CHO/HGPRT GENE MUTATION ASSAY
RESULTS – WITH ACTIVATION
DEFINITIVE

TEST ARTICLE: Verteporfin for Injection SITEK STUDY NO.: 0527-2510
EXPERIMENT NO.: B-1 SOLVENT: 5% Dextrose, H_2O

Test Art. Conc. µg/mL		Ave. No. of C.E.* Colonies Per Plate	Standard Deviation	C.E.	Total Mutants Counted	No. of Plates Seeded	No. of Plates Counted	Mutants/ 10^6 Surviving Cells
SOLVENT	A	131	+/- 4.9	65.5%	5	12	12	3
SOLVENT	B	155	+/- 8.7	77.5%	18	12	12	10
1.0	A	145	+/- 16.0	72.5%	20	12	12	11
1.0	B	165	+/- 21.0	82.5%	2	12	12	1
5.0	A	115	+/- 5.1	57.5%	4	12	12	3
5.0	B	116	+/- 12.2	58.0%	2	12	12	1
10	A	125	+/- 9.0	62.5%	7	12	12	5
10	B	160	+/- 12.9	80.0%	1	12	12	1
50	A	154	+/- 6.1	77.0%	15	12	12	8
50	B	97	+/- 6.5	48.5%	8	12	12	7
100	A	145	+/- 6.9	72.5%	2	12	12	1
100	B	129	+/- 7.5	64.5%	6	12	12	4
DMBA SOL. CONT.	A	134	+/- 18.0	67.0%	6	12	12	4
	B	133	+/- 20.8	66.5%	15	12	12	9
DMBA (5.0 µg/mL)	A	61	+/- 4.6	30.5%	119	12	12	163
	B	63	+/- 3.5	31.5%	119	12	12	157

Solvent for DMBA is acetone

CHO/HGPRT GENE MUTATION ASSAY
RESULTS – WITHOUT ACTIVATION
DEFINITIVE

TEST ARTICLE: Verteporfin for Injection SITEK STUDY NO.: 0527-2510
EXPERIMENT NO.: B-1 SOLVENT: 5% Dextrose, H_2O

Test Art. Conc. µg/mL		Ave. No. of C.E.* Colonies Per Plate	Standard Deviation	C.E.	Total Mutants Counted	No. of Plates Seeded	No. of Plates Counted	Mutants/ 10^6 Surviving Cells
SOLVENT	A	152	+/- 24.2	76.0%	10	12	12	5
SOLVENT	B	149	+/- 7.6	74.5%	9	12	12	5
1.0	A	130	+/- 14.3	65.0%	10	12	12	6
1.0	B	173	+/- 13.7	86.5%	19	12	12	9
5.0	A	132	+/- 3.1	66.0%	9	12	12	6
5.0	B	137	+/- 14.0	68.5%	18	12	12	12
10	A	113	+/- 12.9	56.5%	3	12	12	2
10	B	149	+/- 12.7	74.5%	15	12	12	8
50	A	127	+/- 6.8	63.5%	8	12	12	5
50	B	85	+/- 8.1	42.5%	6	12	12	6
100	A	100	+/- 8.1	50.0%	2	12	11	2
100	B	134	+/- 11.6	67.0%	60	12	12	37
EMS SOL. CONT.	A	133	+/- 9.3	66.5%	1	12	12	1
	B	NA	+/- NA	NA	NA	12	NA	NA
EMS (0.5 µL/mL)	A	65	+/- 2.1	32.5%	224	12	12	287
	B	54	+/- 14.7	27.0%	108	12	12	167

Solvent for EMS is DMSO

C.E. = cloning eficiency = avg. no. of C.E. colonies per plate/200 colonies seeded

- **Confirmatory Assay** – Adequate toxicity was observed in both activated and non-activated systems [Data not shown]. The number of mutants/10^6 surviving cells in the test article treated cells was comparable to the control cells.

Reviewer's Comment [Study Design and Data Presentation] – For the stated objective, these were adequate.

Sponsor's Conclusion and Reviewer's Comment – Verteporfin was not mutagenic in the CHO/HGPRT Gene Mutation Assay under the conditions of the study [e.g. without light activation]. **Reviewer's Comment** – The Reviewer concurs.

f. **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]**

Study Identification: 90079

Site: [redacted]

Study Dates: Dec. 4, 1990 – Jan. 18, 1991

Formulation and Lot No. –BPD-MA- Lot No. H90-120-123- reconstituted in sterile water and diluted with placebo formulation [a white lyophilized powder reconstituted with sterile water]

Vehicle – Placebo formulation

Certificate Analysis: Yes (X) [redacted]

Final Report: May 31, 1991

GLP and QA Statements Signed: Yes (X)

Objective: To evaluate the test article BPD-MA with and without light irradiation for its ability to induce micronucleated polychromatic erythrocytes in bone marrow cells of CD-1 mice".

This study has been previously reviewed by Dr. Oluwadare M. Adeyemo for IND [redacted] Submission [redacted] final review; pp. 25-26. [redacted] Additional comments by the current Reviewer [in italics] are provided below.

1. ***Body Weight*** *- Animals administered 10 mg/kg + irradiation exhibited a 7.2% weight loss at 24 hours post dosing.*

Summary of Genotoxicity

In vitro **Assays-** Under the conditions tested, BPD-MA without irradiation was found to be negative for mutagenicity in the Ames Salmonella/*E. coli* plate incorporation assay and CHO cell/HGPRT mutation assay. Only a single dose without irradiation was evaluated in the CHO cell chromosomal aberration assay and the UDS assay. Therefore, as conducted, these assays provide inadequate data to assess the potential of BPD-MA under dark conditions to induce genotoxicity in these test systems.

Under the conditions tested, BPD-MA with light activation was found to be negative for both mutagenicity and clastogencicity in the Ames Salmonella/*E. coli* plate incorporation assay, UDS assay, CHO cell/HGPRT mutation assay, and CHO chromosomal aberration assay. Although there was no increase in chromosomal aberrations in the CHO chromosomal aberration assay for the BPD-MA + light irradiation groups, there was an increase in the number of chromatid gaps, chromatid breaks, and endoreduplications at various time points and doses compared to the concurrent solvent control. This is suggestive of DNA damage.

There was some concern with the way in which these studies were conducted. These concerns included [1] failure to fully characterize the emission spectra of the light source which was used; [2] failure to include a positive control [e.g. known photogenotoxicant] to insure that the assay could detect mutagenic/clastogenic potential of a photoactive drug under the conditions tested; and/or [3] failure to conduct a confirmatory assay [See ICH Guideline S2B]. In addition, these studies were conducted prior to issuance of ICH guidelines. Therefore, there are deviations from the recommendations presented in both ICH Guideline S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals and ICH Guideline S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. These deviations are listed below.

- **Ames Assay** - According to ICH Guideline S2A, "if no toxicity is observed then the lowest precipitating concentration should be used as the top concentration". The Sponsor utilized concentrations that resulted in precipitation at all but the lowest [15.4 µg/plate] concentration in the definitive and first confirmatory assays. There was a decrease in the number of revertants in the irradiated BPD-MA cultures of TA1535, TA98 and TA100 by up to 50-75% when compared to the concurrent controls. Since the Sponsor indicated that growth was not altered, this finding would suggest that, especially at the higher concentrations, the test compound was decreasing the amount of light reaching the cells.
- **CHO/HGPRT Mutation Assay** - According to ICH Guideline S2A, "[i]n mammalian mutation tests ideally the highest concentration should produce at least 80% toxicity [no more than 20% survival]". This was not achieved in this study.

- **CHO Chromosomal Aberration Assay** - In ICH Guideline S2B, it indicates that exposure to test article should be 3-6 hours not 2 hours as used in this study. Furthermore, if the assay is considered negative, the assay should be repeated with a "continuous treatment without metabolic activation up to the sampling time of approximately 1.5 normal cell cycles is needed".

Photodynamic therapy has been reported to result in DNA damage including DNA strand breaks, alkali-labile sites, DNA degradation, and DNA-protein cross links which may result in chromosomal aberrations, sister chromatid exchanges [SCE], and mutations.[1] However, the literature also indicates that whether or not a PDT is mutagenic or clastogenic appears to be dependent on the cell line evaluated.[1,2] The findings in the studies reviewed here appear to be consistent, therefore, with what has been reported. Hematoporphyrin + light activation has been shown to cause DNA damage and induce sister chromatid exchange but not to increase the incidence of mutations in the CHO/HGPRT mutation assay.[3] Photofrin was negative following light activation in the Ames assay, the CHO/HGPRT mutation assay, and the CHO chromosomal aberration assay. It was, however, reported to result in [1] a significant increase in the incidence of SCE in CHO cells [visible light irradiation] and Chinese hamster lung fibroblasts [near uv light irradiation]; [2] an increase in *tk* mutants and DNA-protein crosslinks in mouse L5178Y cells; and [3] "a light-dose dependent increase in DNA-strand breaks in malignant human cervical carcinoma cells, but not in normal cells".[4] The factors that decide whether or not a PDT is mutagenic or clastogenic do not appear to have been fully elucidated. Therefore, the toxicological and biological significance of the DNA damage associated with PDT is unclear.

***In Vivo* Assay** – Under the conditions tested, BPD-MA, with and without photoactivation, was negative in the mouse micronucleus assay.

Special Toxicology:

I. **Phototoxicity**

A. Mice

a. A single dose range-finding study of benzoporphyrin derivative monoacid [a photodynamic anticancer agent] given I.V. to male mice, followed by exposure to light from a solar simulator [Ref. 325]

Study Identification: 90223

Site: [redacted]

Study Dates: Dosing initiated on November 27 and December 5, 1990

Formulation and Lot No. – liposomal BPD-MA- H90-120-0123

Controls – 5% dextrose

liposomal solution

Certificate Analysis: Yes (X) – [redacted]

Final Report: May 23, 1991

---

[1] Evans, H.H., et. al. [1997]. Mutagenicity of photodynamic therapy as compared to UVC and Ionizing Radiation in human and murine lymphoblast cell lines. *Photochem. Photobiol.* **66[5]**:690-696.

[2] Rerko, R.M., et. al. [1992]. Photofrin II photosensitization is mutagenic at the *tk* locus in mouse L5178Y cells. *Photochem. Photobiol.* **55[1]**:75-80.

[3] Gomer, C.J., et. al. [1983]. Comparison of mutagenicity and induction of sister chromatid exchange in Chinese hamster cells exposed to hematoporphyrin derivative photoradiation, ionizing radiation, or Ultraviolet Radiation. *Cancer Res.*. **43**:2622-2627.

[4] Physicians' Desk Reference. 53rd Edition [1999], Medical Economics Company, Montvale NJ., p. 2796.

GLP and QA Statements Signed: No (X)
Objective: "To identify potential phototoxicity when BPD-MA administration in mice was followed by photo-irradiation with simulated sunlight, and to compare the effects of BPD-MA with that of PHOTOFRIN"

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

1. *Study Design - The 5-minute exposure period was selected based on a preliminary study [phase 1] in which death occurred in all animals dosed at 10 mg/kg and irradiated for 10 minutes. Mice, irradiated for 5 minutes after 10 mg/kg of drug, exhibited squinting eyes, tail edema and necrosis, inactivity, wet perianal area, and brown skin at the site of irradiation.*
2. *Results*

- *The Sponsor states that no significant findings were observed in any mouse administered 2 mg/kg and irradiated 3, 24 or 48 hours post dosing.*
- *Severe dorsal skin changes [brown skin, eschar], tail skin changes [black tip, pink, swollen, missing tip], squinting eyes, cranial alopecia, and very slight edema and/or erythema developed in mice administered 10 mg/kg and photo-irradiated 3 hours post-dosing were similar to those described for the preliminary study. However, no significant findings were observed in mice administered BPD-MA and irradiated 24 hours later.*
- *Two mice administered 20 mg/kg and photo-irradiated 24 hours post dosing developed squinting and/or pink tails.*
- *Skin photosensitivity resulting in severe skin reactions in mice administered Photofrin + irradiation persisted for a longer period than in mice administered BPD-MA + irradiation.*

b. A single dose range-finding study of benzoporphyrin derivative monoacid [a photodynamic anticancer agent] given I.V. to male mice, followed by exposure to light from a solar simulator [Ref. 328]
Study Identification: 90058
Site: [ ]
Study Dates: Dosing initiated on October 9, 1990
Formulation and Lot No. – liposomal BPD-MA- H90-120-0123
Controls – 5% dextrose
liposomal solution
Certificate Analysis: Yes (X) [ ]
Final Report: May 24, 1991
GLP and QA Statements Signed: No (X)
Objective: "To identify potential phototoxicity when BPD-MA administration in mice was followed by photo-irradiation with simulated sunlight, and to compare the effects of BPD-MA with that of PHOTOFRIN"

Dr. Will Coulter previously reviewed this study for IND [ ] Submission [ ] final review; pp. 13-15. [ ] Additional comments by the current Reviewer [in italics] are provided below.

