 109] – Similar to Study PH-92013 [Ref. 104]– Reviewed in Pharmacology Section of this review
PH-91006	Apr. 11, 1991	Photosensitizing Potency of Two Regioisomers of Benzoporphyrin Derivative [BPD-MA] *In Vitro* [Ref. 110] – Similar to Study PH-92013 [Ref. 104]– Reviewed in Pharmacology Section of this review – Reviewed for [redacted] Original Review, p.5, completed Dec. 9, 1991
PH-91004	Mar. 12, 1991	A Comparative Study of the Effects of Two Regioisomers of BPD-MA given i.v. to Tumor-Bearing Mice Followed by Exposure to 690 nm laser light [Ref. 112] - Similar to Study PH-92013 [Ref. 104]– Reviewed in Pharmacology Section of this review – Reviewed for [redacted] Original Review, pp.4-5, completed Dec. 9, 1991
PH-92010	Dec. 15, 1992	A Single Dose Comparative Study of the Anti-Tumour Efficacy of Liposomal BPD-MA and Its Regioisomers Administered to Tumour-Bearing Mice, Followed by Exposure to 690 nm Laser Light [Ref. 114] - Reviewed in Pharmacology Section of this Review – same data as in Ref. 104
PH-93031	Apr. 29, 1994	A Comparison Between BPD-MA and Its 4 Enantiomers with Respect to Photactivation *In Vitro* [Ref. 116]
PH-94002	Apr. 8, 1994	The Effect of Fluence Rate on Skin Phototoxicity and Antitumor Efficacy Produced by 690 nm Light When Tumor Bearing Mice are Treated 30 Minutes After Injection of BPD-MA [Ref. 150]
PH-9901	Mar. 19, 1999	Benzoporphyrin derivative [BPD] Enhances In Vitro and In Vivo Tumor Cell Death Caused by Ionizing Radiation [Ref. 170]
PH-92012	Mar. 12, 1992	Influence of Porphyrins on the Effects of Ionizing Radiation on Normal and Malignant Cells [Ref. 171]
PH-93016	Jul. 26, 1993	Effect of BPD-MA on Hemopoietic Recovery in DBA/2 Mice Following Sublethal Irradiation [Ref. 173]
91003	Apr. 11, 1991	A Dose-Ranging Study of Liposomal BPD-MA Given i.v. to Tumor-Bearing Mice Followed by Exposure to 690 nm Laser Light [Ref. 175]
TX-96007 Interim	Nov. 26, 1996	A Study to Assess the Effects of Various Photosensitizers Using the M1-S Tumor Model in DBA/2 Mice and the Argon Pump Dye Laser [Ref. 176]
PH-93001	Jul. 5, 1993	A Single Dose Comparative Study of the Antitumor Efficacy of Liposomal BPD-MA Administered to tumor-Bearing Mice, Followed by Exposure to various Doses of 690 nm Laser Light at Various Time Postinjection [Ref. 178]
PH-93006	May 27, 1993	Preliminary Experiments on Early Treatment [ET] of Tumor-Bearing Mice with PBD-MA and Light [Ref. 187]
PH-94005	Apr. 20, 1994	A Histopathologic Study of the Time Response of Injected Rhabdomyosarcoma to Photosensitizing Drug and Light Exposure and An Evaluation of Treatment Effect on Skin [Ref. 188]
PH-93020	Jun. 22, 1993	Photodynamic Elimination of Clonogenic Phil Leukemia [CML] Cells [Ref. 212]
PH-93018	Jun. 21, 1993	The Effect of Human Plasma on Photodynamic Treatment of Normal and Remission Samples [Ref. 213]

Report No.	Report Date	Study Title
PH-93019	Jun. 21, 1993	The Sensitivity of Normal Subject and Remission Patient Bone Marrow Samples to Treatment with the Photosensitizer BPD-MA and Red Fluorescent Light [Ref. 217]
PH-95002	Apr. 3, 1995	The Sensitivity of CD34-Enriched Bone Marrow Cells and Mobilized Peripheral Blood Progenitor Cells to Treatment with the Photosensitizer BPD Verteporfin [BPD-MA] and Red Fluorescent Light [Ref. 223]
PHARMACOLOGY/TOXICOLOGY STUDIES - IMMUNOMODULATORY EFFECTS FOLLOWING ACTIVATION		
PH-93014	Ma. 10, 1993	Transdermal Activation of BPD-MA in a Mouse Model [Ref. 157]
PH-94003	Apr. 26, 1995	A Pilot Comparative Safety Study in Hairless Mice [Ref. 160]
PH-94007	Nov. 1994	Transdermal Activation of Liposomal BPD-MA in Hairless Mice [Ref. 161]
PH-98002	Nov. 24, 1998	Systemic Effects of Repetitive, Whole Body PDT with BPD-MA and Blue Light in a Rabbit Model [Ref. 162]
PH-94017	Nov. 30, 1994	Effects of Irradiation of Large Body Surface Areas with LED Light in Pigs Injected Intravenously with BPD-MA [Ref. 163]
PH-95007	June 19, 1996	Skin Photosensitivity Assessment of Partial Body Irradiation Following a Short IV Infusion of Liposomal BPD-MA in Macaca Nemistrina [Pigtail Macaques] [Ref. 164]
PHARAMCOLGOY STUDIES - EXTRACORPOREAL PHOTOPHERESIS/ANTI-VIRAL ACTIVITY		
PH-93022	Apr. 25, 1994	A Comparison of In Vitro Versus In Vivo [Extracorporeal Photopheresis] treatment with BPD-MA and Light in Whole Blood from Yucatan Mini Swine [1] Determination of IgG Binding, Hemolysis and Potassium Loss by Red Cells and [2] Photobleaching of BPD-MA in Plasma [Ref. 165]
PH93023(2)	May 13, 1999	A Comparison of PDT Effect of In Vitro Light Exposure on Red Cell Parameters in Yucatan Miniswine Whole Blood Containing BPD-MA Administered Either Intravenously or In Vitro [Spiked] [Ref. 166]
PH-93024	Nov. 20, 1996	A Pilot Study in Yucatan Miniswine to Determine the Feasibility of an Extracorporeal Photopheresis Treatment of Whole Blood with BPD-MA [Ref. 168]
PH-94018	Apr. 30, 1996	Effect of Light-Activated BPD-MA on Cell-Associated Virus and Cellular Integrity of Blood from SIV-Infected Pig Tail Macaque [Macaca Nemistrina] Monkeys [Ref. 169]
PH-93009	Jun 4, 1993	Effect of BPD-MA and Light on Leukocyte Subpopulations From HIV Infected and Uninfected Individuals, Determined by Flow Cytometric Analysis [Ref. 262]
PH-93010	Jun. 4, 1993	Analysis of BPD-MA and Light Treated Blood Samples From HIV Infected and Uninfected Individuals by Double-Labeled Flow Cytometry [Ref. 263]
PH-93012	Jun. 4, 1993	Effect of BPD-MA and Light on the T Cell Function of HIV Infected and Uninfected Donors [Ref. 264]
PH-93003	Jun. 7, 1993	A Study to Determine the Effects of In Vitro PDT of Normal Donor Blood on White Cells [Ref. 271]
PH-93004	Jun. 7, 1993	Photoinactivation of Spiked VSV in Red Cell Concentrates Using BPD-MA [Ref. 272]
PH-93005	Jun. 7, 1993	Elimination of IgG binding to Red Cell Membranes Post PDT of Red Cell Concentrates [Ref. 273]
PH-93007	May 21, 1993	The Effects of BPD-MA and Red Light at Sub Virucidal Doses on Platelet Concentrates [Ref. 276]
PH-93011	Jun. 7, 1993	Effect of BPD-MA and Light on the Survival of a Laboratory Strain of HIV [LAV-1] in Tissue Culture Medium [Ref. 277]
PH-93015	Jun. 4, 1993	Effect of BPD-MA and Light on the Survival of HIV in Tissue Culture Medium [Ref. 279]
PH-93008	Jun. 1, 1993	Effect of BPD-MA and Light on Cell-Associated HIV in Blood From Infected Human Subjects [Ref. 281]

Report No.	Report Date	Study Title
PH-95008	Jun. 20, 1993	Assessment of In Vivo Inactivation of SIV in Macaca Nemistrina [Pigtail Macaques] Following a Short IV Infusion of BPD-MA and Exposure to Red Light [Ref. 282]
PHARMACOLOGY STUDIES - ARTHRITIS OR AUTOIMMUNE MODELS		
PH-93028	Dec. 1, 1994	Effect of BPD-MA and Red Light on The Progression of CFA-Enhanced Arthritis in MRL-LPR Mice [Ref. 246]
PH-94009	Mar. 29, 1995	Transcutaneous PDT of Antigen Induced Arthritis in Rabbits [Ref. 249]
PH-93017	Jun. 7, 1993	Prophylactic Treatment of Autoimmune Disease in MRL/LPR Mice Using Photodynamically Attenuated Splenocytes from Diseased Animals [Ref. 260]
PH-93025	Sept. 24, 1993	A Preliminary Study of Photodynamic Treatment of Murine Lupus in a Leukapheresis Model [Ref. 261]
PHARMACOLOGY STUDIES - ATHEROSCLEROTIC MODELS		
PK-93001		Plasma Clearance of BPD-MA in Rabbits on an Atherogenic Diets [Ref. 88]
PH-97004	July 7, 1997	Possible Mechanisms of Action of BPD-MA on the Development of Aortic Plaque in Rabbits on a High-Fat Diet [Ref. 106]
TX-94028	Oct. 25, 1995	Effect of Intravenous Administration of BPD-MA in Rabbits on a High Fat Diet [Ref. 107]
PH-94015	Mar. 31, 1998	BPD-MA Inhibition of Intimal Hyperplasia Following Aorta Denudation in Rabbits on a Normal Diet [Ref. 108]
PHARMACOLOGY STUDIES - SKIN PHOTOSENSITIZATION		
PH-92007	Dec. 15, 1992	A Preliminary Study Comparing the Skin Photosensitizing Effects of BPD-MA and Its Two Regioisomers When Administration I.V. to Normal Mice is Followed by Exposure to 690 nm Laser Light [Ref. 113] – Reviewed in Pharmacology Section of this Review – same date as in Ref. 104
PH-93029	Mar. 10, 1994	Skin Photosensitivity During and After Infusion of BPD-MA in Normal Pigs: A Dose Finding Study. [Ref. 136]
PH-95003		Activation of BPD-MA with Light in UVA and Blue Spectral Ranges Using Mouse and Pig Skin Photosensitivity [Ref. 141]
PH-94006	Mar. 18, 1994	Preliminary Experiments on the Effects of Blue Light on the Skin of BPD-MA Injected Mice [Ref. 146]
TX-94005	Sept. 13, 1994	Skin Phototoxicity in Normal Mice After Administration of BPD-MA and Exposure to Either Red or Blue Light [Ref. 147]
PH-94016	Aug. 31, 1994	Effect of Fluence Rate of Red LED Light on Skin Photosensitivity in the Domestic Swine, Following Fast Infusion of BPD-MA [Ref. 151]
PH-93002		Development of Skin Photosensitivity Following IV Administration of BPD-MA to Normal Pigs [Ref. 323]
PH-94008		Skin Photosensitivity in Rabbits-safety studies [Ref. 331]
STUDIES EVALUATING ALTERNATE ROUTES		
TX-95009	Dec. 22, 1995	Oral Administration of BPD-MA Verteporfin in Mice [Ref. 292]
TX-94003	Apr. 20, 1994	Primary Dermal Irritation Study of Three Liquid Topical Formulations of BPD-MA in Rabbits [Ref. 293]
TX-94013	Aug. 5, 1994	Primary Dermal Irritation Study of Three Liquid Topical Formulations of BPD-MA in Rabbits [Ref. 294]
PH-94001	Jun. 6, 1994	A Pilot Study to Assess the Effects of a Topical Formulation of BPD-MA on Rabbit Skin [Ref. 295]
PH-94011	July26, 1994	A Pilot Study to Assess the Effects of Topical Formulations of BPD-MA on Rabbit Skin [Ref. 296]
TX-95003	May 17, 1995	A Study to Assess the Effects of Four Topical Formulations of BPD-MA in NMP with Red Light on Rabbit Skin [Ref. 297]
TX-95005	Apr. 26, 1995	A Study to Assess the Effects of Two Topical Formulations of BPD-MA with Red Light on Rabbit Skin [Ref. 298]

Report No.	Report Date	Study Title
TX-95010	Dec. 13, 1995	A Study to Assess the Effects of Topical Formulations of BPD-MA With and Without Non-Laser Red Light on Rabbit Skin [Ref. 299]
TX-94012	Jun. 21, 1995	A Pilot Study to Determine the Depth of Skin Penetration of Topical Formulations of BPD-MA into Rabbit Skin [Ref. 300]
DEVICE EVALUATION		
PH-93034	Oct. 22, 1993	Comparison of 2 Light Devices by Determining the Effects on Whole Blood [Ref. 70]
PH-93027	Sept. 20, 1993	LED Box for Mice [Ref. 159]

Disclaimer (Use of sponsor's material): Sponsor submitted information was utilized in the preparation of this review.

Introduction/Drug History: Age-related macular degeneration [ARMD] is a leading cause of irreversible vision loss in individuals ≥65 years. ARMD presents as either a "dry" or "nonvascular" form or a "wet" or "vascular" form. The choroidal neovascularization observed with the wet form of this disease is characterized by [1] immature, fragile, and leaky vessels; [2] infiltration of fibrocytes and fibrocellular tissue between the retinal pigmented epithelium (RPE) and photoreceptors; [3] RPE detachment; and [4] subretinal fibrosis. The normal architecture is disrupted, eventually leading to a loss of photoreceptors, RPE, destruction of the macula, and associated vision loss.

The current treatment for neovascular ARMD, photocoagulation, can result in retinal damage, atrophic scarring, and development of visual scotoma, and is essentially nonselective. The Sponsor proposes that BPD-MA plus photoactivation [e.g. photodynamic therapy] is an alternative treatment modality that theoretically results in closure of the choroidal neovasculature [CNV] while minimizing the damage to the overlying neurosensory retina and normal tissues. Although, this treatment would not repair irreversibly diseased tissue, it is predicted that it will prevent the progression of the disease. The Sponsor indicates that BPD-MA tissue concentration at various time points following drug infusion is greater in the CNV than in surrounding normal tissue. Therefore, this treatment potentially results in greater selectivity than photocoagulation, if properly timed. The mechanism of action is similar to that described for other photodynamic therapies. Following photoactivation of BPD-MA, there is the generation of singlet oxygen and other radicals. These moieties perturb cellular structures, including cellular membranes, and result in cytotoxicity. Damage to endothelial cells is also associated with platelet aggregation, degranulation, and thrombus formation, which appears to be a major mechanism for the development of vascular occlusion.

Previous clinical experience: The medical officer, Dr. Wiley Chambers, has reviewed the clinical trials conducted in association with this NDA.

QLT Phototherapeutics, Inc.

INDEX OF STUDIES

QLT Phototherapeutics, Inc.

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1. The following terms were used to refer to the drug product, the regioisomers, and the major metabolic product of verteporfin:

Parent compound = BPD-MA = CL318,952
Regioisomers = BPD-MA_C = BPD-A1 = CL315,555
BPD-MA_D = BPD-A2 = CL315,585
Major Metabolic Product = BPD-DA and diacid

2. The Sponsor provided data to support the stability of the reconstituted drug product for up to 11 hours at room temperature. The Sponsor states that it has been determined that refrigerated reconstituted test article is stable for up to 10 days. The data to support this statement could not be found. This information has been requested from the Sponsor since in several pivotal studies the test article was reconstituted weekly and refrigerated until use.

Pharmacology:

A. **Light Activation** - The Sponsor provided the following graph of the absorption spectrum for verteporfin.

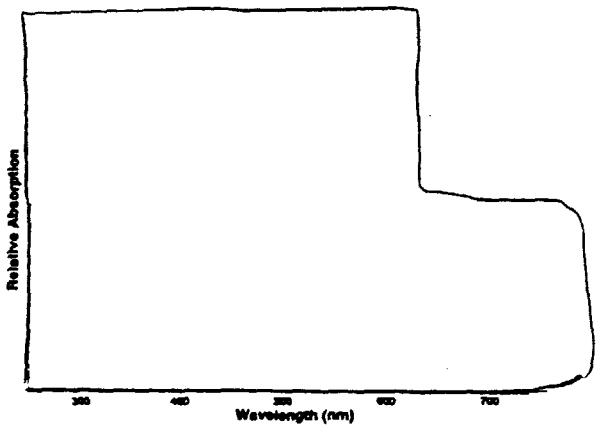

FIGURE 2. Absorption Spectrum of Verteporfin

a. Title: The Photosensitivity Action Spectrum of Liposomal BPD-MA [Ref. 62]
Study Identification: 91002
Site: [redacted]
Study Dates: Not provided
Formulation and Certificate Analysis: No (X) Not provided
Final Report (X) April 11, 1991
GLP and QA Statements Signed: No (X)
Objective: To determine the photosensitivity action spectrum of liposomal BPD-MA in the skin of DBA/2 male mice.

Study Design – Shaved, depilated male DBA/2 mice [N=3] were administered 2 mg/kg of liposomal BPD-MA iv and after 3 hours were exposed to 125 J/cm^2 [argon ion pumped dye laser] at 620-700 nm [increases in increments of 10 nm]. A drug treated/no light exposure group

served as the control. Skin effects were characterized by 3 independent observers on days 0, 1, 2, 3, 6, and 14 post irradiation. Scores ranged from 0 [no observable effect] to 5 [very severe effect].

Results [Note: Photographs were included but the quality did not permit evaluation by the Reviewer.] - All irradiated mice exhibited some degree of a photosensitivity reaction with differences increasing between the various groups as a function of time. By Day 14, healing was observed in all groups, although healing lagged behind in the 680 and 690 nm mice when compared to the other groups. In the table below, the Sponsor provided the average scores through Day 6. [**Note:** Average scores through Day 6 were the only data provided.]

Wavelength (nm)	DAY				
	0	1	2	3	6
0 light	0	0	0	0	0
620	1.3	3.5	3.0	3.45	2.5
630	2.0	3.8	4.3	4.3	4.4
640	2.0	3.7	4.4	4.1	4.1
650	2.0	1.8	2.2	2.4	1.7
660	2.2	1.7	2.5	2.2	1.5
670	2.0	1.5	2.2	1.9	1.0
680	3.5	4.5	4.7	4.8	4.9
690	3.5	4.4	5.0	5.0	5.0
700	2.0	1.2	0.4	0.5	0.73

Sponsor's Conclusion and Reviewer's Comment - The greatest photosensitivity reaction was elicited at wavelengths of 680-690 nm with a lesser effect observed at 630-640 nm. **Reviewer's Comment** - The Reviewer concurs.

b. Ref. 63: Waterfield, E.M. [1994]. **Wavelength-dependent effects of benzoporphyrin derivative monoacid ring A *in vivo* and *in vitro*. *Photchem. Photobiol. Vol.* 60[4]:383-387.** P815 mastocytoma cells were incubated with "various concentrations" of BPD-MA for 1 hour and then washed and irradiated at 678-700 nm [increases of 2 nm increments]. Assessment of cytotoxicity indicated that the LD_{50} for BPD-MA was comparable between 682-694 nm. The LD_{50} at these wavelengths was also less than at other wavelengths. Assessment of photosensitivity reactions in DBA/2 mice administered 2 mg/kg of BPD-MA and irradiated 3 hours later with various wavelengths revealed similar findings to those described in Reference 62 ["a" above]. *In vivo* antitumor efficacy was evaluated in M1-S rhabdomyosarcoma-bearing DBA/2 mice administered 2 mg/kg of BPD-MA and irradiated 3 hours later with wavelengths ranging from 683-695 nm. Approximately 60-90% of the animals irradiated at a wavelength between 685-693 nm were tumor-free on Day 20. A wavelength of 690 nm exhibited significantly more antitumor efficacy than a 630 nm wavelength.

B. Mechanism of Action

a. Ref. 18; Fernandez, J.M., et. al. [1997]. Singlet oxygen generation by photodynamic agents. *J. Photchem. Photobiol. B. Biology*. 37:131-140 - The singlet oxygen quantum yield of several photodynamic agents was measured at 546 nm, 630 nm, and the far-red absorption peak using a technique "in which the photosensitization of lysozyme is used as an internal actinometer". The average quantum yield for hematoporphyrin IX, Photofrin, and BPD-MA was 0.73, 0.89, and 0.84.

b. Ref. 19; Hadjur, C., et. al. [1997]. EPR and spectrophotometric studies of free radical [O_2•, •OH, BPD-MA•⁻] and singlet oxygen [$^1O_2$] generated by irradiation of benzoporphyrin derivative monoacid ring A. *Photochem. Photobiol.* 65(5):818-827. Photodynamic mechanisms have been classified as Type I and Type II. Type I process is mediated through generation of free radicals. Type II process is mediated through the generation of $^1O_2$. The studies described here indicate that after photoactivation of BPD-MA at 690 nm the following products are generated, [1] $^1O_2$; [2] O_2^- that can be converted to •OH by the Fenton reaction; and [3] under anaerobic conditions, BPD-MA⁻. Therefore, the authors conclude that the pharmacological activity of BPD-MA following photoactivation, *in vivo*, may be due to both Type I and Type II reactions.

c. Ref. 21; Aveline, B., et. al. [1994]. Photophysical and photosensitizing properties of benzoporphyrin derivative monoacid ring A [BPD-MA]. *Photochem. Photobiol.* 59[3]:328-335. The authors describe the photophysical characterization of the regioisomeric mixture of BPD-MA following photoactivation. The $^1O_2$ quantum yield was comparable to that reported in Reference 18, above: 0.76 in methanol and 0.79 in benzene. The authors also state that BPD-MA is "a highly photostable compound in solution".

d. Ref. 118: Gillies, R. et. al. [1996]. Spectral characterization of the benzoporphyrin derivative monoacid ring-A photoproduct formed in fetal calf solutions during irradiation with 694 nm continuous-wave radiation. *J. Photochem. Photobiol.* 33:87-90. The authors indicate that aqueous BPD-MA in 10% FCS reacts with $^1O_2$ to generate a photoproduct. However, the mechanism for the formation has not been fully delineated. They also state that this phenomenon has been observed *in vivo* in rabbits.

e. Ref. 119: Jain, A.K., et. al. [1994]. The potential of benzoporphyrin derivative [BPD] as a photosensitizer of blood-borne targets. *Oncol.* 13:33-42. This reference provides data supporting the generation of photoproducts following the activation of BPD-MA. Photoproducts were identified, but not characterized, following irradiation of RBCs incubated with BPD-MA. The reference also describes studies that were conducted to evaluate the potential toxicity of the photoproducts in an *in vitro* P815 cytotoxicity assay. Cytosol was collected from irradiated RBCs that had been incubated with BPD-MA. The authors state that the photoproduct was estimated to be approximately 90% of the drug present in the cytosol. In addition, plasma was incubated with BPD-MA and irradiated. Tumor cells were then incubated with either the cytosol or the plasma. Under the conditions tested, the photoproducts were found to be non-cytotoxic in the presence or absence of irradiation.

f. Ref. 35; Kessel, D. [1989]. *In vitro* photosensitization with a benzoporphyrin derivative. *Photochem. Photobiol.* 49[5]:579-582. This paper describes studies conducted to evaluate some of the photophysical properties of BPD-MA and BPD-DA. The endpoints evaluated were [1] octanol:water partitioning; [2] L1210 leukemia cells:medium distribution; [3] cytotoxicity/viability of leukemia cells; [4] membrane function as determined by membrane transport of cycloleucine; and [5] effects on ATP pool size. The table below outlines the results.