- *Severe skin changes were observed in both the control and treatment groups. Therefore, it is not possible to reach any conclusions on the duration for the potential development of phototoxicity/photosensitivity.*

c. A single dose phototoxicity study of benzoporphyrin derivative monoacid [a photodynamic anticancer agent] given I.V. to mice, followed by exposure to light from a solar simulator [Ref. 326]

Study Identification: 90059
Site:
Study Dates: Dosing initiated December 19, 1990
Formulation and Lot No. – liposomal BPD-MA- H90-120-0123
Controls – 5% dextrose
liposomal solution
Certificate Analysis: Yes (X)
Final Report: May 23, 1991
GLP and QA Statements Signed: Yes (X)
Objective: "To identify potential phototoxicity when BPD-MA administration in mice was followed by photo-irradiation with simulated sunlight, and to compare the effects of BPD-MA with that of PHOTOFRIN"

Dr. Will Coulter previously reviewed this study for IND Submission final review; pp. 16-17. Additional comments by the current Reviewer [in italics] are provided below.

1. Results

- *Very slight erythema of the dorsal skin was observed on Day 2 in 2, 1 and 3 females administered liposomal solution only, 10 and 20 mg/kg, respectively. In addition, pink, scaly and/or swollen tail was observed in 3/10 and 4/10 mice at 10 and 20 mg/kg, respectively.*
- *There was a higher incidence of lesions in females administered 20 mg/kg of BPD-MA [3/5] vs. males [1/5]. This was also true at 10 mg/kg of BPD-MA.*

B. Pigs

c. Development of skin photosensitivity following iv administration of BPD-MA to normal pigs [Ref. 323]

Study Identification: PH-93002
Site:
Study Dates: March 1 – April 15, 1993
Formulation and Lot No. – liposomal BPD-MA- R1186-101
Controls – 5% dextrose
liposomal solution
Certificate Analysis: Yes (X)
Final Report: June 4, 1993
GLP and QA Statements Signed: Yes (X)
Objective: "To determine the time course of development of skin photosensitivity in normal pigs induced by 3 therapeutically relevant laser light [690] nm doses within 4 hours post iv treatment with.... BPD-MA and to determine the concentration of BPD-MA in plasma at various time points after iv administration"

Study Design – A single anesthetized female Yucatan mini swine was administered 0.5 mg/kg of BPD-MA by iv injection. Irradiation at 690 nm at 25, 50, and 100 J/cm^2 was performed at prior to and 2, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after dosing. The procedure was repeated 3 weeks later using a dose of 1.0 mg/kg and irradiation time of prior to and 30, 45, 60, 90, 120, 150, and 180 minutes after dosing. Exposed areas were scored for erythema, induration, and spot size daily X 1 week, then every 3 days.

Blood was collected to determine plasma BPD-MA levels at the time points for irradiation and at 5 and 10 minutes after drug administration. Plasma levels were analyzed using a luminescence spectrophotometer.

Results – The tables below outline the findings in this study.

Skin Reactions of Miniswine #415 Following Injection of 0.5 mg/kg Body Weight

Time (min)	Plasma Level of BPD (μg/mL)	Total Score					
		Day 1 J/cm²			Day 3 J/cm²		
		100	50	25	100	50	25
1	9.82	9.9	7.0	4.5	12.9	7.5	6.0
15	1.35	7.2	4.5	2.0	8.2	5.5	2.5
30	0.62	5.0	1.0	0	5.5	1.3	0.6
45	0.41	3.0	1.5	0	5.2	3.0	0.3
60	0.35	2.5	1.0	0	3.5	3.0	0.3
90	0.26	0	0	0	1.5	1.0	0
120	0.21	0	0	0	1.2	0	0
150	0.19	0.5	0	0	0.5	0	0
180	0.14	2.0	0	0	2.0	0	0

Skin reactions of miniswine #415 following injection of 0.5 mg/kg body weight of BPD-MA at various time points. Scores for reactions were based on erythema (0-5), induration (0-4) and spot size (0.5 for each additional mm in diameter beyond size of radiation diameter). Plasma levels for each time point are also provided.

Skin Reactions of Miniswine #415 Following Injection of 1.0 mg/kg Body Weight

Time (min)	Plasma Level of BPD (μg/mL)	Total Score					
		Day 1 J/cm²			Day 3 J/cm²		
		100	50	25	100	50	25
30	1.325	11.5	10.0	8.7	15.5	13.5	11.5
45	0.775	10.0	7.9	4.7	14.0	10.3	7.0
60	0.516	8.5	7.0	2.7	9.8	8.8	5.8
90	0.356	7.0	2.0	1.3	9.6	5.0	4.7
120	0.269	6.0	5.5	3.0	8.7	6.0	1.3
150	0.229	7.5	6.7	2.7	7.0	8.0	0.7
180	0.221	7.5	4.3	2.7	7.0	0.7	1.0
210	0.179	6.5	2.5	0.3	4.0	0.3	0.3
240	0.168	3.5	0	0	0	0	0

Skin reactions of miniswine #415 following injection of 1.0 mg/kg body weight of BPD-MA at various time points. Scores for skin reaction were based on erythema (0-5), induration (0-4) and spot size (0.5 for each additional mm in diameter beyond the size of the radiation diameter). Plasma levels for each time point are provided.

Sponsor's Conclusions

1. Skin reactions induced by irradiation 90-120 minutes following a dose of BPD-MA tended to be less than those observed following irradiation at 150-180 minutes. These data suggest that the processes involved in skin phototoxicity at the earlier time points [0-60 minutes] are different than at the later time points and reflect changes in distribution. It is postulated that the early skin reactions are secondary to activation of drug in the capillaries and that the later

reactions are secondary to activation of drug in the skin as well as the vasculature. Reviewer's Comment- The Reviewer concurs.

II. Immunogenicity/Immunotoxicity

A. Immunogencity/Antigenicity and Hypersensitivity

a. Assessment of Anti-BPD Immunity in Mice Treated with Liposomal BPD Verteporfin [137]

Study Identification: PH-95011R
Site: [redacted]
Study Dates: August – September 1991 and May-July 1995
Formulation and Lot No. – liposomal BPD-MA- **Lot No.** - L90-120-0216, E93-120-0878
Vehicle – Saline
Certificate Analysis: No (X)
Final Report: Mar. 6, 1996
GLP and QA Statements Signed: No (X)
Objective: "To determine if a single large dose or multiple, smaller doses of liposomally-formulated BPD might induce the formation of a specific cellular response when given to inbred strains of laboratory mice"

Study Design – Single Dose Study- Male DBA/2 mice [N=4; [redacted] Canada; 9 weeks at start] were administered BPD-MA iv at 1 mg/kg. The second group of mice was naive. They were maintained in the dark for 48 hours, then returned to ambient lighting. At 5 and 7 days, 2 mice were sacrificed from each group and the spleen and thymus harvested.

- Multiple Dose Study – Male PL mice [redacted] age 10-13 weeks at start] were administered BPD-MA ip every 2 days at [1] 0.5 mg/kg [N=3] or 2.0 mg/kg [N=3] for 9 injections; [2] 0.5 mg/kg [N=5] for 11 injections; and [3] 0 mg/kg [saline] for 9 injections. Mice were maintained in the dark for 60 minutes then returned to ambient lighting. Naïve mice [N=4] were included in this arm as well. Mice were rested for 26 then administered either BPD-MA or saline at the respective dose level and sacrificed 7 days later. [**Note:** These animals had previously been administered myelin basic protein-sensitized syngeneic spleen cells to induce experimental acute encephalomyelitis (EAE).]

-Spleen and thymus weights and spleen cell number and cellularity were determined for both the single and multiple dose study.

- Evaluation of Cellular Immune Response- Single spleen cell suspensions were prepared and viability assessed by Trypan blue dye exclusion. Spleen cells were cultured in concentrations of BPD-MA ranging from 0-1000 ng/ml or 0-5000 ng/ml ± rIL-2 in the single dose and multiple dose groups, respectively. A T cell mitogenicity assay [ConA] was conducted in parallel for both groups and B cell mitogenicity assay [LPS] was conducted with the repeat dose cell groups. Cells were incubated for 3 days followed by assessment of the activation using MTT reagent.

Results – Single dose - At 5 days, but not 7 days, post BPD administration, spleen weight, cell number and cellularity were decreased by 17%, 45%, and 30%, respectively. Based on MTT activity, there was no difference in activation level of splenocytes in treated vs. untreated animals

in either the presence or absence of rIL-2. ConA response in splenocytes from the control mice sacrificed at Day 5 was lower than that observed in the treated group [approximately 0.66 vs. 0.86 absorbance, respectively]. The significance of this finding is not known, especially since the ConA response in the treated group was comparable to that observed in both the treated and control animals sacrificed Day 7.

-Multiple dose- A concentration of 5000 ng/ml appeared to be toxic. There was no difference in responses in the naïve, vehicle control, and BPD-treated groups.

Reviewer's Comments [Study Design and Data Presentation]

1. There was no positive control.
2. Ideally, the single dose arm should have included a VH control group.
3. Under the conditions tested, there did not appear to be a difference in the measured responses in the naïve mice and those with EAE. However, it would have been preferred that the test system used did not already have an immunological perturbation.
4. Although the iv and ip route of administration frequently have comparable bioavailability, no pharmacokinetic data has been reviewed to support comparable bioavailability of BPD-MA by these routes.
5. Individual data for the single dose study for the immune responses was not provided.
6. The N was small, particularly for the single dose arm of the study.
7. Timing for measurement of immune responses can be critical. The Sponsor did not provide any justification for selection of time points, specifically for sacrifice and incubation of splenocytes with BPD-MA. Typically, specific immune responses are evaluated 4-5 days following exposure to the potential antigen.

Sponsor's Conclusion [numbered] and Reviewer's Comment

1. A single dose of BPD-MA in DBA/2 and in PL mice exhibiting signs of EAE did not elicit a specific cellular immune response. These results suggest that multiple doses of liposomal BPD-MA "can be tolerated without risk of the formation" of a drug-specific immune response. Reviewer's Comment – In general, the Reviewer concurs with the caveat that this true under the conditions tested. [See comments on Study Design and Data Presentation above.]

b. Immunogenicity of BPD-MA – Studies in rabbits [Ref. 138]
Study Identification: PH-95010
Site: QLT Phototherapeutics, Inc.;
Study Dates: July - Aug., 1995
Formulation and Lot No. – liposomal BPD-MA- Lot No. - E93-120-0878; used within 2 weeks of reconstitution
Vehicle – Liposome placebo, saline, 5% dextrose
Certificate Analysis: No (X)
Final Report: Sept. 28, 1998
GLP and QA Statements Signed: No (X)
Objective: "To determine whether repeated dosing with liposomal BPD-MA results in formation of antibodies or sensitized T cells in rabbits."

Study Design - Induction Phase - New Zealand White rabbits [N=3; 3.8-4.8 kg; gender not indicated] were administered 8 injections of BPD-MA at 0.5 or 1.0 mg/kg iv in 2 intervals of once a week for 4 weeks separated by 1 month. Small areas of skin were irradiated with 690 nm with a maximum of 3 spots/treatment.