Table 1. Distribution ratio: *vs* photodamage

	Distribution ratios		ID_{50} values (μg/ml)		
Dye	*octanol* water	*cells* medium	Viability	Transport	ATP Pool Size
BPD-MA	410 ± 30	22 ± 3.5	0.4 ± 0.02	0.5 ± 0.03	0.052 ± 0.004
BPD-DA	73 ± 4.5	11 ± 1.5	2.7 ± 0.3	3.1 ±0.4	0.56 ± 0.04

Octanol/water and cell/medium partition ratios were determined as described in the text. ID_{50} values represent extracellular dye concentrations needed, during 30 min incubations followed by irradiation, to cause 50% decreases in viability, amino acid transport and ATP pool size. Numbers shown above represent the results of 5-7 experiments (mean ± SD).

These studies indicate that [1] BPD-MA is more hydrophobic than BPD-DA; [2] 50% less of the BPD-DA compared to BPD-MA is taken up and retained in tumor cells; [3] BPD-MA is a more potent cytotoxic compound than BPD-DA; [4] based on ID_{50} values and under the conditions of the study, lethal effects correlated with membrane damage; and [5] under the conditions of the study, mitochondria [e.g. ATP pools] were more sensitive to phototoxicity when compared to the membrane changes.

C. **Ocular Pharmacology Studies** – Additional references evaluating efficacy and safety are provided under Ocular Toxicology

a. Ref. 77: Husain, D. et. al., [1996]. Intravenous infusion of liposomal benzoporphyrin derivative for photodynamic therapy of experimental choroidal neovascularization. *Arch. Ophthalmol.* 114:978-985. Cynomolgus monkeys [N=9] were administered 0.375 mg/kg of liposomal BPD-MA by slow infusion [e.g. over 10-32 minutes]. Eleven eyes with laser-induced CNV and 7 normal eyes were irradiated [689 or 692 nm; 600 mW/cm^2, 150 J/cm^2] 30 – 105 minutes after infusion start. Eyes were evaluated by fundus photography, fluorescein angiography, and light and electron microscopy 24 hours following irradiation. Fundus photography was also performed immediately after irradiation, and the eyes of 1 monkey were evaluated 4 weeks after irradiation. The tables below summarize the efficacy in the CNV eyes and the selectivity in the normal eyes. [The grading system was based on damage to the neurosensory retina as well as medium and large choroidal vessels.] Damage to the choriocapillaris and RPE as well as "some damage to the photoreceptors throughout" all grades was observed in all normal eyes. [Note: The Sponsor indicates that they had some focusing problems at 55 minutes that may have adversely affected efficacy.]

Table 2. Efficacy of Photodynamic Therapy (Choroidal Neovascularization Closure) With Slow Infusion

Time of Treatment After Start of Infusion, min	No. of Choroidal Neovascularizations	
	Treated	Closed
32	3	3
45	5	5
55	5	3
65	5	2
75	4	0 (71)
85	3	0
95	3	0
105	2	0

Table 3. Effect of Photodynamic Therapy on Normal Retina and Choroid With Slow Infusion*

Time of PDT After Start of Flash Infusion, min	No. of PDT Spots on Normal Choroid	Grade				
		1	2	3	4	5
32	3	1	0	0	1	1
45	3	1	1	0	1	0
55	3	1	2	0	0	0
65	3	1	2	0	0	0
75	3	2	1	0	0	0
85	3	1	1	1	0	0
95	3	2	1	0	0	0
105	2	1	0	1	0	0

Summary of Pharmacology: These studies indicate that BPD-MA absorbs light within the red light [approximately 700 nm], blue light [approximately 430-450 nm], and UVA [approximately 350 nm] portion of the light spectrum. An activation wavelength of 690 nm was selected for the photodynamic therapy application since this wavelength penetrates tissues better than wavelengths within the UVA and blue light portion of the spectrum. In addition, 690 nm does not interact with hemoglobin.

In vitro studies further indicated that activation of BPD-MA resulted in the generation of singlet oxygen [$^1O_2$] and free radicals. Therefore, the Sponsor suggested that the cytotoxic mechanisms *in vivo* may be due to both Type I [generation of free radicals] and Type II [generation of $^1O_2$] processes. The quantum yield of $^1O_2$ for BPD-MA was comparable to that described for both Hematoporphyrin 1X and Photofrin. These studies suggest that the cytotoxic effects are in part due to membrane damage. Following activation of BPD-MA, a photoproduct was also generated, which was found to be noncytotoxic in the presence or absence of irradiation in an *in vitro* tumor cytotoxicity assay.

Activated BPD-MA was shown to have efficacy in numerous animal models, both *in vitro* and *in vivo*. These included [1] tumor models; [2] photosensitivity models; and [3] CNV and choriocapillaris closure models. LDL-complexed BPD-MA initially distributed to choroidal tissue in normal eyes. Over time, levels of BPD-MA distributed to the RPE and photoreceptors. Closure of experimentally induced CNV was observed in all cynomolgus monkeys administered 0.375 mg/kg of liposomal BPD-MA and irradiated 32-45 after initiation of drug infusion with a laser light at 692-nm [600 mW/cm^2, 160 J/cm^2]. Closure was observed in 3/5 monkeys when eyes were irradiated at 55 minutes. [**Note:** The Sponsor maintained that efficacy at this time point may have been adversely affected due to focussing problems.] In normal eyes from cynomolgus monkeys administered the same dose of BPD-MA, some degree of damage to the choriocapillaris, retinal pigmented epithelium, and photoreceptors was observed in all eyes. When eyes were irradiated at ≤45 minutes from the start of infusion, there was marked to severe damage of the outer nuclear layer in 1-2 of 3 eyes evaluated. When irradiation was performed from ≥55 minutes following the start of infusion, the damage to the outer nuclear layer was slight to minimal. [Additional discussion of efficacy and toxicity are provided in the Toxicology Portion.]

Safety Pharmacology:

I. General Pharmacology

a. Title: General pharmacology of L-BPD-MA [Ref. 9?]
Study Identification: TX-97007
Site:
Study Dates: Aug. 1, 1996 – May 13, 1997
Formulation and Certificate Analysis: No (X) Lot # G93-120-0891 – dissolved in sterile water and diluted with 5% dextrose; No certificate of analysis
Vehicle – 5% dextrose
Sterile distilled water for injection
Final Report (X) May 13, 1997
GLP and QA Statements Signed: No (X)
Objective: To evaluate the general pharmacological effects of L-BPD-MA in various animal models.

Study Design and Results

General parameters for study design

Species	Dose*			Strain
	mg/kg	ml/kg	Route	
Mice	0.2, 2, and 20	10	iv	ICR [Crj:CD-
Rats		5		Sprague-Dawley [Crj:CD-
Rabbits		2		Japanese native white
Guinea pigs – isolated ileum	-	-		

*No irradiation

I. General State and Behavior

1. Irwin Test – Male mice [N=3/group, 8 weeks, 34.7-37.1 g] were administered either vehicle or test article. Gross observations based on Irwin's method were conducted predosing and 0.5, 1, 2, and 6 hours post drug administration. Cageside observations were conducted at 24, 48, and 72 hours post drug administration. No changes or mortality were observed [No data presented].

II. CNS Evaluation

1. Spontaneous Movement – Male mice [N=5/group; 8 weeks, 31.5-36.1 g] were administered either vehicle, positive control [chlorpromazine HCl, 5 mg/kg, sc], or test article. Spontaneous movement was determined [automatic locomotion activity counter] for 15 hours after administration of the test article. The positive control resulted in decreased spontaneous movement compared to the vehicle control. Increases in spontaneous movement were observed in treatment groups at various time points. Although activity was slightly increased in the mid- and high-dose animals, there was considerable inter-animal variability and a clear dose- and time-dependent response was not demonstrated. The relationship of the changes to drug administration is questionable.

2. **Sleeping time** - Male mice [N=5/group; 8 weeks, 30.1-38.1 g] were administered either vehicle, positive control [chlorpromazine HCl, 4 mg/kg, sc], or test article. Thiopental [40 mg/kg, iv] was administered either 10 minutes [VH, BPD-MA] or 30 minutes [chlorpromazine] post drug administration and sleeping time was measured. The positive control significantly lengthened sleeping time compared to the vehicle control. There were no treatment-related effects with BPD-MA.

3. **Acetic acid-induced writhing** - Male mice [N=5/group; 8 wks, 28.8-37.5 g] were administered either vehicle, positive control [ketoprofen, 20 mg/kg, pc], or test article. Acetic acid [0.7%, 10 ml/kg, ip] was administered either 10 minutes [VH, BPD-MA] or 60 minutes [ketoprofen] post drug administration and writhing responses were counted for 20 minutes. The positive control significantly decreased the number of writhings compared to the vehicle control. There were no treatment-related effects with BPD-MA.

4. **Anticonvulsant effects** - Male mice [N=5/group, 8 weeks, 29.5-36.6 g] were administered either vehicle, positive control [phenobarbital Na, 50 mg/kg, sc], or test article. Direct current electroshock [30 mA for 0.2 sec.] was applied through the cornea of both eyes either 10 minutes [VH, BPD-MA] or 30 minutes [phenobarbital] post drug administration and duration of tonic body flexion and hind limb extension, seizure incidence, and lethality incidence were determined. The positive control significantly decreased the mortality incidence and the incidence and duration of tonic flexion and extension compared to the vehicle control. Although there were no deaths at the high dose of L-BPD-MA, there no effects that were considered to be treatment related.

5. **Anticonvulsant effects [pentetrazol-induced]**- Male mice [N=5/group, 8 weeks, 30.1-35.0 g] were administered either vehicle, positive control [phenobarbital Na, 50 mg/kg, sc], or test article. Pentetrazol [120 mg/kg, ip] was administered either 10 minutes [VH, BPD-MA] or 30 minutes [phenobarbital] post drug administration and latency to seizure onset was determined. The positive control significantly decreased the incidence of tonic convulsions, clonic convulsions and mortality compared to the vehicle control. There were no treatment-related effects with BPD-MA.

6. **Anticonvulsant effects [strychnine-induced]**- Male mice [N=5/group; 8 wks, 30.1-34.5 g] were administered either vehicle, positive control [phenobarbital Na, 50 mg/kg, sc], or test article. Strychnine [2 mg/kg, ip] was administered either 10 minutes [VH, BPD-MA] or 30 minutes [phenobarbital] post drug administration and latency to seizure onset was determined. The positive control significantly decreased the incidence of tonic convulsions, clonic convulsions and mortality compared to the vehicle control. There were no treatment-related effects with BPD-MA.

7. **Proconvulsant effects - Anticonvulsant effects [pentetrazol-induced]**- Male mice [N=5/group, 8 weeks, 29.8-33.4 g] were administered either vehicle or test article. Pentetrazol [45 mg/kg, ip] was administered 10 minutes post drug administration and latency to seizure onset, seizure incidence, and lethality were determined. There were no treatment-related effects with BPD-MA.

8. **Rectal Temperature Effects** - Male rabbits [N=3/group, 8-9 weeks, 1.6-2.0 kg] were administered either vehicle, positive control [chlorpromazine HCl, 5 mg/kg, sc], or test article. Rectal temperature was measured every hour for 4 hours post drug administration. Temperature

was decreased by 0.9° C in the positive control compared to the vehicle control. The increase in temperature was comparable across the VH and treated groups.

III. Respiratory and Cardiovascular Effects

1. ***In vivo* Respiratory and Cardiovascular Effects** – Anesthetized male rabbits [N=3/group; 8-9 weeks, 1.5-2.3 kg; phenobarbital Na, 30 mg/kg, iv] were administered either vehicle or test article. Respiratory rate and relative intensity [intubation, pneumotachometer and amplifier], blood pressure [femoral artery cannulation; pressure transducer], heart rate [tachometer], blood flow [carotid artery noncannulating probe; electromagnetic flow meter], and ECG [Lead II] were measured for 1 hour post drug administration. There were no treatment-related effects. [Note: QT interval was not determined].

IV. Gastrointestinal Effects

1. **Isolated Guinea Pig Ileum** – Ileum was obtained from male guinea pigs [N=6, 5-6 weeks, 414.7-536.4 g]. Contractions of ileal segments were determined in response to treatment with either acetylcholine [10^{-9} – 10^{-5}M], histamine [10^{-8} – 10^{-5} M], serotonin [10^{-8}-10^{-5} M], or barium [10^{-6} – $3X1^{-3}$ M] 5 minutes after treatment with either VH or test article [5 concentrations ranging from 10^{-8}-10^{-4} M]. Responses to each stimulant were examined in 5 preparations. There were no treatment-related effects with BPD-MA.