- Elicitation Phase - Two months after the last dose of BPD-MA was administered, the animals were challenged with an intradermal injections of 35 µl of BPD-MA

diluted 1:10, 1:100, 1:1,000, and 1:10,000; liposomal placebo, saline, and 5% dextrose and the sites protected from the light. [Concentrations were selected based on a result of a pilot study conducted in a single animal.] Skin reactions were observed at 15 min., 24 and 48 hours post challenge. A few days after the challenge [exact number of days not provided], rabbits were administered a single dose of either 0.5 or 1 mg/kg iv. The skin test was repeated 9 days later.

Results – Results were negative in all rabbits.

Reviewer's Comment [Study Design and Data Presentation]

1. There was no positive control.
2. The Sponsor did not justify the induction protocol. Although it is acceptable to vary the number and timing of exposures, weak sensitizers may necessitate a more frequent administration than once weekly.
3. The Reviewer agrees with the Sponsor that challenging 2 months after the last exposure to BPD-MA may have been too long.
4. The Sponsor should have used the maximum nonirritating concentration of BPD-MA to conduct the elicitation phase of this study. In this pilot study barely perceptible redness was observed at a 1:10 concentration at 24 and 48 hours. Therefore, it is likely that a more concentrated preparation than 1:100 could have been used.

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

1. "BPD-MA was found to be non-immunogenic in rabbits and [PDT did not] induce immunogenicity of liposomal BPD-MA". **Reviewer's Comment**- Under the conditions tested, BPD-MA did not elicit and hypersensitivity/immunogenic reaction in rabbits. There were, however, several concerns with respect to the study design.

c. <u>Antigenicity study of liposomal BPD-MA [verteporfin] in guinea pigs [Ref. 140]</u>
Study Identification: TX-99001
Site: [redacted]
Study Dates: February 19 – March 24, 1998
Formulation and Lot No. – liposomal BPD-MA- **Lot No. - TC0715**; used within 4 days of reconstitution; Sponsor indicates all solutions were within 94-99% of nominal concentrations
Vehicle – Saline
Certificate Analysis: No (X)
Final Report: January 9, 1999
GLP and QA Statements Signed: Yes (X); GLP standards according to the Ministry of Health and Welfare Ordinance No. 21; Japan
Objective: "To investigate the potential antigenicity of Liposomal BPD-MA [verteporfin] by examining active systemic anaphylaxis [ASA] and passive cutaneous anaphylaxis [PCA] reactions in guinea pigs"
Study Design – <u>Sensitization for ASA</u> - Male Slc[redacted] guinea pigs [N = 6; 7-8 weeks; wt 392-494 g] were assigned to treatment groups as indicated below.

Table 1 [Antigen levels and groups]

Group	Sensitizing substance	Route	Antigen level (mg/kg)	Dose vol. (mL/kg)
I	Saline + FCA (FIA)	s.c.	–	1
II	Liposomal BPD-MA	i.v.	1.0	1
III	Liposomal BPD-MA + FCA (FIA)	s.c.	1.0	1
IV	OVA + FCA (FIA)	s.c.	5	1

Sensitization by the iv and the sc routes were performed 5X/week for 2 consecutive weeks and 1X/week for 3 consecutive weeks, respectively. FCA was used for the first sensitization and FIA for the second and third sensitizations. Sera were collected from these animals 12 days following the final sensitization.

- Elicitation Phase of the ASA – Challenge antigen [e.g. BPD-MA, saline, or OVA] were administered intravenously to the appropriate group at the same antigen level as in the sensitization phase. Animals were monitored for anaphylactic symptoms for 1 hour after challenge and observed for mortality for 24 hours.

- PCA Reaction - Male [redacted] guinea pigs [N = 2; 12 weeks; [redacted] wt 502-672 g]. Serum collected Day 12 from the ASA group was diluted 4-fold for 5 separate dilutions starting from a 2-fold dilution for the test article animals and a 64-fold dilution for the OVA group. The dilutions were injected intradermally [0.05 ml/site]. Challenge antigens [1 mg/kg for BPD-MA and 5 mg/kg OVA] mixed with Evans Blue were injected iv four hours after sensitization. The size of the pigmented spots was measured 1 hour later and a mean axis ≥5 mm was considered positive.

Results – Animals in the test article or saline groups demonstrated no signs of anaphylaxis. In addition, the PCA was negative. Strong positive reactions were obtained with ovalbumin.

Reviewer's Comment [Study Design and Data Presentation] – These were adequate.

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

1. Under the conditions tested, BPD-MA "was considered to have no antigenicitity following either sensitization via intravenous or subcutaneous administration at 1.0 mg/kg". **Reviewer's Comment** – The Reviewer concurs.

d. The Sponsor has conducted several studies and provided several references in which the effect of BPD-MA ± activation on delayed type hypersensitivity reactions was evaluated.

i. Ref. 254 – Report No. PH-96001; **The effect of BPD-MA activated by UVA light on contact hypersensitivity to 2,4 dinitrofluorobenzene [DNFB] in mice [Report Date – Jan. 13, 1997; QLT Inc.; Vancouver, B.C.; Lot No. E9-120-0878 and H92-902-017]** –*Dr. Javier Avalos previously reviewed this study for IND [redacted] Submission [redacted] final review; pp. 13-15.* [redacted] DTH was evaluated in 3 strains of mice [male and female DBA/2; male SKH.1 hairless; and female hairless (HRS/J-hr/t)] administered BPD-MA ± UVA photoactivation. These studies indicated that BPD-MA alone did not suppress the DTH response to DNFB. UVA alone, however, did. Combination of BPD-MA + UVA photoactivation did not enhance the DTH suppression of UVA only in the DBA/2 and female HRS/J-hr/t mice. There was minimal enhancement [25% vs. 39%] in the SKH/1 mice.

ii. Ref. 255- PH-97005; The effect of BPD activated by broad spectrum light 24 hours post injection on contact hypersensitivity to 2,4 dinitrofluorobenze [DNFB] in mice [Report Date – Aug. 1, 1997; QLT, Inc., Vancouver, B.C.] – The findings in this study are consistent with those described above. DHT response in SKH.1 hairless mice administered 1 mg/kg iv ± irradiation [white light, 400-700 nm with a peak of 575 nm; variable doses]. Animals, which were not irradiated, were kept in dark cages. No suppression in ear swelling compared to

controls was observed in the BPD-MA group only. There was approximately a 30-40% suppression in BPD-MA + irradiation.

iii. Ref. 253 – QLT Report PH-94026: Effect of BPD-MA [verteporfin] on a murine model of delayed type hypersensitivity [QLT Inc., Vancouver, B.C., March – Oct. 1994; Lot No. N-011-1, N-011-13, H92-902017] – The findings in this study appeared to differ slightly from those described in References 254 and 255. The DTH response in female Hairless [HRS/J-hr/+] mice was suppressed [40-60% reduction in ear swelling compared to control] following administration of BPD-MA ± photoactivation prior to or during the sensitization phase. [**Note:** Animals in the dark group were maintained under dark conditions for 1 hour before being returned to ambient light]. There was <20-30% suppression in ear swelling when the exposure to BPD-MA occurred after sensitzation. DTH suppression was transient as demonstrated by the induction of a cutaneous hypersensitivity reaction following sensitization with a second antigen.

1. **Ref. 256 –Simkin, G.O., et. al. [1997] Inhibition of contact hypersensitivity with different analogs of benzoporphyrin derivative. *Immunopharmacol.* 37:221-230.** – This paper discusses several experiments in SKH.1 hairless mice. In general the effects on DHT response to DNFB are consistent with those previously described. The Sponsor conducted additional studies in BALB/c mice treated with BPD-MA and then sensitized to DNFB which indicated that the DTH response was decreased compared to controls following treatment with BPD-MA and exposure to ambient light but the response was comparable to that in control animals following treatment with BPD-MA and protected from light. This may explain the results described in Ref. 253 in which BPD-MA alone appeared to induce a suppression of the DTH response. Animals were returned to ambient light 1 hour following administration of BPD-MA, which may not have been of sufficient duration. BPD-DA treatment caused less suppression of ear swelling than did BPD-MA treatment.

B. Immunotoxicity

a. Effects of BPD on parameters of the immune response in naïve mice in the presence or absence of light applied transdermally [Ref. 267]

Study Identification: TX-94037
Site: QLT, Inc., Vancouver, B.C.
Study Dates: January - March 1994
Formulation and Lot No. – liposomal BPD-MA- **Lot No.** - H92-902-017;
Vehicle – 5% Dextrose
Certificate Analysis: No (X)
Final Report: September 28, 1994
GLP and QA Statements Signed: No (X)
Objective: "To determine the effects of treatment of normal naïve animals, with BPD alone or BPD and light delivered transcutaneously, on unactivated cells in the immune system."

Study Design – Unshaved male and female C57/Bl6 mice [N=3; 9-12 weeks; [redacted] were administered BPD-MA ± irradiation. For mice irradiated [560-850 nm; 30 mW/cm^2; 90 minutes], BPD-MA doses were 0, 0.1, 0.5, and 1 mg/kg. For mice that were not irradiated, BPD-MA doses were 0, 0.5, 2.5, and 10 mg/kg. Following test article administration by iv injection, animals were either maintained in the dark for 3 hours or were irradiated 1 hour after dosing. Mice were then exposed to antigen, sheep red blood cells [sRBC] via an i.p. injection. Animals were maintained under ambient light for the remainder of the experiment. Four days post immunization, animals were sacrificed, the spleens were excised and single cell suspensions prepared. The following assays were conducted: [1] a standard ^{51}Cr-release NK Cell

Assay; [2] a standard Mixed Lymphocyte Reaction [MLR] Assay; [3] a Cell Mediated Lysis Assay using; and [4] Plaque Forming Cell Assay [PFC] to measure antibody response sRBC. Each assay was conducted 3 times.

Results and Conclusions – The Sponsor concludes that at doses up to 10 mg/kg without irradiation and up to 1 mg/kg with irradiation that there were no significant effects on any of the measured parameters. The Sponsor concluded that this suggested that "in situ immune responses and in vitro immune responses have not been compromised by the treatment with BPD-MA". However, in Ref. 268 and 266, the Sponsor states that there was a limited effect [e.g. suppression] on cell-mediated lympholysis. There were, however, a number of concerns with respect to the conduct and result of these studies and, therefore, the studies should be interpretted cautiously. These major concerns included the following. [1] No positive controls were included to insure that the assay was working. [2] There was considerable variability in the levels of lysis in both the NK assay and CML assay within and between experiments. It would have been helpful had the Sponsor provided historical control data. [3] It is preferred if the animals used in the NK and CML assays had not had their immune systems stimulated [e.g. injection of sRBC] prior to the assays being conducted. [4] It would have been more appropriate if the NK and CML assays had been performed within 24 hours of exposure to BPD-MA instead of 4 days later when the drug had been cleared for several days and effects had the potential to reverse. Finally, since mice were unshaven, it is unclear as to how much of the irradiation dose reached the skin and activated drug.

b. Studies on the effect of BPD-MA on primary and secondary immune responses to soluble protein antigens [Ref. 266]

Study Identification: TX-94019
Site: QLT, Inc., Vancouver, B.C.
Study Dates: March -June, 1994
Formulation and Lot No. – liposomal BPD-MA- **Lot No.** - H92-902-017; stored for a maximum of 2 weeks
Vehicle – Saline
Certificate Analysis: No (X)
Final Report: May 1, 1995
GLP and QA Statements Signed: No (X)
Objective: "[1] To investigate the effect of BPD-MA on memory [secondary] responses to soluble protein antigens, both in the absence of light and after light treatment and [2] to investigate the selectivity of transdermal therapy in primary responses to soluble protein antigens."

Study Design - [1] Assessment of Memory Response - C57Bl/6 mice [male and female, 6 weeks ( ) were primed with a sc injection of OVA [100 µg sc]. After 3 weeks, unshaved animals were administered 0, 0.5, 2.5, and 1 mg/kg of BPD-MA and were either returned to the dark for 3 hours followed by ambient light or returned to the dark for 1 hour then irradiated [560-800 nm; 162 J/cm^2] and returned to ambient light. They were then challenged with OVA [20 µg sc]. Blood samples were collected on Days 4, 6, and 8 post challenge and antigen-specific antibody was measured via an ELISA.