2. **Charcoal Transit Time** - Male mice [N=5/group, 8 wks, 29.4-34.6 g], fasted for 18 hours, were administered either vehicle, positive control [morphine HCl, 10 mg/kg, sc], or test article. A charcoal suspension [0.2 ml; po] was administered either 10 minutes [VH, BPD-MA] or 30 minutes [morphine] post drug administration. Thirty minutes after the charcoal gavage, mice were sacrificed, and the distance the charcoal had traveled was measured. The positive control significantly decreased the charcoal transit compared to the vehicle control. There were no treatment-related effects with BPD-MA.

3. **Plasma BSP Clearance** - Male rats [N=5/group, 8 weeks, 233.4-276.3 g] were administered either vehicle or test article [0.002, 0.02, 0.2, 2, and 20 mg/kg]. BSP [50 mg/kg, iv] was administered 10 minutes post drug administration. Blood samples were collected under anesthesia 15 minutes after BSP administration, plasma was harvested, and BSP concentration was measured. There were no treatment-related effects at ≤2 mg/kg of L-BPD-MA. At 20 mg/kg there was a statistically significant increase in the plasma concentration of BSP compared to control values [39.81 ± 7.34 (SE) vs. 139.29 ± 13.58 µg/ml].

V. Renal System

1. ***In Vivo* Renal Function** - Male rats [N=5/group, 8 weeks, 233.4-276.3 g] were administered either vehicle, positive control [furosemide, 50 mg/kg, po], or test article. Saline [30 ml/kg, po] was administered either 10 minutes [VH, BPD-MA] or 30 minutes [furosemide] post drug administration. Urine was collected for 5 hours and urine volume and electrolyte concentrations [Na, K, Cl] were measured. The positive control significantly increased urine volume and electrolyte excretion compared to the vehicle control. There were no treatment-related effects with BPD-MA.

Reviewer's Comment – Study Design and Data Presentation – These are adequate.

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

1. L-BPD-MA administration did not induce any significant CNS effects based on the parameters evaluated.
2. L-BPD-MA administration did not induce any significant cardiovascular or respiratory effects based on the parameters evaluated.
3. With the exception of hepatic function, L-BPD-MA administration did not induce any significant gastrointestinal effects based on the parameters evaluated. There was a significant decrease in BSP excretion at 20 mg/kg.
4. L-BPD-MA administration did not induce any significant renal effects based on the parameters evaluated.

Reviewer's Comment – The Reviewer concurs. The mechanism for the alteration in BSP clearance was not identified.

II. Cardiovascular Safety Pharmacology

A. Dogs

a. Title: Hemodynamic effects of benzoporphyin derivative-monoacid ring A [BPD-MA] cryodessicated powder in anesthetized dogs [Ref. 120]
Study Identification: 36624
Site:
Study Dates [In-life]: March 11-26, 1991
Formulation and Lot No. – not provided, liposomal formulation
Certificate of Analysis: No (X)
Final Report (X) April 23, 1991
GLP and QA Statements Signed: No (X)
Objective: "To determine the cardiovascular hemodynamic effects of BPD-MA in the anesthetized beagle dog"

Dr. Will Coulter previously reviewed this study for IND Submission review completed Dec. 9, 1991; pp. 5-6. Comments by the current Reviewer are in italics.

- *Several cardiovascular changes were observed but it is not possible to determine whether these are treatment related–effects. First, there were no vehicle or saline controls included to assess volume effects. Second, several of the dogs exhibited signs of photosensitivity. It is unclear as to how this impacted the results.*

b. Title: Cardiovascular effects of benzoporphyrin derivative monoacid ring A [BPD-MA], a photodynamic therapy agent, without photoactivation in the beagle dog [Ref. 121]
Study Identification: PH-93032
Site:
Study Dates [In-life]: January 16 – February 1, 1994
Formulation and Lot No. – F93-120-0880 - verteporfin was reconstituted in sterile water for injection [2.0 mg/ml.]
Vehicle - 0.9% NaCl

QLT Phototherapeutics, Inc.

Certificate of Analysis: Yes (X)
Final Report (X) November 7, 1994
GLP and QA Statements Signed: Yes (X)
Objective: "To determine the effects of intravenous administration of BPD-MA without photoactivation on [cardiovascular parameters] in conscious beagle dogs"

Dr. Javier Avalos previously reviewed this study for IND [redacted] Submission [redacted] review completed May 16, 1995; pp. 50-52. This review is provided below. Comments by the current Reviewer are in italics. Strikethroughs were included by the current Reviewer as clarification.

Laboratory: [redacted]
(QLT Report PH-93032)

Date of Study: Dosing of the animals commenced on January 16, 1994, and the last necropsy was performed on February 1, 1994.

Four male and 4 female beagle dogs (Canis familiaris) were intravenously administered with various doses (2, 10, and 20 mg/kg) of BPD-MA (Batch No. F93-120-0880) at a rate of 5ml/min. The animals (5 to 7 months old on arrival December 21, 1994) weighed 5.9 to 10.3 kg and were housed individually after they were received from [redacted] Additional animals were transferred from the "spare" colony to ensure an adequate number of suitable animals for the study. These animals were obtained from [redacted] Throughout the treatment period, the animals were exposed to less than 20 footcandles from a GE Cool light F40CW source. Prior to test and saline administrations, animals were surgically implanted with catheters to measure heart rate, *and* cardiac output blood pressure, ~~and pulmonary artery pressure~~. These parameters were recorded for 30 minutes prior to test article administration and up to 2 hours *continuously* post administration [*data were presented as averages over 30 sec.]*. Blood samples (2 ml) were obtained for clinical pathology testing prior to the first treatment to determine health status of the animals. Blood samples were also collected for the CH50 (2 ml) at predose, 5, 30, and 60 minutes after the infusion of test material. Electrocardiogram recording were obtained for each administration 3 times prior to the saline dose; *up to 18 minutes after start of either saline or test article; up to 30 minutes after finish of saline injection; and* 1, 15, 30, 60, 90, and 120 minutes after the *end* of the test article injection. *Clinical observations/mortality checks were conducted BID. Body weights were obtained 2X/week and food consumption was measured daily.* Animals were subjected to external and internal gross examinations on the completion of the observation period. Heart, lungs, and any other affected tissue were examined histopathologically.

Saline was administered initially to determine potential volume effects. One male (#101) and female (#151) animal were treated at 2 mg/kg [*1 ml/kg*]. A second set of animals (#102 and #152) was treated with 10 mg/kg [*5 ml/kg*] and following a 72 hour washout period, was subsequently dosed at 20 mg/kg [*10 ml/kg*]. An additional 2 males (#103 and #104) and 2 females (#153 and #154) were dosed at 20 mg/kg. [*Solubility factors, e.g. final concentration of test article, limit dosage.*]

Results

Mortality - All animals survived until the scheduled necropsy. However, animals 102 and 152 were not sacrificed following the 20 mg/kg dose, but were transferred to another study for the same Sponsor.

Clinical Observations – There were no treatment-related effects. [No summary table provided.]

Body Weight and Food Consumption - There were no apparent treatment-related effects. However, no control animals were included in this study for comparison.

Cardiovascular Effects - No treatment related effects in mean blood pressure, heart rate, cardiac output, or electrocardiograms were reported. [*ECGs were evaluated by Dr. D.K. Detweiler, Diplomate, A.C.V.I.M, Cardiology]*

Complement Activation – [*Samples were obtained from dog #154 during this study and from dogs #101 and 151 in conjunction with Study PH-94004. One dog was conscious and 2 were anesthetized.*] Complement activity was depleted in serum from all three dogs (#101, #151, and #154) administered a dose of 20 mg/kg. The depletion was greatest at 5 minutes from the start of infusion (30-39%) and was still observed at 60 minutes post-infusion (10-26%).

Gross and Histopathological Effects -Dark or raised areas on the heart were reported after gross examination of the animals. In addition, various combinations of dark or depressed areas, pale discoloration, firmness and nodule in the lung were reported. Histological findings were not reported. The changes observed were described as not being test article related, but due to catheter implantation. Pulmonary findings were found in all examined animals [*4/6*], but were considered to be background changes in dogs. However, historical data and proper controls were not included in the report.

Conclusion - The administration of 20 mg/kg BPD-MA by intravenous injection to Beagle dogs resulted in no cardiovascular changes. Complement activation was decreased primarily 5minutes after administration of 20 mg/kg at a rate of 5 ml/min.

b. Title: <u>Cardiovascular Effects of Benzoporphyrin Derivative Monoacid Ring A [BPD-MA], A Photodynamic Therapy Agent, Without Photoactivation in the Anesthetized Beagle Dog [Ref. 122]</u>
Study Identification: PH-94004
Site: [redacted]
Study Dates: February 4 – 7, 1994
Formulation and Lot No. – F93-120-0880 - reconstituted in sterile water for injection [2.0 mg/ml.]
Vehicle - 0.9% NaCl
Certificate Analysis: Yes (X) [redacted]
Final Report (X) November 11, 1994
GLP and QA Statements Signed: Yes (X)
Objective: "To determine the effects of 20 mg/kg intravenous administration of BPD-MA without photoactivation on [cardiovascular parameters] in anesthetized beagle dogs"

QLT Phototherapeutics, Inc.

Dr. Javier Avalos previously reviewed this study for IND [redacted] Submission [redacted] review completed May 16, 1995; pp. 52-53. This review is provided below with comments by the current Reviewer in italics. Strikethroughs were included by the current Reviewer as clarification.

Laboratory: [redacted]
(QLT Report PH-94004)

Date of Study: Dosing of the animals commenced on February 4, 1994, and the last necropsy was performed on February 7, 1994.

One male and 1 female beagle dogs (Canis familiaris) were intravenously administered with a dose of 20 mg/kg of BPD-MA (Batch No. F93-120-0880) at a rate of 5 ml/min. *Dogs were maintained under isoflurane anesthesia.* The animals (17 months old) weighed 8.7 and 7.6 kg and were housed individually after they were transferred from an existing colony at [redacted] Throughout the treatment period, the animals were exposed to less than 20 footcandles from a GE Cool light F40CW source. Prior to test and saline administrations, animals were surgically implanted with catheters to measure heart rate, cardiac output, *and* blood pressure, and ~~pulmonary artery pressure~~. These parameters were recorded for 30 minutes prior to test article administration and up to 2 hours *continuously* post administration [*data were presented as averages over 30 sec.]*. Blood samples (2 ml) were obtained for clinical pathology testing prior to the first treatment to determine health status of the animals. Blood samples were also collected for the evaluation of complement activation [CH50] (5 ml) at predose, 5, 10, 30, and 60 minutes after the infusion of test material. Electrocardiogram recordings were obtained for each administration *4* times prior to the saline dose; *up to 18 minutes after start of either saline or test article; up to 30 minutes after finish of saline injection;* and 1, 15, 30, 60, 90, and 120 minutes after the *end* of the test article injection. Animals were subjected to external and internal gross examinations on the completion of the observation period. Heart, lungs, and any other affected tissue were examined histopathologically.

Results

Mortality - All animals survived until the scheduled necropsy.

Cardiovascular Effects - No treatment related effects in mean blood pressure, heart rate, cardiac output, or electrocardiograms were reported. *According to Dr. Detweiler, QT wave lengthening was observed in one animal in response to the anesthesia.*

Complement Activation - The data reported for this study are identical to the data reported in the previous study (PH-94032). However, these two studies are not identical. [*Samples were obtained from dog #154 during Study PH-94032 and from dogs #101 and 151 in conjunction with Study PH-94004. One dog was conscious and 2 were anesthetized.*]

Gross and Histopathological Effects -Dark or raised areas on the heart were reported after gross examination of the animals. In addition, various combinations of dark or depressed areas, pale discoloration, firmness and nodule in the lung were reported. Histological findings were not reported. The changes observed were described as not being test article related, but due to catheter implantation. Pulmonary findings were found in all examined animals, but were

considered to be background changes in *dogs*. However, historical data and proper controls were not included in the report.

Conclusion - If the data obtained are indeed from the anesthetized animals, the administration of 20 mg/kg BPD-MA by intravenous injection to anesthetized Beagle dogs resulted in no cardiovascular changes. *Due to a small N [N=1/sex], the results should be interpreted cautiously.*

B. Pigs - The Sponsor conducted studies to support the safety of bolus administration in humans [rate of 0.2 mg/kg/min]. Some of the cardiovascular studies were conducted in Yucatan microswine. In one study in anesthetized microswine, in which liposomal BPD-MA was administered at 0.08-0.5 mg/kg/min, 5/9 animals exhibited cardiovascular effects. These effects included ST segment depression and/or cardiorespiratory arrest within 2-5 minutes after bolus injection. It was postulated that this effect was due to the liposomal portion of the drug and secondary to complement activation. According to the Sponsor, the literature describes [1] anaphylactoid-like reactions in swine to liposomal products and [2] complement activation by negatively charged phospholipids such as egg phosphatidylglycerol. [**Note:** The Sponsor provided a reference (Ref. 133; Wassef, N.M., et. al. [1989] Anaphylactoid reactions mediated by autoantibodies to cholesterol in miniature pigs. *J. Immunol.* 143(9):2990-2995) in which the author proposes that anti-cholesterol antibodies may be involved in induction of anaphylactoid-type reactions in mini-swine. Verteporfin does not contain cholesterol.]

A number of studies were conducted in an effort to define the pathogenesis of the cardiopulmonary effects observed in the anesthetized swine. Unfortunately, a liposomal control was not included in these studies, which would have provided strong support for their argument. A summary of the cardiovascular findings in pigs, rats, dogs, and monkeys is provided in Ref. 124 [Safety Assessment of Liposomal BPD-MA Verteporfin to Support Fast Infusion in Humans]. Dr. Javier Avalos has previously reviewed the pig studies [anesthetized and conscious] for IND [ ] Submission [ ] review completed May 16, 1995; pp. 27-29 and 42-54. [ ] Based on these studies it was concluded that there was a correlation between the rate of infusion, the rate and magnitude of complement activation, and the onset of cardiovascular events in anesthetized swine. Preadministration of Benadryl did not block complement activation, but did block the development of the profound cardiovascular events. Although complement activation did occur in conscious swine, it was to a much lesser degree than that observed in anesthetized animals. Although significant complement activation did occur in several animals following a slow infusion [e.g. over 45-minutes], the peak tended to be at later time points. This suggests that the rate of complement activation is also important in the etiology of CV effects.

i. Study Title - Preliminary investigations on the effects of bolus injection and infusion of liposomally formulated BPD and its constituents [Ref. 123]

Dose/Species - 2 mg/kg at a rate of 1 ml/min; 1 ml/min for the 1st 10 min then 3 ml/min; or 7 ml/min; Yorkshire X Landrace swine; ± Benadryl pretreatment

Summary Results -

1- ECG changes consistent with hypoxia were observed when BPD-MA was injected as a bolus [7 ml/min]; other factors associated with the types of changes observed in the ECG were ruled out [normal blood gases and electrolytes]

2 – Complement depletion [4-88%] observed within 5 min. of injection had returned to baseline within 60 minutes

3 – CV effects were abrogated by pretreatment with Benadryl although complement was

still activated

ii. Study Title - Studies of complement interaction with a liposomal formulation of BPD [Ref. 125]
Dose/Species - *in vivo* and *in vitro*; pig, rat, rabbit, dog, human
Summary Results -
1 - rank order of *in vivo* activation after bolus infusion in anesthetized animals: rabbit <dog=rat<pig
2 – Considerable variation in complement activation was observed in the pig
3 - Preliminary results in humans administered BPD-MA by slow infusion - no ↑ in C3a
4 – In humans with antiphospholipid antibodies – significant and variable ↑ in complement activation

iii. Study Title - Cardiovascular effects after a 45 minute infusion of benzoporphyrin derivative monoacid ring A (BPD-MA), a photodynamic therapy agent without photoactivation in the conscious micro swine [Ref. 126]
Dose/Species - 2 mg/kg at 1 ml/min X 10 min, then 3 ml/min X 35 min. 1X/wk for 3 weeks microswine
Summary Results
1 - No treatment-related CV effects
2 – Hematology revealed a slight anemia and giant platelets. Sponsor attributes this to sampling

iv. Study Title - Cardiovascular effects after an intravenous bolus of benzoporphyrin derivative monoacid ring A (BPD-MA), a photodynamic therapy agent without photoactivation in the conscious micro swine [Ref. 127]
Dose/Species - 2 mg/kg at 7 ml/min; microswine
Summary Results –
1 - No treatment-related CV effects with the exception of a single female exhibiting a slight, transient ↓ in CO
2 - ↑ in pulmonary artery pressure in the majority of animals at 10 minutes post injection; Sponsor attributes change to artifact; basis for this determination was not indicated.
3 – Several changes in clinical pathology: ↓RBC counts, PCV, and Hb [app.15-25%]; ↑ WBC counts; ↑ AST [app. 55-140%]; ↑ BUN [app. 25-35%]; Sponsor considered changes to be a "consequence of the experimental design"

v. Study Title - Complement activation in Montreal Pigs [Ref. 128]
Dose/Species - 2 mg/kg –slow infusion –1X/wk for 3 weeks; 2 mg/kg bolus - no anesthesia; 2 mg/kg bolus + anesthesia; Montreal pigs
Summary Results
1 – Following repeat slow infusions - <40% complement activation after all treatments, <20% in 11/16 treatments
2 – Following bolus with no anesthesia - <20% complement activation in 6/6 pigs
3 – Following bolus plus anesthesia –1/6 mortality, statistically significant ↑ in complement activation compared to unanesthetized animals, complement activation ranged from 23-41% by 3 min post injection

vi. Study Title - Cardiovascular effects after an intravenous bolus of benzoporphyrin derivative monoacid ring A (BPD-MA), a photodynamic therapy agent without photoactivation in the anesthetized micro swine [Ref. 129]
Dose/Species - 2 mg/kg as bolus, Yucatan micro swine
Summary Results
1 – 1 male died 10 min. post-injection – sharp ↓in HR and BP, marked ECG conduction changes
2 – In males, marked transient ↓ in mean systemic BP, HR, CO, and ECG changes within 1-min post-infusion, baseline in 10 minutes
3 – In females, transient ↑ in mean systemic BP and pulmonary artery pressure, other effects were variable
4 - Several changes in clinical pathology: ↓ RBC counts, PCV, and Hb [app.35-45%]; ↓ albumin and globulin [app. 15-35%]; ↑ BUN [app. 10-50%]; Sponsor considered changes to be a "consequence of the experimental design"

[Note: Animals were not pre-treated with Benadryl as indicated in the review.]

vii. Study Title - Cardiovascular effects after a 45 minute infusion and/or bolus injection of benzoporphyrin derivative monoacid ring A (BPD-MA), a photodynamic therapy agent without photoactivation in the anesthetized micro swine [Ref. 130]
Dose/Species – 2 mg/kg – infusion of 1 ml/min X 10 min, then 3 ml/min X 35 min; 1 week later – pretreatment with Benadryl and bolus [7 ml/min]
Summary Results
1 – Variable but mild effects on heart rate and/or blood pressure in some males.
2 - Following the bolus injection, there were no treatment-related CV changes with the exception of a mild, transient ↑ then ↓ in BP and ↑ HR in 3/3 males and slight ↑ in BP and HR in 1/2 females within 1 minute, values returned to baseline by 1 hour
3 - QT prolongation was considered an expected effect of anesthesia [assessment by Dr. D.K. Detweiler, VMD, Diplomate, ACVIM, Cardiology]

viii. Study Title - Cardiovascular effects after a rapid intravenous bolus of benzoporphyrin derivative monoacid ring A (BPD-MA), a photodynamic therapy agent without photoactivation in the conscious micro swine [Ref. 131]
Dose/Species – 2 mg/kg at a rate of 28 ml/min.
Summary Results
1 – transient ↓ in CO and stroke volume and ↑ in peripheral resistance shortly after dosing with return to baseline by 15 min.
2 – Ventricular extrasystoles observed after both test article and saline – attributed to intracardiac catheter presence [assessment by Dr. D.K. Detweiler, VMD, Diplomate, ACVIM, Cardiology]
3 - Several changes in clinical pathology: ↓ RBC counts, PCV, and Hb [app.15-40%]; ↓ albumin and globulin [app. 15-25%]; ↑ AST [app. 50%]; ↑ glucose [app. 50-70%]; Sponsor considered changes to be a "consequence of the experimental design"

ix. Study Title - Effect on complement level [CH50] of rapid bolus iv injection of BPD-MA samples from Bio-research [Ref. 132]

Dose/Species – 2 mg/kg at a rate of 28 ml/min. – conscious pigs [strain not indicated

Summary Results – Complement activation ↑ 0-20% at 3 min, ↑ 0-35% at 5 min., and generally ↑ approximately 0-25% at ≥15 min.

Summary of Safety Pharmacology-

Studies were conducted in mice, rats, rabbits, and guinea pigs to evaluate the general pharmacological effects of BPD-MA. Under the conditions tested, BPD-MA was negative in the Irwin Test and in tests evaluating the CNS, respiratory, cardiovascular, and renal systems. With the exception of a significant decrease in the plasma BSP clearance in rats at 20 mg/kg, BPD-MA did not demonstrate any GI effects. No change in BSP clearance was observed at 2 mg/kg of BPD-MA. The mechanism for the alteration in BSP clearance was not identified.

Cardiovascular effects were further evaluated in both dogs and pigs. In conscious and anesthetized dogs [N=1-2/sex] at doses ranging from 2-20 mg/kg [5 ml/min.] without photoactivation, there were no effects on mean blood pressure, heart rate, cardiac output or ECGs. QT wave prolongation was observed in one animal which, according to Dr. Detweiler, was in response to anesthesia. There was complement depletion in both conscious and anesthetized dogs administered 20 mg/kg by 5 minutes post injection with peak depletion of approximately 30-40%. Levels were still depleted by 10-26% at 60 minutes. The results of these studies should be interpreted cautiously because of the small N. A second study was conducted in anesthetized beagles that indicated at ≥10 mg/kg, there was a decrease in CO and an increase in MAP. However, determination of the relationship of these findings to BPD-MA is not possible. First, there were no vehicle or saline controls included to assess volume effects. Second, several of the dogs exhibited signs of photosensitivity. It is unclear as to how the photosensitivity impacted the results.

A series of studies were conducted in pigs, which were intended to support bolus administration in humans. Bolus administration of BPD-MA in anesthetized pigs resulted in profound cardiovascular events including marked decreases in BP, HR, CO as well as cardiovascular collapse. ECG changes, such as ST segment depression, were consistent with hypoxia. Similar CV effects were not observed in conscious pigs or if the drug was administered as an infusion in anesthetized pigs. As in the dog, BPD-MA administration was associated with an increase in complement activation. The degree and rate of complement activation were greater in anesthetized vs. conscious pigs. Pretreatment with Benadryl resulted in an abrogation of the CV effects, although complement activation was unaltered. The Sponsor suggested, therefore, that there was a correlation between the rate of infusion, the rate and magnitude of complement activation, and the onset of cardiovascular events in anesthetized swine. The Sponsor cites an article that indicates that complement is activated by negatively charged phospholipids such as egg phosphatidylglycerol [Cohnn, A. et. al. [1991]. The role of surface charges in the activation of the classical and alternative pathways of complement by liposomes. *J. Immunol.* 146:4234-4241]. Unfortunately, the Sponsor did not utilize a liposomal control in any of these studies, which would have provided strong support for their argument.

References 127, 129 and 131 [bolus injection in conscious and anesthetized pigs] indicated that there were changes in several clinical pathology parameters when compared to baseline

values. These changes included [1] decreases in RBC, PCV, and Hb of approximately 20-40% with decreases apparently greater in anesthetized animals; [2] decreases in albumin and globulin of approximately 15-20%; [3] increases in AST ranging from approximately 50-140%; [4] mild increases in BUN ranging from approximately 20-50%; and/or [5] increases in glucose of approximately 50%. The Sponsor attributed these changes to experimental design. However, since no concurrent controls were provided and similar findings were observed in the toxicology studies, a treatment-related effect is considered possible.