- [2] Selectivity of Potential Immune Effects – a. Mice were primed with OVA and exposed 5 days later to BPD-MA ± irradiation. After 3 weeks, animals were challenged with an ip dose of OVA or sc dose of keyhole limpet hemocyanin [KLH]. Blood samples were collected on Days 4, 6, and 8 post challenge. Antigen-specific antibody was measured via an ELISA.

- b. Five days following administration of BPD-MA ± irradiation, mice were primed with KLH [sc]. Blood samples were collected on Days 4, 6, and 8 post challenge and antigen-specific antibody was measured via an ELISA. After 11 days, mice were challenged with OVA. Blood samples were collected on Days 4, 6, and 8 post challenge. Antigen-specific antibody was measured via an ELISA.

Results – There were no statistically significant effects on the levels of IgM or IgG in any of the treated groups when compared to the control groups.

Reviewer's Comment [Study Design and Data Presentation] –

1. The Sponsor should have included a positive control to demonstrate the validity of the assay.
2. N was not indicated although it appears as though samples may have been pooled.
3. Since mice were unshaven, it is unclear as to how much of the irradiation dose reached the skin and activated drug.

Conclusions – Under the conditions tested, BPD-MA ± irradiation did not alter the primary or secondary response to antigen when compared to control animals. As the Sponsor notes, if the treatment were to take place at earlier time points or closer to the induction of the primary response, the activated cells could be affected. Due to the absence of the appropriate positive controls, the results should be interpreted cautiously.

C. ***In Vitro*** **Special Toxicology Studies**

a. Homogeneity, stability and dosing system compatibility of CL 315,555/315,585 [BPD-MA] formulation reconstituted with sterile water for injection USP[Ref. 338]
Study Identification: 91067
Site: [redacted]
Study Dates: Not provided
Formulation and Lot No. – liposomal **BPD-MA- Lot No. – H90-120-0123**
Certificate Analysis: Yes (X)
Final Report: March 5, 1991
GLP and QA Statements Signed: Yes (X) [21 CFR 58]
Objective: To determine the uniformity, stability and dosing system compatibility of the ...(BPD-MA)... formulation reconstituted with sterile water."

Dr. Will Coulter previously reviewed this study for IND [redacted] Submission [redacted] final review; p. 20. [redacted]

The methodology for analyzing the drug concentration was not provided.

b. Homogeneity, stability and dosing system compatibility of CL 315,555/315,585 [BPD-MA] formulation reconstituted with 5% dextrose injection USP[Ref. 338]
Study Identification: 91067
Site: [redacted]
Study Dates: Not provided

Formulation and Lot No. – liposomal BPD-MA- Lot No. – J90-120-0175
Certificate Analysis: No (X)
Final Report: March 18, 1994
GLP and QA Statements Signed: Yes (X) [21 CFR 58]
Objective: To determine the uniformity, stability and dosing system compatibility of BPD-MA (formulation reconstituted with sterile water) in 5% dextrose."

Study Design – Stock solution [25 mg lyophilized BPD-MA reconstituted in sterile water] was diluted with 5% dextrose to yield concentrations of 0.1 mg/ml and 1.0 mg/ml. Homogeneity was determined immediately after preparation. Samples allowed to stand at room temperature were analyzed for stability at 0, 6, and 11 hours and samples frozen [-10 to –20° C] were analyzed for stability on study days 14, 34, and 90. Compatibility with the dosing apparatus was also evaluated.

Results – The percent of expected concentration, from [1] any third of the solution; [2] after 6 and 11 hours at room temperature; [3] and after 14, 13, and 90 days of frozen storage; and [4] dispensing through dosing apparatus, ranged [redacted]

c. **In vitro human blood compatibility of CL 315,555/315,585 benzoporphyrin derivative monoacid; [A photodynamic anti-cancer agent) [Ref. 337]**
Study Identification: 90057
Site: [redacted]
Study Dates: Not provided
Formulation and Lot No. – liposomal BPD-MA- Lot No. – H90-120-0123
Certificate Analysis: No (X)
Final Report: March 15, 1991
GLP and QA Statements Signed: Yes (X) [21 CFR 58]
Objective: To determine "the potential of ... BPD-MA to effect human red blood cell hemolysis and to flocculate protein, in the presence or absence of light."

Dr. Will Coulter previously reviewed this study for IND [redacted] Submission [redacted] final review; p. 20. [redacted] Additional comments by the current Reviewer are provided below [in italics].

- ***BPD-MA did not induce hemolysis under the conditions tested. However, hemolysis was observed in the repeat dose studies in both rats and dogs.***

Summary of Special Toxicology-

Phototoxicity – Studies conducted in mice demonstrated that a dose of 2 mg/kg of BPD-MA followed in 3, 24, and 48 hours by irradiation with a solar simulator did not result in any significant phototoxicity. However, following irradiation at 3 hours after administration of 10 mg/kg, severe skin lesions developed including edema, erythema, and/or eschar and after administration of 20 mg/kg death occurred. Solar simulator irradiation at 24 hours after administration of 10 and 20 mg/kg resulted in less severe reactions including pink, scaly, and/or swollen tail. A pilot study, conducted in beagle dogs administered 20 mg/kg indicated that the most severe phototoxicity occurred in animals exposed to sunlight 24 hours post-dosing. Animals exposed to sunlight at ≥48 hours post drug administration, exhibited phototoxicity for 3-4 days including slight to moderate erythema of the shaved forelimb and perinostril area. Study PH-

93002 in Yucatan mini swine suggested that there were two phases to the phototoxicity. The first phase may be due to activation of drug in the microvasculature of the skin, and, therefore, severity was a function of plasma levels. The second, later, phase may be due to activation of drug in the skin and vasculature, and, therefore, severity was a function of both plasma and tissue levels. The severity of and susceptibility to phototoxicity appears to be dose-related.

Immune System Effects - The Sponsor conducted several studies to evaluate [1] the potential immunogenicity of BPD-MA in rats, rabbits, and guinea pigs; [2] the effect of BPD-MA ± irradiation on delayed type hypersensitivity in mice; and [3] the effect of BPD-MA on immunocompetency in mice. These studies should be interpreted cautiously since there were several concerns with respect to study design. One of the major concerns is that the Sponsor did not include appropriate controls. [**Note:** The deficiencies in these studies are delineated above.] These studies, however, suggested that BPD-MA was neither immunogenic nor immunosuppressive. BPD-MA alone did not appear to modify the DTH response. However, in conjunction with irradiation, the DTH response was suppressed. These studies suggested that UVA alone or in combination with BPD-MA were equally effective in certain strains of mice. The Sponsor's do, however, note that activated immune cells appear to have greater uptake of drug than nonactivated cells. Theoretically, under certain experimental conditions, activated/stimulated immune cells would be more sensitive to the cytotoxic effects of activated BPD-MA.

In Vitro Human Blood Compatibility - BPD-MA did not induce hemolysis under the conditions tested in Study 90057. However, hemolysis was observed in the repeat dose studies in both rats and dogs. In addition, literature provided by the Sponsor describes studies in which RBCs were lysed following incubation with phosphatidylcholine in humans, rabbits, rats, and mice. The authors cited literature that suggested that cholesterol was removed from RBCs membranes when the RBCs are incubated with egg yolk phosphatidylcholine liposomes.

Homogeneity, stability and dosing system compatibility – These studies indicate that the verteporfin was stable for up to 11 hours at room temperature and for up to 3 months if frozen.

Overall Summary:

General Pharmacology - BPD-MA is a photodynamic agent that requires light activation to exert its pharmacological effects. 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 indicate that activation of BPD-MA results in the generation of singlet oxygen [$^1O_2$] and free radicals with a quantum yield of $^1O_2$ for BPD-MA comparable to that described for both Hematoporphyrin 1X and Photofrin.

In vitro studies also suggest that liposomes of BPD-MA are disrupted in the presence of plasma and the drug is transferred to lipoproteins. At <6 hours, drug was largely associated with lipoproteins, primarily with HDL and to a lesser extent with VLDL and LDL. Only a small percentage [e.g. approximately 6%] was associated with albumin. By 24 hours of incubation, drug was fairly evenly distributed between each of the 3 lipoprotein fractions. *In vitro* studies also indicate that uptake by cells was rapid. The rank order for 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. Cytotoxicity appeared to be related to both intracellular and membrane damage in the *in vitro* systems.

Activated BPD-MA was shown to have efficacy in numerous models, both *in vitro* and *in vivo*. These included [1] tumor models; [2] photosensitivity models; and [3] CNV and choriocapillaris closure.

Pharmacokinetics - Clearance of BPD-MA from the blood and plasma was rapid in mice. Fifteen minutes following administration of liposomal BPD-MA, the highest concentrations, based on radioactivity, were observed in the gall bladder followed by the liver, adrenals, kidney, lung, heart, spleen, small intestine, then fat, salivary glands, pancreas, and tumor. At 3 hours post drug administration, radioactivity was decreasing in most organs. The exceptions were the gall bladder and small intestine, in which tissue levels had increased, and skin and pancreas, in which tissue concentration was essentially unchanged. By 24 hours in the gall bladder and liver, the drug concentration, based on radioactivity, was 2-3 µg/g tissue. By 168 hours following drug administration, all tissue levels were <0.4 µg/g tissue.

The primary route of excretion was through the bile in both the mouse [approximately 60% of the radioactive dose] and the rat [approximately 90% of the radioactive dose]. Approximately 80% of the excreted drug in the bile in the rat was unchanged. Urinary excretion accounted for approximately 4% in the mouse and <1% in the rat. The drug that was eliminated in the urine in the rat was predominantly metabolized. In the rat, approximately 3% of the radioactivity was left in the carcass after 168 hours.

Following intravenous administration, there was rapid distribution of the drug followed by a slower elimination phase with all analytes exhibiting a bi-exponential decline. In both the rat and cynomolgus monkey, there was a stereospecific disposition of regioisomers. In addition, studies suggested that there was some stereospecific disposition of the enantiomers in the rat [This was not evaluated in the monkey.] The relative exposure to BPD-MA_D was approximately 2-3X BPD-MA_C in both species. The $t_{1/2}$ for BPD-MA_C was approximately 3, 7, and 5 hours in the male monkey, and male and female rat, respectively. The $t_{1/2}$ for BPD-MA_D was approximately 5, 7, and 3 hours in the male monkey, and male and female rat, respectively. Exposure to BPD-DA in rats appeared to represent <10% of the overall exposure to test article.

Following intravenous administration, BPD-MA rapidly distributed to the iris, ciliary body, retina, and choroid in rabbits. In addition, these tissues demonstrated the greatest drug concentrations. Minimal drug accumulated in the avascular portions of the eye [e.g. cornea, lens, and vitreous]. Maximum drug concentration, following an iv injection of 6 mg/kg verteporfin, was observed at 30-60 minutes in the choroid, anterior segment, and the ciliary body and process. By 2 hours, the drug concentration had begun to gradually decrease. Drug concentration in the retina, however, continued to increase up to 2 hours. By 24 hours, drug concentration had significantly decreased with the highest levels still present in the retina and the choroid.

There were at least 3 proposed mechanisms for drug uptake into tissues: [1] LDL receptors; [2] "scavenger" receptors; and [3] diffusion. The LDL receptor is expressed on endothelial cells and is upregulated in neovascular endothelium. Therefore, this would theoretically increase the drug accumulation in CNV compared to other tissues and increase specificity of PDT with BPD-MA. However, as the Sponsor noted, LDL receptors are also expressed on normal endothelium and retinal pigmented epithelium. In addition, scavenger receptors are also present on RPE.

Therefore, the literature suggested that the iris, ciliary body, retina [including RPE and neurosensory retina], and choroid would also be potentially susceptible to PDT toxicity.

BPD-MA is metabolized to varying degrees in the plasma and liver with the rate and the stereospecificity of the metabolizing enzymes both species and tissue dependent. The rate of metabolism in the human hepatic preparations and human plasma generally was less than that observed in the animal species. In addition, stereospecifity of metabolism was generally greater in the rat, dog and/or mouse hepatic and plasma preparation than it was in the human samples in which metabolism was similar for each regioisomer. There did not appear to be any stereospecificity for metabolism of the enantiomers. However, these results were considered preliminary.