Several gross and histopathological findings were described that were attributed to background incidence or catheter implantation. Although this would be plausible, neither historical nor concurrent control data were provided to support this conclusion.

Pharmacokinetics/Toxicokinetics:

I. Pharmacokinetics/Biodistribution

A. *In Vitro* Studies

a. Uptake and Release Kinetics -Two studies were conducted which evaluated the uptake and release kinetics of BPD-MA and the results will only be summarized here. These results, as well as additional *in vivo* data, are discussed in the literature citation [iii].

i. Ref. 39: Uptake and release kinetics of the photosensitizers liposomal BPD-MA, NC0060, and NC0069 [Study Identification: PH-97006]

ii. Ref. 36: Kinetics of uptake and release of liposomal [L-BPD] and aqueously [A-BPD] formulated BPD by tumor and normal cells in vitro [Study Identification: PH-93013] *Dr. Javier Avalos previously reviewed this study for IND Submission pp. 29-31; review completed May 16, 1995.*

iii. Ref. 37. Richter, A.M., et. al. [1994]. Kinetics of cellular uptake and retention of the benzoporphyrin derivative [BPD]; relevance to photodynamic therapy. *SPIE*, **2325:189-197.**

Results - The *in vitro* systems for the 2 studies were human endothelial cell line, human and mouse leukemia cell lines, and normal mouse spleen cells ± activation. There were some differences between the two studies that the Sponsor attributed to differences in experimental conditions and methods of detection. However, the following generalizations seem appropriate. Uptake was rapid with a maximum concentration [saturation] observed within 20-30 minutes. In one study [PH-93013], within 30 minutes after washing cells and placing them in medium only, the majority of the drug was present in the medium. However, in the second study after 24 minutes, the concentration of BPD-MA was decreased by only 10% [based on fluorescence intensity]. 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 longer 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 at 15 minutes, 1, 3, 24, 48, and 72 hours following a 10 mg/kg iv injection of BPD-MA and irradiation, was decreased by approximately 50-60% at all time points.

b. Title: Demonstration of the transient existence of liposomes in the presence of plasma [Ref. 46]
Study Identification: PH-97009
Site: [redacted]
Study Dates: September - December 1997
Formulation and Certificate Analysis: Not provided
Final Report (X) December 17, 1997 [Date signed off]
GLP and QA Statements Signed: No (X)
Objective: To determine [1] the mechanism of drug transfer from the liposome to the plasma components and [2] the fate of the liposome following drug administration.

Study Design – [1] Liposomal BPD-MA [0.37 mg/ml] was incubated for 5 minutes with human plasma [0-50% v/v]. Samples were run through agarose gel electrophoresis. [2] Sub-micron particle sizing was conducted following a 5 minute incubation of liposomal BPD-MA [37 μg/ml] with either 9.5% lactose or plasma, or plasma only.

Results and Conclusions – The electrophoresis results indicated that both BPD-MA and the lipid portion of the drug were "acquired by plasma lipoproteins". This suggested that the liposomes were disrupted in the presence of plasma. The sizing experiment supported the electrophoresis results, e.g. "intact liposomes will not be present in the circulation under physiological conditions".

Reviewer's Comment - The Reviewer concurs.

c. **Distribution of BPD-MA in the Plasma** - The following two studies and two literature citations discuss the distribution of BPD-MA in plasma in *in vitro* studies. [It would appear that the literature citations are summaries of the data presented in the study reports.] The findings are summarized below.

i. Ref. 47: The distribution of BPD-MA and liposomal lipid among plasma proteins **[Study Identification: PH-94024]**

ii. Ref.284: Tissue distribution of radioactivity following a single intravenous administration of ^{14}C-BPD-MA in the mouse **[Study Identification: PH-151090]**

iii. **Ref. 52: Alison, B.A., et. al. [1990]. The plasma distribution of benzoporphyrin derivative and the effects of plasma lipoproteins on its biodistribution. *Photochem. Photobiol.* 52[3]:501-507.**

iv. **Ref. 48: Richter, A.M., et. al. [1993] Liposomal delivery of a photosensitizer, benzoporphyrin derivative monoacid ring A [BPD], to tumor tissue in a mouse tumor model. *Photchem photobiol.* 57[6]:1000-1006**

Results – When liposomes were incubated with lipoproteins at low liposome:lipoprotein ratios, the liposomes appeared to be disrupted primarily through interaction with LDL. Agarose gel electrophoresis resolution of liposomal BPD-MA immediately following mixture with human plasma, indicated that the test article was primarily associated with the HDL and/or albumin fraction. Appendix IV of Ref. 284 contains results from an *in vitro* analysis of the distribution of aqueous vs. liposomal radiolabeled BPD-MA in human whole blood and plasma. The greatest

percentage of radiolabel was associated with plasma [approximately 85-95%] after 1, 6, and 24 hours of incubation of ^{14}C-BPD-MA with human whole blood. Less than 10% of the radiolabel was associated with WBCs or RBCs. Of the liposomal BPD-MA in the plasma, approximately 90% was associated with the lipoproteins and approximately 6% with albumin. However, after a one-hour incubation, aqueous BPD-MA was reported to associate with albumin at higher levels [approximately 35%]. The rate of association for aqueous BPD-MA has been reported [Ref. 52] to be 38% of radioactivity with HDL, and 17-18% with LDL and VLDL, each in human plasma without albumin. After 24 hours of incubation, liposomal drug was fairly evenly distributed between each of the 3 lipoprotein fractions.

B. *In Vivo* Studies

a. Mice

i. Title: Richter, et. al. [1990] Biodistribution of tritiated benzoporphyrin derivative [^{3}H-BPD-MA], a new potent photosensitizer, in normal and tumor bearing mice; *J. Photochem. Photobiol.* 5:231-244 [Ref. 360]
Study Identification: NA
Site: NA
Study Dates: NA
Formulation and Certificate of Analysis: NA
Final Report (X) NA
GLP and QA Statements Signed: No (X)
Objective: To determine the biodistribution [blood, brain, heart, intestine, kidney, lung, liver, muscle, skin, stomach, spleen, thymus, and tumor] in normal and tumor-bearing mice.

Dr. Will Coulter previously reviewed this literature citation for IND [redacted] Submission [redacted] final review; p. 25.[redacted] Additional comments by the current Reviewer are provided below in italics.

- *Biodistribution was comparable in normal and tumor-bearing mice. Preliminary clearance data in 5 mice indicated that 60% of the dose was cleared via the bile and approximately 4% via the kidney.*

ii. Title: Tissue distribution of radioactivity following a single intravenous administration of ^{14}C-BPD-MA in the mouse [Ref. 284]
Study Identification: PH-151090
Site: [redacted]
Study Dates: October 11 – December 10, 1990
Formulation and Lot No.: ^{14}C-BPD-MA; No lot number provided
Certificate of Analysis: No (X) Analysis of radiopurity was provided
Final Report (X) December 17, 1990
GLP and QA Statements Signed: No (X)
Objective: "To characterize the distribution and elimination of drug-related radioactivity in tissues of tumor bearing mice, following a single intravenous dose of ^{14}C-BPD-MA, administered in a liposomal formulation".

Dr. Will Coulter previously reviewed this study for IND [redacted] Submission [redacted] final review; p. 24.[redacted] Comments by the current Reviewer are in italics. Results [redacted] are discussed above under Distribution of drug in plasma.

The following table outlines the biodistribution in the mouse.

Table 1 Concentration of radioactivity in mouse tissues (eq equivalent/g tissue) at various times post intravenous injection of ^{14}C-DPD-MA in liposomal formulation at a dose of 4 mg/kg body weight. Each value represents mean ± S.D. of determinations on 3 mice.

TISSUE	15 min.	3 h	24 h	168 h
BLOOD	7.189 ± 0.389	3.584 ± 1.046	0.304 ± 0.029	0.016 ± 0.004
PLASMA	8.948 ± 0.304	4.444 ± 1.088	0.366 ± 0.018	0.021 ± 0.005
ADRENALS	13.191 ± 2.607	4.038 ± 1.195	0.576 ± 0.170	0.225 ± 0.110
BLADDER	0.817 ± 0.195	0.644 ± 0.043	0.119 ± 0.032	0.035 ± 0.004
BONE	0.657 ± 0.073	0.302 ± 0.171	0.050 ± 0.014	0.027 ± 0.004
BONE AND MARROW	1.943 ± 0.221	1.225 ± 0.283	0.140 ± 0.011	0.063 ± 0.009
BRAIN	0.134 ± 0.026	0.064 ± 0.009	0.007 ± 0.002	0.000 ± 0.000
EAR (SKIN)	0.559 ± 0.053	0.591 ± 0.142	0.191 ± 0.033	0.063 ± 0.006
EPIDIDYMIS	0.654 ± 0.186	0.511 ± 0.059	0.142 ± 0.011	0.064 ± 0.008
ESOPHAGUS	1.550 ± 0.330	1.085 ± 0.645	0.169 ± 0.034	0.047 ± 0.017
EYE	0.437 ± 0.156	0.258 ± 0.071	0.061 ± 0.012	0.009 ± 0.002
FAT	2.573 ± 0.609	1.603 ± 0.412	0.098 ± 0.033	0.049 ± 0.011
GALL BLADDER	120.265 ± 57.653	167.823 ± 84.650	3.015 ± 0.678	0.164 ± 0.085
HEART	6.247 ± 1.534	2.366 ± 0.729	0.162 ± 0.012	0.103 ± 0.035
LARGE INTESTINE	1.468 ± 0.241	1.301 ± 0.017	0.300 ± 0.058	0.036 ± 0.009
SMALL INTESTINE	3.069 ± 0.759	7.094 ± 1.005	0.256 ± 0.051	0.052 ± 0.013
KIDNEY	8.345 ± 2.172	5.407 ± 1.466	0.543 ± 0.001	0.178 ± 0.024
LIVER	49.065 ± 6.548	19.696 ± 7.670	2.010 ± 0.173	0.816 ± 0.098
LUNG	7.575 ± 0.878	3.150 ± 0.959	0.201 ± 0.033	0.120 ± 0.054
LYMPH NODES	2.850 ± 0.819	2.410 ± 0.170	0.620 ± 0.201	0.237 ± 0.026
MUSCLE	1.241 ± 0.216	0.750 ± 0.225	0.038 ± 0.013	0.012 ± 0.002
PANCREAS	2.438 ± 0.251	2.461 ± 0.315	0.205 ± 0.022	0.037 ± 0.003
SALIVARY GLANDS	2.131 ± 0.056	1.789 ± 0.438	0.210 ± 0.030	0.091 ± 0.051
SPLEEN	4.817 ± 1.023	2.759 ± 0.493	0.412 ± 0.018	0.154 ± 0.041
STOMACH	1.403 ± 0.370	1.259 ± 0.189	0.215 ± 0.117	0.040 ± 0.004
TESTIS	0.175 ± 0.080	0.201 ± 0.056	0.094 ± 0.021	0.058 ± 0.011
TUMOR	2.003 ± 0.294	1.882 ± 0.768	0.713 ± 0.218	0.066 ± 0.017

The Sponsor indicated that 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.

iii. Ref. 48: Richter, A.M., et. al. [1993] Liposomal delivery of a photosensitizer, benzoporphyrin derivative monoacid ring A [BPD], to tumor tissue in a mouse tumor model. *Photchem photobiol.* 57[6]:1000-1006 The following tissue half-lives were calculated between 15 minutes and 24 hours in mice administered radiolabeled aqueous or liposomal BPD-MA at 4 mg/kg.

Table 1. Half-lives of the radioactivity in mouse tissues following intravenous injection of liposomal (L) and aqueously formulated (A) ^{14}C-BPD, at 4 mg/kg, calculated for a period between 15 min and 24 h postinjection

	Tissue half-lives (h)	
Tissue	L-^{14}C-BPD	A-^{14}C-BPD
Liver	5.2	5.13
Tumor	16.1	16.9
Skin	15.4	22.3
Lung	4.6	5.5
Fat	5.1	6.5
Spleen	6.8	6.0

b. Rats

i. Title: <u>Excretion and mass balance of radioactivity following a single intravenous administration of 4.0 mg/kg ^{14}C- BPD-MA in the rat [Ref. 354]</u>
Study Identification: PH-011090
Site: [redacted]
Study Dates: October 30 – December 7, 1990
Formulation and Lot No.: ^{14}C-BPD-MA; Lot No. not found
Certificate of Analysis: No (X)
Final Report (X) December 14, 1990
GLP and QA Statements Signed: No (X) [QA statement for study portion conducted at Phoenix International Life Sciences]
Objective: To determine the rate, route and elimination, based on radioactivity, of an intravenous dose of BPD-MA in male and female Sprague-Dawley rats.

Dr. Will Coulter previously reviewed this study for IND [redacted] Submission [redacted] final review; pp. 23-24 [redacted]

ii. Title: <u>Mass balance and excretion of radioactivity following a single intravenous dose of ^{14}C-CL 318,952 [BPD-MA] in the rat [Ref. 288]</u>
Study Identification: TX-94038
Site: [redacted]
Study Dates: September 22 – November 30, 1993
Formulation and Lot No.: Liposomal BPD-MA; ^{14}C-BPD-MA; Lot No. D93-120-0850
Certificate of Analysis: Yes (X)
Final Report (X) March 4, 1994
GLP and QA Statements Signed: Signed QA Statement - Yes (X); Sponsor indicates GLP study but no signed statement was included

Objective: "[1] To assess the rate, route, and extent of elimination of radioactivity following a single 4.0 mg/kg intravenous dose of ^{14}C-CL 318,952 [BPD-MA] in the rat".

Dr. Javier Avalos previously reviewed this study for IND [redacted] Submission [redacted] review completed May 16, 1995. This review is provided below.

Mass balance and excretion of radioactivity following a single intravenous dose of ^{14}C-CL318,592 (BPD-MA) in the rat [GLP study]

Laboratory: [redacted]
(Study Number HWI 6123-197)

Animal Strain: Crl:CD (SD)BR rats [redacted]

Animal Starting Weight: 257-274 g

No. of Animals: 5 male animals used through out study; individually housed

Test Materials: 25 mg sample of lyophilized BPD-MA (batch number D93-120-0850) was reconstituted with 11.5 ml of sterile water for injection and 2.7 ml of this solution were removed and added to 7.6 ml of 14C-CL318,592 yielding a total dosing solution volume of 10.3 ml. The final solution concentration was 2.43 mg/ml and corresponded to 1.04 mg/g (5.325 x 107 dpm/g) of radioactive CL318,592.

Route: Intravenous infusion (rate not specified)

Dose: 4 mg/kg

Methods: Each animal received a single 4 mg/kg intravenous dose of label BPD-MA dissolved in sterile water. The animals were housed in individual glass metabolism cages for the separation and collection of expired air, urine, and feces. Urine was collected at 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours postdose. Expired air and feces were collected at 24-hour intervals up to 168 hours postdose. Carcasses were retained and analyzed for radioactivity content. After proper preparation, all material was analyzed by liquid scintillation.

Results: *The total recovery of radioactivity was 94.5%.* Feces was the major route of elimination with 90.3% of the dose being excreted via this route. Fifty-three and seven tenths percent was eliminated within the first 24 hours. An additional 28.7% was eliminated in the 24-48 hour interval. Recovery from the urine represented less than 1% of the administered dose and the carcass retained 3.25% of the dose. No radioactivity was detected in the expired air.

iii. Title: An intravenous single dose pharmacokinetic study of verteporfin in Sprague-Dawley rats including assessment of BPD-DA and enantiomer ratios [Ref. 361]
Study Identification: PK-98001
Site: [redacted]
Study Dates: March 27, 1998 – May 7, 1999
Formulation and Lot No.: Liposomal BPD-MA; Lot No. TC0715; dosing solution was prepared on the day of dose administration
Certificate of Analysis: Yes (X)

QLT Phototherapeutics, Inc.

Method Validation Report: [redacted]
Final Report (X) May 7, 1999
GLP and QA Statements Signed: Yes (X)
Objective: "[1] To assess the pharmacokinetic profile of verteporfin and its main metabolite BPD-DA following single intravenous administration... and to assess the time course of the enantiomer ratios of verteporfin regioisomers and BPD-DA after injection".

Study Design- Sprague-Dawley rats [N= 6/sex/group] were administered verteporfin by an iv bolus at 2 mg/kg. Animals were maintained in light of <20 foot candles. Blood was collected via femoral cannulation at 0, 1, 15, 60, 240, and 480 minutes post dosing. Three animals per sex were used for the first 3 time points and the remaining 3 animals/sex for the second set of time points. Plasma samples were analyzed for BPD-DA, BPD-MA_c and BPD-MA_d by HPLC with UV detection. Concentration of enantiomers was determined using capillary electrophoresis with laser-induced fluorescence [CE-LIF].

Results – The table below delineates the AUC_{0-t}, AUC_{0-inf}, C_{max}, t_{max}, $t_{1/2}$, clearance, and Vss for the BPD-DA, BPD-MA_c and BPD-MA_d in males and females.

Parameter	AUC_{0-t} µg•hr/ml	AUC_{0-inf} µg•hr/ml	C_{max} µg /ml	t_{max} hrs	$t_{1/2}$ hrs	Clearance ml/min/kg	Vss L/kg
BPD-MA_c							
Males	2.533	3.475	7.460	0.017	6.94	4.80	1.00
Females	2.870	3.438	10.237	0.017	4.69	4.85	0.70
BPD-MA_D							
Males	6.764	10.182	12.90	0.017	7.29	1.64	0.4
Females	8.451	9.310	14.967	0.017	2.67	1.79	0.24
BPD-DA							
Males	1.087	-	0.848	0.017	-	-	-
Females	1.246	2.031	0.941	0.017	6.41	-	-
Verteporfin							
Males	9.297	13.653	20.360	0.017	7.21	2.44	0.57
Female	11.323	12.628	25.203	0.017	2.94	2.64	0.35

The enantiomer data were presented graphically and indicated that there was some stereospecific disposition of the enantiomers.

Sponsor's Conclusions

1. All analytes exhibit a bi-exponential decline characterized by a "very rapid distribution phase followed by a slower elimination phase".

2. BPD-DA disposition is similar to the regioisomers and there is no accumulation. Exposure to this metabolite represents <10% of verteporfin exposure based on AUC.

3. Relative exposure to BPD-MA_D is greater than to BPD-MA_C.

4. AUC_{0-t} is slightly [approximately 20-25%] greater in females than in males. However, the AUC_{inf} is similar. A significant portion of the AUC_{inf} is extrapolated since the final sampling time point was at 8 hours following drug administration. This appears to have resulted in a longer $t_{1/2}$ than previously reported.

5. There is some stereospecific disposition of enantiomers.

Reviewer's Comment – In the toxicokinetic studies in rats, the exposure tended to be greater in males than in females. The reason that exposure is greater in females in the current study is not known. Despite this discrepancy and the imprecision resulting from extrapolation of AUC_{inf} from a final sampling time point of 8 hours, the values for C_{max} and AUC_{inf} in this study are comparable to those observed in Study 92020 [14-day repeat dose rat toxicity study]. In the current study, the C_{max} is slightly greater and the AUC_{inf} is less [approximately 30-40%] than these values in Study TX-90601 [28-day repeat dose rat toxicity study].

c. Microswine

i. Title: Plasma clearance of liposomal BPD in Yucatan micro swine [Ref. 362]
Study Identification: TX-94029
Site: [redacted]
Study Dates: May 1994 –January 1995
Formulation and Lot No.: Liposomal BPD-MA; Not provided [test materials contained ±10% of expected concentration]
Certificate of Analysis: Not provided
Final Report (X) January 30, 1995
GLP and QA Statements Signed: No (X)
Objective: "To determine the plasma clearance of liposomal BPD in male and female conscious and anesthetized Yucatan micro swine after a slow, fast or rapid intravenous infusion"

Study Design – Blood samples were collected from male and female Yucatan micro swine administered 2 mg/kg of liposomal BPD-MA [These animals were evaluated for CV effects without photoactivation in studies TX-94007 [93003], TX-94009 [93004], TX-94010 [93005], TX-94014 [93006], TX-94018 [93019]. Animals [generally N=3/sex] were either anesthetized (TX-94010 [93005], TX-94014 [93006]) or conscious (TX-94007 [93003], TX-94009 [93004], TX-94018 [93019]). The rate of drug administration was slow over 45 minutes (TX-94007 [93003], TX-94014 [93006]), fast at 7 ml/min (TX-94009 [93004], TX-94010 [93005]), or rapid at 15-19 ml/min (TX-94018 [93019]). Blood samples were collected prior to dosing, 5, 15, 30, 45, 60, 90, and 120 minutes following start of slow infusion; or prior to dosing, 3, 5, 15, 30, 45, 60 minutes following start of fast or rapid infusion

Results – The table below delineates the results

Study No.	No. Animals	Concentration of BPD (ug/mL) From The Start of Infusion (minutes)							C_{max} ug/mL	AUC ug.min/mL
		5	15	30	45[2]	60	90	120		
93003 Week 1	M - 3 F - 3	.649 .809	2.88 3.21	2.83 4.13	2.67 4.63	.683 1.21	.379 .491	.262 .303	2.7 4.6	176 243
93003 Week 2	M - 3 F - 3	.710 .820	2.65 3.21	3.00 4.19	3.00 4.67	.868 1.33	.439 .655	.326 .415	3.0 4.7	200 262
93003 Week 3	M - 3 F - 3	.660 1.04	2.76 3.60	3.11 4.76	2.76 4.97	1.03 1.62	.538 .768	.381 .517	2.8 4.9	205 306
93006	M - 3 F - 3	1.27 1.15	4.94 4.75	7.13 7.03	6.52 6.96	3.24 3.44	1.74 1.97	1.18 1.38	6.5 7.0	514 553
		Concentration of BPD (ug/mL) After Injection (minutes)								
		3	5	15	30	45	60			
93004	M - 3 F - 3	19.83 14.86	8.16 5.95	1.83 1.51	.936 .839	.719 .693	.517 .487		93 119	252 234
93005	M - 3 F - 3	47.9 33.6	16.15 17.02	7.18 4.76	3.94 2.60	2.78 1.92	2.18 1.52		503 112	953 476
93006	M - 3 F - 3	24.7 27.1	16.12 14.10	4.75 3.54	2.33 2.15	1.55 1.43	1.17 1.10		50 89	359 381
93019	M - 2 F - 3	11.39 10.15	5.31 4.88	1.36 1.46	.792 .876	.580 .628	.382 .502		44 38	156 152

[1] All animals were dosed with 2.0 mg/kg BPD-MA

[2] End of infusion

Sponsor's Conclusions

1. Values for the various PK parameters in anesthetized animals were increased over those observed in conscious animals.

2. PK values were comparable following a fast and rapid infusion in conscious animals.

Reviewer's Comment - The Reviewer concurs with the following caveat. The calculation of AUC based on a final sampling time of either 60 or 120 minutes may introduce significant imprecision.

d. Nonhuman Primates

i. Title: A single dose intravenous pharmacokinetic study of liposomal benzoporphyrin derivative monoacid ring A [BPD-MA; CL-318,952] in male cynomolgus monkeys [Ref. 334]
Study Identification: PK-94001
Site:
Study Dates [in-life]: March 29-30, 1994
Formulation and Lot No.: Liposomal BPD-MA; Batch No.F93-120-0880
Certificate of Analysis: Yes [X]
Final Report (X) October 18, 1994
GLP and QA Statements Signed: Yes (X)

Objective: "To determine the pharmacokinetics of Liposomal Benzoporphyrin Derivative Monoacid Ring A ...., verteporfin, in male cynomolgus monkeys after a single intravenous dose."