The major metabolite identified, BPD-DA, was formed quickly in all species in both the *in vivo* and *in vitro* studies. These studies indicated that BPD-DA did not accumulate in the plasma and that the diacid was further metabolized at a fairly rapid rate by hepatic but not plasma enzymes. The Sponsor stated that no other peaks besides BPD-MA_c, BPD-MA_D, and BPD-DA were observed and that any other metabolite would account for approximately 5-10% of the initial BPD-MA. Microsomal NADPH enzymes did not appear to contribute to BPD-MA metabolism *in vitro*. Conjugation of BPD-MA and BPD-DA did not appear to occur *in vitro*. Liver metabolism appeared to be carried out by esterases.

All regioisomers and enantiomers exhibited generally comparable activity both in *in vivo* and *in vitro* systems. The activity of BPD-DA compared to BPD-MA was dependent on the test system in which it was evaluated. However, in *in vivo* tumor cytotoxicity and photosensitivity studies, BPD-MA appeared to be at least five times more potent than BPD-DA. The comparability of BPD-MA and BPD-DA in CNV models was not evaluated.

Safety Pharmacology - 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.

In conscious and anesthetized dogs at doses ranging from 2-20 mg/kg without photoactivation, there were no effects on mean blood pressure, heart rate, cardiac output or ECGs. The results of these studies should be interpreted cautiously because of the small N. It was not possible to reach conclusions in second study in anesthetized dogs with respect to the relationship of changes in CO and MAP to BPD-MA effects since the proper controls were not included and some animals exhibited signs of phototoxicity. Bolus administration of BPD-MA in anesthetized pigs resulted in profound cardiovascular events including marked decreases in BP, HR, CO and 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.

Complement activation and complement depletion was observed in both conscious and anesthetized dogs and pigs. In the dogs administered 20 mg/kg of BPD-MA, peak depletion reached approximately 30-40% of control values with depletion of 10-26% still observed at 60 minutes. 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 cited an article that indicated that complement was activated by negatively phospholipids such as egg phosphatidylglycerol [Cohn, 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.

Ocular Toxicity - The results from the ocular toxicity studies in cynomolgus monkeys, dogs, and rabbits should be interpreted keeping in mind the following concerns. The studies in the rabbits were provided primarily as literature citations. Consequently only selected summary data were provided. One of the literature citations conducted studies with LDL-complexed BPD-MA instead of clinical formulation. Localization of BPD-MA in corneal neovascularization was evaluated. The normal cornea, unlike the retina, is an avascular structure. Furthermore, the blood supply to the retina in the rabbit is different than in nonhuman and human primates. The presentation of the histopathological data provided for individual animals in the single and repeat dose studies in monkeys was vague, inconsistent, and did not utilize proper terminology. The grading system was weighted primarily towards changes in the ONL [e.g. pyknosis] and damage/closure of the medium and large choroidal vessels. The grading system did not indicate the severity of changes observed in the INL, RPE, and photoreceptors nor was the severity of the lesions in the individual animal data always indicated. It was not clear as to who read the slides or the qualifications of the individual reading the slides. However, it appeared from the terminology used that the individual was not trained in veterinary pathology. In addition, it was not clear as to whether the read was blinded or peer reviewed. [Note: The Sponsor has been requested to provide the rationale for their grading system and to provide the qualifications of the individual conducting the histopathological evaluation.] The N was small, generally ranging from 1-2 animals, although there were multiple lesions per eye. Frequently, there was only 1 lesion evaluated at a given test article dose and light dose. Therefore, this was essentially an N of 1, which is inadequate for a pivotal study. The study was not conducted in compliance with GLP according to 21 CFR 58. Consequently, these studies in the monkey are considered inadequate for regulatory purposes. Despite these limitations, the data suggested the following: [1] Efficacy as determined by CNV closure and toxicity to the retinal/choroidal tissue is dependent primarily on dose of verteporfin and time to irradiation. However, due to study design it was not possible to determine, with any confidence, the effects of fluence or irradiance. [2] The ocular toxicity is an extension of the pharmacological activity of verteporfin + irradiation. [3] No ocular toxicity was associated with drug only or irradiation only.

No retinal or ocular lesions were observed using fundus photography, fluorescein angiography, direct and indirect ophthalmoscopy and/or histopathology in monkeys following irradiation only or BPD-MA only and in dogs following administration of BPD-MA and exposure to sunlight 24 hours later. Although no retinal lesions were observed in the dog, the animals did exhibit signs that were consistent with dermal phototoxicity up to the 96-hour time point. However, evaluation of the eyes was not conducted until Day 13 and 15 following PDT. Consequently, the only reasonable conclusion is that this experimental paradigm did not result in any irreversible damage to the retina.

Studies suggested that efficacy of CNV closure following a single treatment of BPD-MA plus photoactivation in monkeys with laser-induced CNV was dependent on the dose of verteporfin and time interval between dosing and irradiation. The most common lesion reported in these monkeys, apart from CNV or choriocapillaris closure, was damage to the ONL. ONL damage ranged from minimal to >50% pyknosis. Other findings that were reported at a dose of 1 mg/kg included inner nuclear layer (INL) damage and photoreceptor damage. Serous detachment was reported at 1 mg/kg and irradiation [600 mW/cm^2; 150 J/cm^2] at 5 minutes as well as at 0.375 mg/kg with fluences of 400-600 J/cm^2 and light intensity of 600 mW/cm^2. Other findings did not clearly demonstrate a clear relationship to drug dose, irradiation dose, or timing and included [1] damage/congestion and/or closure of medium or large choroid vessels; [2] retinal damage; and [3] possible break in Bruch's membrane. Histopathological evaluation 4 weeks post irradiation suggested that the lesions were resolving, although RPE damage and macrophage infiltration persisted regardless of timing of irradiation and choriocapillaris closure was not always observed. It was not clear what histopathological lesions were attributable to the laser-induction of CNV since control lesions were not included in the histopathological evaluation.

A single dose of verteporfin + irradiation resulted in a number of lesions in the normal retina/choroid of monkeys and rabbits. Lesions varied from mild to severe, depending on the experimental conditions. In the monkey at doses of 0.375-1 mg/kg [600 mW/cm^2; 150 J/cm^2] and generally at all time points evaluated for each dose, there was closure or damage to the choriocapillaris as well as some degree of damage to the RPE, the ONL, and the outer and inner segments of the photoreceptors. Damage to the medium and large choroidal vessels [platelets, congestion, occlusion] and varying degrees of INL damage tended to be observed at doses of 0.5, 0.75, and 1.0 mg/kg. At a dose of 0.375 mg/kg, the lesions tended to become more severe as the irradiance, but not the fluence, increased. The Sponsor considered a dose of 1 mg/kg to lead to unacceptable toxicity to the normal retina and choroid. Similar lesions were observed in rabbits administered 2 mg/kg and irradiated within 30 minutes or 3 hours post dosing [10, 50, 100 J/cm^2]. In rabbits, under these experimental conditions, serous retinal detachment was observed more frequently.

In monkeys, repeat administration [once a week X 3 weeks] of verteporfin with photoactivation resulted in the development of several dose-dependent lesions. Based on histopathology, mild damage to the retina, choroid, and optic nerve were reported at doses of 6 mg/m^2 followed in 20 minutes with photoactivation at an irradiance of 600 mW/cm^2 and a fluence of 100 J/cm^2. Significant retinal, choroidal, and optic nerve damage was observed at doses of 12 and 18 mg/m^2.

Systemic Toxicity – The Sponsor conducted GLP acute and repeat dose toxicity studies in rats and dogs with and without photoactivation of BPD-MA. The primary toxicities observed in these studies included [1] local effects; [2] hematopoietic effects; [3] hepatic effects; and [4] renal effects.

Local Effects - In the single and repeat dose studies, the local toxicity observed following administration of BPD-MA and treatment of the hindlimb skin with filtered light of wavelength 687-713 nm was a function of both test article and light dose. The findings at the site of irradiation included both gross lesions [e.g. erythema, edema, scabbing, skin discoloration, and open wounds] and histopathological lesions [e.g. ulceration, necrosis extending into the muscle, granulation tissue, varying degrees of epidermal regeneration, and myositis]. In the single dose

studies, the NOAEL in the rat was 0.5 mg/kg + 50-100 J/cm^2 and in the dog was 0.1 mg/kg + 50 J/cm^2. In the intermittent dose studies [e.g. BPD-MA with photoactivation q 72 hrs for 4 treatments], the NOAEL for local effects was 0.5 mg/kg and 0.1 mg/kg + 50 J/cm^2 in the rat and dog, respectively.

Hematopoietic Effects - RBC Parameters - Decreases in RBC indices [RBC, Hb, Hct] were observed in multiple studies. The magnitude of the decreases tended to be dose and time dependent. The Sponsor stated that the decrease in these parameters was due to hemolysis induced by the liposomes. This is supported by the studies in which a negative and a liposomal control were included. In addition, this effect has been reported in the literature [**Ref. 315:** Kobayashi, T., et. al. [1983]. Lysis of erythrocytes by phosphatidylcholine containing polyunsaturated fatty acid. *J. Biochem.* 93:675-680.]. In the intermittent study in rats, but not in dogs, decreases in RBC indices were also observed on Day 13 including a 6-15% dose-responsive decrease in RBC count at ≥0.5 mg/kg. In the two week studies RBC indices were decreased by 5-26% at 25 mg/kg/day and 30-40% at 10/25 mg/kg in the rat and dog, respectively. [**Note:** Although there was no change in Hb, Hct, and RBC counts in the rat at 10 mg/kg, there was a 2X increase in percent reticulocyte suggesting a perturbation at this dose.] In rats administered verteporfin for 28 days [≥10 mg/kg/day in males and ≥2 mg/kg/day in females], RBC counts, Hb, and Hct were decreased by approximately 15-40% in a dose-dependent fashion. After a 28-day recovery, a number of the RBC indices were higher than concurrent controls. References 127, 129 and 131 [bolus injection in conscious and anesthetized pigs] demonstrated decreases in RBC counts, PCV, and Hb of approximately 20-40% with change apparently greater in anesthetized animals. 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.

There were a number of other findings described that were considered secondary to the effect of BPD-MA and the liposomal control on RBCs. In general, the magnitude of the change was dose and time dependent. These included the following. [1] **Hemolysis** was noted in blood samples in all test article groups and in the liposomal control group in the intermittent rat study. [2] There was a significant **increase in absolute and relative spleen weights** in rats ranging from 40-160% in the intermittent study and 14-day studies at 25 mg/kg and in the 28 day study in males at ≥10 mg/kg/day and in females at 25 mg/kg/day. Although returning towards baseline values, spleen weights were still increased after the 28-day recovery period. [3] There was also a 2-6X **increase in bilirubin** in the intermittent rat study at ≥1mg/kg, in the 14 day rat and dog studies at ≥10 mg/kg and 25/10 mg/kg, respectively, and in the 28-day rat study at ≥10 mg/kg/day in males and 25 mg/kg/day in females. Levels were still increased in the males at 25 mg/kg/day following the 28-day recovery. [4] A number of **RBC morphological changes** were described in the animals with decreased RBC counts including an increased incidence and/or severity of polychromasia, anisocytes, poikilocytes, target cells, spherocytes, macrocytes, crenation, and/or nRBCs. [5] **Percent reticulocytes were also significantly increased** by 2-40X in the 14 and 28-day rat and 14-day dog studies ≥10 mg/kg in rats and 25/10 mg/kg in dogs. [6] **Histopathological changes** included an increase in the severity of extramedullary hematopoiesis in the spleen and/or liver and bone marrow erythroid hyperplasia. These changes were observed in all repeat dose studies with the exception of the intermittent dog study. Following the 28-day recovery in the rat, the splenic extramedullary hematopoiesis was still observed although the incidence and severity was decreased compared to the rats sacrificed the day after 28-day dosing. [7] Finally in the 28-day rat study, there was a dose-dependent **decrease in the myeloid:erythroid ratio** at ≥10 mg/kg/day in males and females. This change was attributed to

erythroid hyperplasia, a decrease in the number of band and mature neutrophils, and a decrease in the number of lymphocytes. In general, comparable findings were noted in the liposomal control animals. These data suggested that verteporfin or liposomal vehicle administration results in a dose and dosing duration dependent regenerative anemia secondary to hemolysis.

- **WBC Parameters** – With the exception of the intermittent dose study in dogs, there tended to be a treatment-related increase in WBC counts characterized by a neutrophilia in dogs and rats and a lymphocytosis in rats. In studies without photoactivation, WBC counts were increased ranging from approximately 35-120% in the 14-day study in rats and dogs at 25 mg/kg/day and 25/10 mg/kg/day, respectively. The increases ranged from 50-300% in male and female rats administered 10 and 25 mg/kg/day, respectively, for 28 days. In general, comparable findings were noted in the liposomal control animals.

Hepatic Effects –Changes in enzymes were not consistent and did not suggest significant hepatotoxicity. Clinical pathology demonstrated sporadic increases in **AST, ALT** and/or SAP in rats and dogs. Increases in AST were also observed in conscious and anesthetized swine compared to baseline. However, no controls were provided in the pig studies and therefore, these studies should be interpreted cautiously. Histopathological changes were observed only in the liver in the 2-week dog study and 28-day rat study. Almost all dogs at 10/25 mg/kg/day exhibited globular brown pigment [positive for iron with Perl's Iron stain] and perivascular neutrophilic infiltration in the portal areas. Kupffer cell pigment accumulation was observed in rats at ≥10 mg/kg/day in males and 25 mg/kg/day in females. *In vitro* studies in human liver slices suggested that BPD-MA may induce biochemical changes associated with toxicity based on decreases in cellular ATP and K^+ concentration, and protein synthesis. In general, these effects were observed at higher concentrations and longer incubation periods.

The fact that the pigment observed histopathologically was positive for iron indicates that the hepatic lesions, including the perivascular neutrophilic infiltration observed in dogs, are secondary to the destruction of RBCs. AST is not liver specific. Since both AST and ALT are leakage enzymes, one would expect a concomitant increase with liver toxicity. However, this was not the case in these studies. Dogs did exhibit a concomitant increase in AST and SAP in the 14-day study. Rats exhibited a mild increase in ALT and SAP in the 14-day but not the 28-day study. These studies do not indicate a clear pattern of primary drug-induced hepatotoxicity. Any changes observed *in vivo* appeared to occur at the higher doses, only following multiple daily drug administration, and in conjunction with changes in RBC indices.

Renal Effects – Increases in **BUN and creatinine** were not consistent, generally did not occur concomitantly, and the increase in BUN in any study was minimal ranging from approximately 10-50%. No changes in either BUN or creatinine were observed in the 28-day rat study. Changes in **urinalysis** were uncommon and included a 2X increase in urine volume associated with a decrease in SpG and an increase in the mean concentration of urobilinogen in males and females administered 25 mg/kg/day in the 28-day rat study. After the 28-day recovery period, urine volume was increased in all treated males. **Histopathology** in the 2 week repeat dose dog study revealed an increase in incidence and severity of interstitial nephritis [slight to moderate], tubular basophilia [minimal to moderate], and granular pigment in the tubules [slight]. The pigment was iron positive and was generally associated with the interstitial nephritis and tubular basophilia. Histopathology in the 28-day rat study, revealed tubular pigment accumulation at 25 mg/kg/day in both males and females.

The fact that the pigment was positive for iron would indicate that these changes are secondary to the destruction of RBCs. The findings in the dog suggest that the presence of this iron pigment may lead to renal damage [e.g. interstitial nephritis and tubular basophilia]. The presence of the pigment may be associated with the increase in urine volume observed in the 28-day rat study. The findings in these studies do not indicate a clear pattern of primary drug-induced renal toxicity. Any changes observed *in vivo* appeared to occur at the higher doses, only following multiple daily drug administration, and in conjunction with changes in RBC indices.

Pulmonary Findings- There were trends towards increases in the incidence in interstitial pneumonia in single dose rat studies and an increase in the incidence of lung granuloma in males and females in the 28-day rat study at 25 mg/kg/day and in female dogs at ≥5 mg/kg/day. The relationship to treatment is not known.

Toxicokinetics – In both the rat and the dog, exposure to BPD-MA_D, based on AUC, was approximately two to four times greater than for BPD-MA_C. In dogs, there was no apparent difference in exposure to either regioisomer. In rats, there was no apparent gender difference in exposure to BPD-MA_C. However, exposure to BPD-MA_D, and consequently to BPD-MA, was approximately 30-45% greater in males than in females. For both the rat and the dog, exposure was comparable following single or multiple doses indicating that drug did not accumulate in the plasma. Photoirradiation did not affect exposure in the one study in rats in which a comparison was made. The table below delineates the AUC data in the rat and dog following a single exposure.

Dose [mg/kg]	CL 315,555 –AUC_{0-24} [µg•hr/ml]			CL 315,585 –AUC_{0-24} [µg•hr/ml]		
	Day 1			Day 1		
	Rat[a]		Dog[b]	Rat		Dog
	Male	Female	M +F	Male	Female	M +F
0.5	-	-	2.0	-	-	3.9
2.0	2.51	NC	-	10.7	7.43	-
5.0	-	-	19.4	-	-	42.5
10[c]	19.4	14.5	36.8	76.9	54.1	84.8
25[c]	55.9	41.4	88.6	245	166	218

NC = not calculated due to insufficient data

[a]Values reflect data from Study 92020; A two-week intravenous toxicity study of CL 318,592 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rat [Ref. 312]

[b] Values reflect data from Study TX 93004: A two-week intravenous toxicity study of CL 318,592 (Benzoporphyrin derivative monoacid ring A, a photodynamic therapeutic agent) in dog [Ref. 312]

[c]For dogs, the 25 mg/kg/day dose was administered on Day 0 and 1 and then decreased to 10 mg/kg/day. The 10 mg/kg/day PK data were obtained on Day 14 only.

Theoretically, exposure to BPD-MA, based on AUC, should be equal or comparable to the sum of the exposure of the two regioisomers. However, there was a 50-80% difference between the AUC values obtained from the 14-day rat study in which AUC values were obtained by summing the value for BPD-MA_C and BPD-MA_D and the AUC values from the 28-day rat study for which exposure to BPD-MA only was provided by the Sponsor. These differences are delineated in the table below. [Note: The Sponsor has been asked to discuss the potential source[s] of this variation.]

Dose [mg/kg]	BPD-MA –AUC_{0-24} [µg•hr/ml]			
	14-Day Study[a]		28-Days Study[b]	
	Male	Female	Male	Female
2.0	13.21	NC	23.20	18.23
10	96.3	68.6	147.61	168.97
25	300.9	207.4	441.13	371.80

[a]Values reflect data from Study 92020; A two-week intravenous toxicity study of CL 318,592 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rat [Ref. 312]. Exposure to BPD-MA was calculated by adding the AUC values of the two regioisomers

[b] Values reflect data from Study 92020; A 28-day intravenous toxicity study [with a 28-day recovery] of benzoporphyrin derivative monoacid [BPD-MA] in the albino rat [Ref. 317].

The NOAELs for the studies conducted without irradiation are as follows: [1] 2 mg/kg/day in the 14-day rat study. This represents 8X and 64X in male and female rats, respectively, human exposure based on AUC at a dose of 6 mg/m^2. [**Note:** Values were based on adding the AUC for BPD-MA_C and BPD-MA_D. The AUC for BPD-MA_C was not calculated in females at 2 mg/kg due to insufficient data. Therefore, the value for males was used since AUC for this regioisomer is similar in males and females at 10 and 25 mg/kg/day. A human AUC of 1.63 µg/ml was obtained from Study BPD PK001A]. [2] 5 mg/kg/day in the 14-day dog study. This represents approximately 38X human exposure based on AUC at a dose of 6 mg/m^2. [3] <2 mg/kg/day in the 28-day rat study – A dose of 2 mg/kg in male and female rats, respectively, represents approximately 14X and 11X human exposure based on AUC at a dose of 6 mg/m^2. In general, NOAELs were based on clinical pathology results, specifically changes in RBC indices and associated changes.

Reproductive Toxicology – There were no adverse effects on male or female fertility at doses up to 10 mg/kg/day. This dose in male and female rats represents approximately 60 and 40 times, respectively, the human dose of 6 mg/m^2 based on AUC. [**Note:** The human AUC data are from Study No. BPD PK 001A and the rat AUC data are from Study no. TX-92020: a 14-day repeat dose study. These values will be used throughout this section for extrapolating exposure.]

For the developmental studies, the Sponsor stated that the NOAEL for maternal toxicity in rats was 2 mg/kg/day based on changes in RBC indices. This NOAEL was not identified in the definitive rat developmental toxicity study [Study TX-93002] but was extrapolated from the range-finding study [3151.11]. This type of extrpolation of NOAEL across studies is considered inappropriate. Therefore, based on the endpoints evaluated in the definitive rat developmental study, a NOAEL for maternal toxicity would be ≥25 mg/kg/day. The NOAEL for maternal toxicity in the definitive rabbit developmental toxicity study [TX-93001] was 3 mg/kg/day based on a weight loss on GD 6-9 and a decrease in weight gain of 75% at 10 mg/kg compared to the controls on GD 9-12. This would suggest that the high dose used in the rabbit study was appropriate. Based on the degree of change in the RBC parameters in the dose range-finding studies, the rabbit appeared to be more sensitive than the rat.

The NOAEL for developmental toxicity in the rat was 2 mg/kg/day. This was based on an increased incidence of anopthalmia/microphthalmia that was observed in 0, 1, and 5 fetuses and 0, 1, and 4 litters at 0, 10, and 25 mg/kg/day, respectively. The historical controls provided by the laboratory indicated that the mean fetal incidence for anophthalmia and/or microphthalmia was 0.09% [range of 0-0.6%] and the mean litter incidence was 1.2% [range of 0-4.3%]. The mean fetal and litter incidence at 25 mg/kg/day exceeded these values. The mean fetal incidence

at 10 mg/kg/day was within historical control ranges [0.3%] and the mean litter incidence was only slightly greater than the mean litter range [4.5%]. However, based on the findings at the higher dose, a treatment-related effect can not be ruled out. The Sponsor suggested that this lesion might be related to maternal toxicity. However, based on the endpoints evaluated in this study, no maternal toxicity was demonstrated. Therefore, direct support for this conclusion by the Sponsor is not available. An indirect, rather than a direct, fetal effect was supported by results of the placental transfer study [A9301], which indicated that <1% of the total dose [based on radioactivity] crossed the placenta and was found in fetal tissue. The 2 and 10-mg/kg/day dose in female rats represents approximately 6 and 40 times the human dose of 6 mg/m^2 based on AUC. There were no data that indicated any change in embryofetal survivability.

The NOAEL for teratogenic effects and embryofetal toxicity in the rabbit was 10 mg/kg/day. A treatment-related effect, with respect to the slight increase in total resorptions, pre-implantation losses, and early resorptions, can not be totally ruled out. However, it is considered unlikely based on considerations outlined in the Reproductive Toxicology section. The 10 mg/kg/day dose represents approximately 20 times the human dose of 6 mg/m^2 [approximately 0.15 mg/kg] based on surface area.

In the pre and postnatal studies, there were no effects considered related to treatment on F_1 maternal performance, physical, reflexological and behavioral development, or F_1 and F_2 survival and development. The NOAEL for pre and postnatal development was 10 mg/kg/day. The 10-mg/kg/day dose in female rats represents approximately 40 times the human dose of 6 mg/m^2 based on AUC.

Genotoxicity - Under the conditions tested, BPD-MA without irradiation was found to be negative for mutagenicity in the Ames Salmonella/*E. coli* plate incorporation assay and CHO cell/HGPRT mutation assay. Only a single dose without irradiation was evaluated in the CHO cell chromosomal aberration assay and the UDS assay. Therefore, as conducted, these assays provide inadequate data to assess the potential of BPD-MA under dark conditions to induce genotoxicity in these test systems.