Dr. Javier Avalos previously reviewed this study for IND [ ] Submission [ ] review completed May 16, 1995. This review is provided below. Comments by the current Reviewer are provided in italics.

A single dose intravenous pharmacokinetic study of BPD-MA in male cynomolgus monkeys (GLP study)

Laboratory: [redacted]
(Study Number 940211)

Animal Strain: male Cynomolgus monkeys [redacted]

Animal Starting Weight: 4.96-7.02 kg

No. of Animals: 8 animals; individually housed

Test Materials: Thirty mg of lyophilized powder of BPD-MA (batch number F93-120-0880) was reconstituted with 14 ml of sterile water for injection (batch number 330291).

Route: Single intravenous bolus dose

Study Design:

Group Number	Dose Level (mg/kg)	Dose Concentration (mg/ml)
1	0.375	2
2	1.0	2

Methods: Animals were given an intravenous dose of BPD-MA based on body weight. Animals were transferred into private restraint chairs and a catheter inserted into the saphenous or cephalic vein for dose administration. During the experiment, animals were observed twice daily for pharmacologic and toxicologic signs and general well being. To facilitate blood sampling, animals remained in the restraint chairs for 3.5 hours postdose. The animals were bled at predose, 5, 10, 15, 20, 25, 30, 80, 140, and 210 minutes, and 24 hours after dosing. Blood samples were centrifuged and plasma separated from cellular components. Plasma was divided into two samples and shipped [redacted] for analysis. No animals were scheduled for sacrifice. However, one animal (PR0750) died 3.5 hours after dosing. For this animal, gross and histopathological examinations were performed.

Results:

Mortality: No treatment-related deaths were reported. The death observed at 3.5 hours after BPD-MA injection was concluded to be a result of the animal being "an extreme responder to stress-stimuli (chair-restraint)."

Clinical Observations: Normal observations were reported for the animals assigned to this study with the exception of one animal from Group 2 (PR0750). Within 1 hour after dosing, this

animal was hypoactive and pale in color. During the next hourly observation the animal vomited. At the 3-hour observation, PR0750 was observed to be hypoactive, pale in color, and have dilated pupils. The animal died approximately 3.5 hours after dosing. Similar responses were noted for this animal in a previous pharmacokinetic study.

Body Weights: Body weights were used in dose calculations.

Gross and Microscopic Pathology: Moderate diffuse chronic pleuritis was observed for the lung suggesting a condition which predated the start of this study. Marked diffuse lymphoid depletion of the thymus observed macroscopically and confirmed histopathologically provided evidence to support the longstanding nature of the pulmonary lesion. Multifocal endocardial hemorrhage for the heart observed histopathologically correlated with the macroscopic observation of mild multifocal endocardial red discoloration. This was interpreted as an agonal event attributed to terminal hypotension and not an effect of administration of BPD-MA.

Table 11. Plasma levels of CL315,555 in male cynomolgus monkeys administered a bolus intravenous injection of BPD-MA.

Nominal time	Group I (0.375 mg/kg)/µg per mL					
(h)	PR0207	PR0222	PR0688	PR0181	Mean ±	SD
0.00	0.000	0.000	0.000	0.000	0.000	0.000
0.08	2.160	0.913	0.467	1.490	1.258	0.733
0.17	0.702	0.664	0.222	0.547	0.534	0.218
0.25	0.355	0.359	0.151	0.266	0.283	0.098
0.33	0.210	0.214	0.106	0.192	0.181	0.051
0.42	0.173	0.200	0.091	0.180	0.161	0.048
0.50	0.129	0.146	0.074	0.110	0.115	0.031
1.33	0.114	0.072	0.000	0.000	0.047	0.056
2.33	0.081	0.000	0.000	0.000	0.020	0.041
3.50	0.000	0.000	0.000	0.000	0.000	0.000
24.00	0.000	0.000	0.000	0.000	0.000	0.000

[a] *Quantity of blood sample obtained was not sufficient for plasma harvest, therefore no sample was shipped for analysis.*
[b] *Limit of quantitation (0.0511 µg/mL) adjusted for sample dilution. Result is < 0.102 µg/mL.*
[c] *Animal was an observed mortality approximatey 3.5 h after dose administration.*
[d] *Sample quantity was insufficient for analysis.*

Nominal time	Group II (1.0 mg/kg)/µg per mL					
(h)	PR0660	PR0750	PR0756	PR0764	Mean ±	SD
0.00	0.000	0.000	0.000	0.000	0.000	0.000
0.08	a	2.220	3.760	3.350	3.110	0.798
0.17	1.430	1.300	1.340	1.410	1.370	0.061
0.25	0.919	0.917	0.912	0.805	0.888	0.056
0.33	0.526	0.653	0.599	0.656	0.609	0.061
0.42	0.458	0.623	d	0.531	0.537	0.083
0.50	0.393	0.559	0.178	0.667	0.449	0.213
1.33	0.149	0.351	0.403	0.226	0.282	0.116
2.33	0.074	0.236	0.232	0.165	0.177	0.076
3.50	0.093	0.242	0.080	0.127	0.135	0.074
24.00	b	c	0.000	0.000	0.000	0.000

[a] *Quantity of blood sample obtained was not sufficient for plasma harvest, therefore no sample was shipped for analysis.*
[b] *Limit of quantitation (0.0511 µg/mL) adjusted for sample dilution. Result is < 0.102 µg/mL.*
[c] *Animal was an observed mortality approximatey 3.5 h after dose administration.*
[d] *Sample quantity was insufficient for analysis.*

Table 12. Plasma levels of CL315,585 [*BPD-MA$_D$*] in male cynomolgus monkeys administered a bolus intravenous injection of BPD-MA.

Nominal time	Group I (0.375 mg/kg)/ µg per mL					
(h)	PR0207	PR0222	PR0688	PR0161	Mean ±	SD
0.00	0.000	0.000	0.000	0.000	0.000	0.000
0.08	3.330	1.870	1.100	2.770	2.268	0.984
0.17	1.460	1.470	0.555	1.320	1.201	0.436
0.25	0.757	0.825	0.338	0.694	0.654	0.217
0.33	0.386	0.461	0.213	0.444	0.376	0.113
0.42	0.265	0.402	0.161	0.394	0.306	0.115
0.50	0.156	0.267	0.131	0.218	0.193	0.061
1.33	0.112	0.105	0.053	0.064	0.084	0.029
2.33	0.081	0.000	0.000	0.000	0.020	0.041
3.50	0.050	0.000	0.000	0.000	0.013	0.025
24.00	0.000	0.000	0.000	0.000	0.000	0.000

[a] *Quantity of sample obtained was not sufficient for plasma harvest, therefore no sample was shipped for analysis.*
[b] *Limit of quantitation (0.0489 µg/mL) adjusted for sample dilution. Result is < 0.0978 µg/mL.*
[c] *Animal was an observed mortality approximatey 3.5 h after dose administration.*
[d] *Sample quantity was insufficient for analysis.*

Nominal time	Group II (1.0 mg/kg)/ µg per mL					
(h)	PR0660	PR0750	PR0756	PR0764	Mean ±	SD
0.00	0.000	0.000	0.000	0.000	0.000	0.000
0.08	a	4.380	5.770	5.860	5.337	0.830
0.17	3.060	2.880	2.710	3.160	2.953	0.199
0.25	2.030	2.000	1.820	1.850	1.925	0.105
0.33	1.130	1.250	1.050	1.400	1.208	0.152
0.42	0.860	1.030	d	1.030	0.973	0.098
0.50	0.068	0.867	0.247	1.210	0.598	0.533
1.33	0.191	0.409	0.626	0.295	0.380	0.186
2.33	0.094	0.329	0.257	0.200	0.220	0.099
3.50	0.113	0.431	0.124	0.151	0.205	0.152
24.00	b	c	0.059	0.000	0.030	

[a] *Quantity of sample obtained was not sufficient for plasma harvest, therefore no sample was shipped for analysis.*
[b] *Limit of quantitation (0.0489 µg/mL) adjusted for sample dilution. Result is < 0.0978 µg/mL.*
[c] *Animal was an observed mortality approximatey 3.5 h after dose administration.*
[d] *Sample quantity was insufficient for analysis.*

Quantitative Levels of BPD Isomers (CL315,555 [*BPD-MA$_C$*] and CL315,585 [*BPD-MA$_D$*]) in plasma: The results were supplied by the Sponsor and are listed in Tables 11 and 12. The maximum concentration of each isomer was detected 5 minutes after injection of BPD-MA. The isomer CL315,555 was completely excreted by 2.33 hours after a dose of 0.375 mg/kg BPD-MA while CL315,585 was not completely excreted until 3.5 hours following the same injection dose. A higher dose of BPD-MA (1 mg/kg) resulted in both isomers being retained longer by the animals. At this higher dose, CL315,555 was completed excreted by 3.5 hours after dosing while CL315,585 was still present 24 hours after injection.

The pharmacokinetic parameters for this study are delineated in the following tables. The Sponsor indicates that the long interval between the 3.5-hour and 24-hour samples might have

"lead to an overestimation of AUC for those subjects with a non-zero concentration value at 3.5 hours".

NONCOMPARTMENTAL PARAMETERS BY ISOMER AND DOSE GROUP

BPDMA-1:

ID	GROUP	C_{MAX}	AUC	CL_T	V_{SS}	$t_{1/2}$
		µg/ml.	µg·min/ml	ml/min/kg	l/kg	hr
PRO181	0.375 mg/kg	1.49	16.40	11.43	0.15	
PRO207	0.375 mg/kg	2.16	32.08	5.67	0.26	2.69
PRO222	0.375 mg/kg	0.91	19.74	9.50	0.27	
PRO688	0.375 mg/kg	0.47	7.23	25.95	0.42	
	Mean	1.26	19.11	13.14	0.28	
	SD	0.73	10.71	8.67	0.11	
PRO660	1.0 mg/kg	1.43	47.28	10.57	0.59	3.32
PRO750	1.0 mg/kg	2.22	87.05	5.74	0.41	4.17
PRO756	1.0 mg/kg	3.76	129.01	3.88	0.46	2.13
PRO764	1.0 mg/kg	3.35	157.81	3.17	0.42	2.62
	Mean	2.69	105.29	5.84	0.47	3.06
	SD	1.06	48.37	3.34	0.08	0.89

BPDMA-2:

ID	GROUP	C_{MAX}	AUC	CL_T	V_{SS}	$t_{1/2}$
		µg/ml	µg·min/ml	ml/min/kg	l/kg	hr
PRO181	0.375 mg/kg	2.77	37.60	4.99	0.10	
PRO207	0.375 mg/kg	3.33	79.36	2.36	0.25	1.86
PRO222	0.375 mg/kg	1.87	38.26	4.90	0.12	
PRO688	0.375 mg/kg	1.10	18.37	10.20	0.26	
	Mean	2.27	43.40	5.61	0.18	
	SD	0.98	25.69	3.29	0.08	
PRO660	1.0 mg/kg	3.06	82.29	6.08	0.27	3.00
PRO750	1.0 mg/kg	4.38	140.51	3.56	0.24	
PRO756	1.0 mg/kg	5.77	234.62	2.13	0.68	9.71
PRO764	1.0 mg/kg	5.86	227.15	2.20	0.25	2.26
	Mean	4.77	171.14	3.49	0.36	4.99
	SD	1.32	73.03	1.84	0.21	4.10

ii. Title: Pharmacokinetic studies in pigtail macaque monkeys [Macaca nemistrina], following fast infusion of liposomal BPD-MA [Ref. 363]
Study Identification: PH-94023
Site: [redacted]
Study Dates: May 1992 –July 1992
Formulation and Lot No.: Liposomal BPD-MA; Batch No.F93-120-0887
Certificate of Analysis: No (X)
Final Report (X) December 12, 1994
GLP and QA Statements Signed: No (X)
Objective: "To determine the pharmacokinetics of BPD-MA in monkey plasma, following fast infusion."

Dr. Javier Avalos has reviewed this study for IND [redacted] Submission [redacted] review completed May 16, 1995. This review is provided below.

Pharmacokinetic studies in pigtail macaques monkeys following fast infusion of liposomal BPD-MA (non-GLP study)

Laboratory: [redacted]