Under the conditions tested, BPD-MA with light activation was found to be negative for both mutagenicity and clastogencicity in the Ames Salmonella/*E. coli* plate incorporation assay, UDS assay, CHO cell/HGPRT mutation assay, and CHO chromosomal aberration assay. Although there was no increase in chromosomal aberrations in the CHO chromosomal aberration assay for the BPD-MA + light irradiation groups, there was an increase in the number of chromatid gaps, chromatid breaks, and endoreduplications at various time points and doses compared to the concurrent solvent control. This was suggestive of DNA damage.

There was some concern with the way in which these studies were conducted. These concerns included [1] failure to fully characterize the emission spectra of the light source which was used; [2] failure to include a positive control [e.g. known photogenotoxicant] to insure that the assay could detect mutagenic/clastogenic potential of a photoactive drug under the conditions tested; and/or [3] failure to conduct a confirmatory assay [See ICH Guideline S2B]. In addition, these studies were conducted prior to issuance of ICH guidelines. Therefore, there were deviations from the recommendations presented in both ICH Guideline S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals and ICH Guideline S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. For details see Summary of Genotoxicity.

In Vivo Assay – Under the conditions tested, BPD-MA, with and without photoactivation, was negative in the mouse micronucleus assay.

Photodynamic therapy has been reported to result in DNA damage including DNA strand breaks, alkali-labile sites, DNA degradation, and DNA-protein cross links which may result in chromosomal aberrations, sister chromatid exchanges [SCE], and mutations.[1] However, the literature also indicates that whether or not a PDT is mutagenic or clastogenic appears to be dependent on the cell line evaluated.[1,2] The findings in the studies reviewed here appear to be consistent, therefore, with what has been reported. Hematoporphyrin + light activation has been shown to cause DNA damage and induce sister chromatid exchange but not to increase the incidence of mutations in the CHO/HGPRT mutation assay.[3] Photofrin was negative following light activation in the Ames assay, the CHO/HGPRT mutation assay, and the CHO chromosomal aberration assay. It was, however, reported to result in [1] a significant increase in the incidence of SCE in CHO cells [visible light irradiation] and Chinese hamster lung fibroblasts [near uv light irradiation]; [2] an increase in *tk* mutants and DNA-protein crosslinks in mouse L5178Y cells; and [3] "a light-dose dependent increase in DNA-strand breaks in malignant human cervical carcinoma cells, but not in normal cells".[4] The factors that determine whether or not a PDT is mutagenic or clastogenic do not appear to have been fully elucidated. Therefore, the toxicological and biological significance of the DNA damage associated with PDT is unclear.

Phototoxicity – Studies conducted in mice demonstrated that a dose of 2 mg/kg of BPD-MA followed in 3, 24, and 48 hours by irradiation with a solar simulator did not result in any significant phototoxicity. However, following irradiation at 3 hours after administration of 10 mg/kg, severe skin lesions developed including edema, erythema, and/or eschar and after administration of 20 mg/kg death occurred. Solar simulator irradiation at 24 hours after administration of 10 and 20 mg/kg resulted in less severe reactions including pink, scaly, and/or swollen tail. A pilot study, conducted in beagle dogs administered 20 mg/kg indicated that the most severe phototoxicity occurred in animals exposed to sunlight 24 hours post-dosing. Animals exposed to sunlight at ≥48 hours post drug administration, exhibited phototoxicity for 3-4 days including slight to moderate erythema of the shaved forelimb and perinostril area. Study PH-93002 in Yucatan mini swine suggested that there were two phases to the phototoxicity. The first phase may be due to activation of drug in the microvasculature of the skin, and, therefore, severity was a function of plasma levels. The second, later, phase may be due to activation of drug in the skin and vasculature, and, therefore, severity was a function of both plasma and tissue levels. The severity of and susceptibility to phototoxicity appears to be dose-related.

Immune System Effects - The Sponsor conducted several studies to evaluate [1] the potential immunogenicity of BPD-MA in rats, rabbits, and guinea pigs; [2] the effect of BPD-

---

[1] Evans, H.H., et. al. [1997]. Mutagenicity of photodynamic therapy as compared to UVC and Ionizing Radiation in human and murine lymphoblast cell lines. *Photochem. Photobiol.* 66[5]:690-696.

[2] Rerko, R.M., et. al. [1992]. Photofrin II photosensitization is mutagenic at the *tk* locus in mouse L5178Y cells. *Photochem. Photobiol.* 55[1]:75-80.

[3] Gomer, C.J., et. al. [1983]. Comparison of mutagenicity and induction of sister chromatid exchange in Chinese hamster cells exposed to hematoporphyrin derivative photoradiation, ionizing radiation, or Ultraviolet Radiation. *Cancer Res.*. 43:2622-2627.

[4] Physicians' Desk Reference. 53rd Edition [1999], Medical Economics Company, Montvale NJ., p. 2796.

MA ± irradiation on delayed type hypersensitivity in mice; and [3] the effect of BPD-MA on immunocompetency in mice. These studies should be interpreted cautiously since there were several concerns with respect to study design. One of the major concerns was that the Sponsor did not include appropriate controls. The deficiencies in these studies are delineated under the Special Toxicology section. These studies, however, suggested that BPD-MA was neither immunogenic nor immunosuppressive. BPD-MA alone did not appear to modify the DTH response. However, in conjunction with irradiation, the DTH response was suppressed. These studies suggested that UVA alone or in combination with BPD-MA were equally effective in certain strains of mice. The Sponsors noted that activated immune cells appear to have greater uptake of drug than nonactivated cells. Theoretically, under certain experimental conditions, activated/ stimulated immune cells, therefore, should be more sensitive to the cytotoxic effects of activated BPD-MA.

In Vitro Human Blood Compatibility - BPD-MA did not induce hemolysis under the conditions tested in Study 90057. However, hemolysis was observed in the repeat dose studies in both rats and dogs. In addition, literature provided by the Sponsor described studies in which RBCs were lysed following incubation with phosphatidylcholine in humans, rabbits, rats, and mice. The authors cited literature that suggested that cholesterol was removed from RBCs membranes when the RBCs were incubated with egg yolk phosphatidylcholine liposomes.

Homogeneity, stability and dosing system compatibility – These studies indicate that verteporfin is stable for up to 11 hours at room temperature and for up to 3 months if frozen.

Recommendations: These will be provided as an addendum to the review.

NDA Issues:

Labeling Review:

1. Clinical Pharmacology

Mechanism of Action

VISUDYNE therapy is a two-stage process requiring administration of both verteporfin for injection and nonthermal red light

Verteporfin is transported in the plasma primarily by [redacted] Once verteporfin is activated by light in the presence of oxygen, highly reactive, short-lived singlet oxygen [redacted] are generated. Light activation of verteporfin results in local damage to neovascular endothelium, resulting in vessel occlusion. Damaged endothelium is known to release procoagulant and vasoactive factors through the lipo-oxygenase [leukotriene] and cyclo-oxygenase [eicosanoids such as thromboxane pathways, resulting in platelet aggregation; fibrin clot formation and vasoconstriction]. [redacted]

The temporary occlusion of choroidal neovasculature (CNV) following VISUDYNE therapy has been confirmed in humans by fluorescein angiography.

Pharmacokinetics

Follow intravenous infusion, verteporfin exhibits a elimination half-life approximately 5-6 hours. Extent of exposure and the maximal plasma concentration are proportional to the dose between 6 and 20 mg/m^2. At the intended dose, pharmacokinetic parameters are not significantly affected by gender

Verteporfin is to its diacid metabolite by liver and plasma esterases. NADPH-dependent liver enzyme systems [including the cytochrome P450 isozymes] do not appear to play a role in the metabolism of verteporfin. Elimination is by the fecal route, with less of the dose recovered in the urine.

Precautions

There is no clinical data related to the use of VISUDYNE in anesthetized patients. At a >10-fold higher dose given by bolus injection to anesthetized pigs verteporfin caused severe hemodynamic effects, including death, probably as a result of complement activation. These effects were diminished or abolished by pretreatment with antihistamine and they were not seen in conscious pigs or in any other species, whether conscious or under general anesthesia.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been conducted to evaluate the carcinogenic potential of

No effect on male or female fertility in rats following intravenous administration at up to 10 mg/kg/day

(approximately 60 and 40 fold human exposure at 6 mg/m^2 based on AUC_{inf} in male and female rats, respectively[1]).

[1 The multiple of the human exposure was extrapolated from rat AUC data from Study TX-92020: A two-week intravenous toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rats. The human value for AUC_{inf} was 1.63 μg•hr/ml obtained from healthy male Caucasians in Study BPD PK 001A.]

Pregnancy

Teratogenic Effects

Pregnancy Category C – There are no adequate and well-controlled studies in pregnant women. VISUDYNE should be used during pregnancy only if the benefit justifies the potential risk to the fetus.

Rat fetuses of dams administered at ≥10 mg/kg/day during organogenesis (approximately 40 fold human exposure at 6 mg/m^2 based on AUC_{0-24} in female rats[2]) exhibited an increase in the incidence of anophthalmia/microphthalmia. Rat fetuses of dams given 25 mg/kg/day had an increased incidence of wavy ribs and

In pregnant rabbits, a decrease in body weight gain and food consumption was observed in animals that received intravenously at 10 mg/kg/day during organogenesis. The no observed adverse effect level (NOAEL) for maternal toxicity was 3 mg/kg/day (approximately 7 fold human exposure at 6 mg/m^2 based on body surface area). There were no teratogenic effects observed in rabbits at doses up to 10 mg/kg/day.

*[2 The multiple of the human exposure was extrapolated from rat AUC data from Study TX-92020: **A two-week intravenous toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rats. The human value for AUC was 1.63 μg•hr/ml obtained from healthy male Caucasians in Study BPD PK 001A.]***

[The multiple of the human exposure was extrapolated from rat AUC data from Study TX-92020: A two-week intravenous toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, a photodynamic therapeutic agent) in rats. The human value for AUC_{inf} was 1.63 μg•hr/ml obtained from healthy male Caucasians in Study BPD PK 001A.]

Nursing Mothers

It is not known whether [redacted] is excreted in human milk. Because many drugs are excreted in human milk [redacted]

N21-119 **VISUDYNE**

QLT Phototherapeutics, Inc.

Investigator's Brochure/Informed Consent Review:

Reviewer's Signature: /S/

Susan D. Wilson, D.V.M., Ph.D.

11-30-99

Date

Team Leader Concurrence: /S/

Andrea Weir, Ph.D., D.A.B.T.

11-30-99

Date

CC: list:

cc:Original

HFD-550:Division Files
HFD-550:Div.Dir/KMidthun
HFD-550/Pharm/SDWilson
HFD-550/CSO/LGorski
HFD-550/MO/Dep.Dir/ WChambers
HFD-550/CHEM/AFenselau
HFD-550:PK/VTandon

Appendix:

Draft Date:

Addendum:

134 *pages have been removed here because they contain confidential information that will not be included in the redacted portion of the document for the public to obtain.*

ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW - NDA 21-119

TO:	**Lori Gorski**
FROM:	**Susan Wilson**
THROUGH:	**Andrea Weir**
DATE:	**Dec. 7, 1999**
RE:	**N21-119 – VISUDYNE** **QLT Phototherapeutics** **Addendum to NDA Review**

INTERNAL RECOMMENDATIONS

I. **SAFETY PHARMACOLOGY** – Profound cardiovascular effects were observed in anesthetized, but not conscious, swine following iv bolus administration of VISUDYNE. These effects included marked decreases in CO, HR, and systemic BP, as well as cardiorespiratory arrest and death. In addition, ECG changes were consistent with hypoxia [e.g. ST segment depression]. Additional studies suggested that the cardiovascular effects may be related to not only the magnitude of complement activation but also to the rate of activation. Pretreatment with Benadryl did not block complement activation but did block the development of profound cardiovascular events. The Sponsor suggests that this response was species specific since it was not observed in other species and was secondary to complement activation by negatively charged phospholipids [e.g. egg phosphatidylglycerol]. Unfortunately, the Sponsor did not evaluate the effects of the liposomal formulation without verteporfin, which would have supported their argument. Although the studies conducted in anesthetized dogs did not demonstrate similar findings, these studies had deficiencies. The relevance of the cardiovascular effects observed in the pig to humans is not known.

II. **OCULAR TOXICOLOGY** – From a regulatory perspective, the studies provided by the Sponsor were not considered adequate to assess the ocular toxicity of VISUDYNE plus irradiation. The Sponsor conducted several studies in the cynomolgus monkeys, which is considered an appropriate model due to similarities between the blood supply to nonhuman and human primates. However, the histopathological descriptions were inconsistent, vague, and did not utilize proper terminology. The grading system devised by the Sponsors was weighted primarily towards changes in the ONL and damage/closure of the medium and large choroidal vessels. The grading did not consistently indicate the severity of changes observed in the INL, RPE, and photoreceptors. It was not clear as to who read the slides nor the qualifications of the individual reading the slides. However, it appeared from the terminology used that the individual was not trained in veterinary pathology. In addition, it was not clear as to whether the read was blinded or peer reviewed. The N was small, generally ranging from 1-2 animals. Although there

were multiple lesions per eye, there was often only 1 lesion evaluated under a given test article and light dose regimen. The study was not conducted in compliance with GLP according to 21 CFR 58. Despite these limitations, the following conclusions seem appropriate from the data provided: [1] efficacy, as determined by closure of laser induced CNV closure, and toxicity to the normal retinal and choroidal tissue is dependent primarily on the dose of verteporfin and time to irradiation; [2] the ocular toxicity is an extension of the pharmacological activity of verteporfin plus irradiation; [3] no ocular toxicity appears to be associated with drug only or irradiation only.

III. SYSTEMIC TOXICOLOGY –

A. Hematopoietic Effects – Statistically and biologically significant decreases observed in RBC indices [RBC count, Hb, Hct], increases in percent reticulocytes, and alterations in RBC morphology were consistently observed in both rats and dogs. Studies conducted in pigs also suggested changes in RBC indices following a single dose. However, the appropriate controls were not included, which could have ruled in or out a treatment-related effect. The magnitude of the change in RBC parameters tended to be dose and time dependent. The Sponsor states that the decrease in these parameters is due to hemolysis induced by the liposomes. This is supported by the studies in which a negative and a liposomal control were included and by literature provided by the Sponsor [Ref. 315]. Associated changes included an increase in bilirubin, an increase in absolute and relative spleen weights in rats, and histopathological changes [e.g. splenic and/or hepatic extramedullary hematopoiesis, bone marrow erythroid hyperplasia.] There also tended to be an increase in WBC counts characterized by a neutrophilia in both rats and dogs. The NOAEL for hematological parameters in the 14 day study in male rats, female rats, and dogs was 8X, 6X, and 38X, respectively, the human exposure based on AUC.

B. Hepatic Effects – Histopathological changes in the liver were observed in the 14-day dog study and the 28-day rat study at 10/25 mg/kg/day and ≥10 mg/kg/day, respectively. The changes in the dogs included accumulation of globular brown pigment, which was positive for iron with Perl's iron stain, and neutrophilic infiltration in the portal areas. In the rat, pigment accumulation was observed in the Kupffer cells. Since the pigment was positive for iron, it would appear that these lesions are secondary to RBC hemolysis. Changes in enzymes [e.g. AST, ALT, and SAP] were not consistent. *In vitro* data suggest that BPD-MA may induce biochemical changes associated with toxicity. The toxicology studies do not indicate a clear pattern of primary drug-induced hepatotoxicity. The changes observed appear to occur at higher doses, generally following multiple daily drug administration, and generally seen in conjunction with RBC changes.

C. Renal Effects – Histopathological changes in the kidney in the 14-day repeat dose dog study included an increase in the incidence and severity of interstitial nephritis [slight to moderate], tubular basophilia [minimal to moderate], and granular pigment in the tubules [slight]. This pigment was positive for iron and generally seen in association with the nephritis and basophilia. In the 28-day rat study, pigment accumulation in the tubules was noted. Increases in BUN and creatinine were inconsistent, generally did not occur concomitantly, and tended to be mild. A 2X increase in urine volume associated with a decrease in SpG was measured in the 28-day rat study. These studies do not indicate a clear pattern of primary drug-induced renal toxicity. The changes observed tended to occur at the higher doses, generally following multiple daily drug administration, and in conjunction with RBC changes.

D. Skin Phototoxicity – Phototoxicity was observed in dogs for up to 96-hours following administration of 20 mg/kg of verteporfin and exposed to sunlight. Phototoxicity was severe in dogs exposed at 24 hours after drug administration. In animals exposed ≥48 hours, erythema was

slight to moderate on the shaved forelimb and perinostril area. In mice administered 2 mg/kg of verteporfin did not demonstrate any significant phototoxicity when exposed to a solar simulator 3, 24, and 48 hours later. However, significant phototoxicity was observed at 10 and 20 mg/kg. The severity of and duration of susceptibility to phototoxicity appears to be dose-related.

E. Immune System Effects – Studies suggested that verteporfin was not antigenic or capable of eliciting a hypersensitivity response in rabbits and guinea pigs. However, it was shown that verteporfin with activation can suppress a delayed type hypersensitivity reaction. In addition, the Sponsor indicates that verteporfin has been investigated for its potential utility for the treatment of several autoimmune disorders. Therefore, under the correct conditions, verteporfin with photoactivation can be immunomodulatory/immunosuppressive. Data also indicated that activated immune cells are potentially more susceptible to PDT than quiescent cells. Under the proposed usage, it is felt that this is unlikely to pose a significant risk, but a treatment-related effect can not be ruled out.

F. Local Tolerance – No studies have apparently been conducted to address the potential for local irritation following accidental extravasation.

G. Pulmonary Effects – There was an increase in the incidence of interstitial pneumonia in the single dose rat study and an increase in the incidence of lung granuloma in the 28-day rat study and in female dogs in the 14-day study. The relationship to treatment is not known.

IV. Reproductive Toxicology – There were no effects on fertility in either males or females. There was an increase in the incidence of anopthalmia/microphthalmia at ≥10 mg/kg/day and an increase in bent ribs and fetal alterations at 25 mg/kg/day in fetuses from rats administered verteporfin daily from GD 6-15. There were no developmental abnormalities observed in the fetuses from rabbits administered up to 10 mg/kg/day of verteporfin from GD 6-18. There was a slight increase in the incidence of total resorptions, pre-implantation loss, and early resorptions. The incidence of total resorptions 1 and 2 does out of 18 at both 3 and 10 mg/kg/day exceeded the mean historical control value. However, the Sponsor will be requested to provide the historical range for this occurrence for the laboratory in which the study was conducted. There were no effects on pre and post natal development at doses up to 10 mg/kg/day.

V. Genotoxicity – There were several deficiencies in these studies which are discussed above. Under the conditions tested, verteporfin without irradiation was found to be negative for mutagenicity in the Ames Salmonella/*E. coli* plate incorporation assay and CHO cell/HGPRT mutation assay and negative in the rat micronucleus assay. The UDS assay and CHO cell chromosomal aberration assay were considered inadequate to assess the potential of verteporfin to induce genotoxicity under dark conditions. Under the conditions tests, verteporfin with light activation was found to be negative for both mutagenicity and clastogenicity in the Ames Salmonella/*E. coli* plate incorporation assay, CHO cell/HGPRT mutation assay, the UDS assay and the CHO cell chromosomal aberration assay, and the rat micronucleus assay. In the CHO cell chromosomal aberration assay with irradiation, there did appear to be an increase in the number of chromatid gaps, chromatid breaks, and endoreduplications at various time points and doses. Photofrin was also negative in these studies. However, Photofrin did exhibit mutagenic effects in other *in vitro* systems in which verteporfin was not evaluated.

The deficiencies have been discussed with the medical officer, Dr. Wiley Chambers. Based on these discussions, approval is recommended contingent on resolution of labeling concerns.

forwarded to sponsor 1/6/00

EXTERNAL RECOMMENDATIONS

1. Please provide the range of the historical control data for the incidence of total resorptions in rabbits from the laboratory in which study TX 93001. The data should be within 5 years of the year in which the study was conducted [e.g. 1993]

Reviewer's Signature

/S/

Susan D. Wilson, DVM, Ph.D.

7 Dec 99

Date

Team Leader Concurrence

/S/

Andrea Weir, Ph.D., D.A.B.T.

7 Dec 99

Date

CC:
Original/Div. File
HFD-550/DivDir/KMidthun
HFD-550/DepDir/MO/WChambers
HFD-550/CSO/LGorski
HFD-550/PharmTox/SWilson
HFD-550/Chem/AFenselau

APPEARS THIS WAY
ON ORIGINAL

NDA 21-119 Visudyne
Review #2 QLT Phototherapeutics

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.
Review #3

NDA Number: N21-119

Serial Number: No serial numbers were provided for the submissions reviewed here

Submission [Letter] Date:	Oct. 12, 1999	Nov. 29, 1999	Nov. 30, 1999	Dec. 10, 1999
Type of Submission:	BP	BP	BP	BP

Information to Sponsor: Yes(X)

Completion Date: February 2, 2000

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) of drug substance:

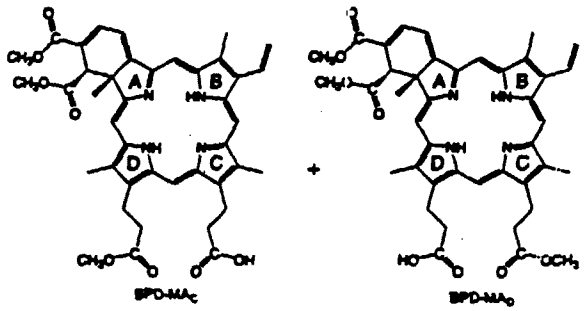
Drug name:
1° - VISUDYNE™
2° - verteporfin
3° - BPD-MA
4° - benzoporphyrin derivative monoacid ring A
5° - CL 318,952

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
TX-99004	Aug. 18, 1999	An acute arterial irritancy study of verteporfin for injection in the rabbit [correspondence date of 11-29-99, Appendix 1, p. 7-53]	TC0631

Studies not reviewed within this submission

Report No.	Report Date	Study Title
TX-98008	Jul. 19, 1999	Test for chemical induction of gene mutation at the HGPRT locus in cultured Chinese hamster ovary [CHO] cells with and without metabolic activation with a confirmatory assay [correspondence date of 11-30-99, Appendix 4, p. 43-111]*

This study has previously been reviewed [draft report]. The Sponsor will be asked to provide a statement that indicates whether or not there were any changes in the final report.

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

Introduction/Drug History:

I. The following pharmacology/toxicology comments were forwarded to the Sponsor on September 22, 1999. The initial comment, Sponsor's response [dated October 12, 1999], and Reviewer's comment are provided below:

1. **Comment** - Please clarify the following. Results from dogs identified as #101 and 151 appear in both References 121 [PH-93032; Cardiovascular Effects of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent, Without Photoactivation in the Beagle Dog], and 122 [PH-940014; Cardiovascular Effects of Benzoporphyrin Derivative Monoacid Ring A (BPD-MA), a Photodynamic Therapy Agent, Without Photoactivation in the Anesthetized Beagle Dog]. Please indicate if these are unique identifiers [e.g. does 101 and 151 refer to a specific animal] or are the same identifiers used for different studies. It was also noted that two animals [102 and 152] used in the study reported in Reference 121 were not euthanized but used in subsequent studies. Please indicate, if applicable to this submission, in which study they were used.
Sponsor's Response – The animal identifiers used by [redacted] were not unique identifiers. The animals are assigned numbers at the start of each study with males starting at 101 and females at 151. Animals 102 and 152 were not used in any other study reported in the NDA submission.
Reviewer's Comment – This response is adequate.

2. **Comment** - In Study TX93-9003; Pilot Study: A Single Dose Intravenous Study in Dogs of Liposomal Benzoporphyrin Derivative Monoacid Ring A (BPD-MA; CL-318,952), it is indicated that female DA0113 had a sore on the scrotum. Please clarify this discrepancy.

Sponsor's Response – This discrepancy was due to a coding error that will be corrected and a final report amendment will be issued.
Reviewer's Comment – This response is adequate. Appendix 5 of the submission dated November 30, 1999 is an amendment to the final report, which states that this entry was changed in the final report to "accurately reflect the raw data".

II. The submission dated November 26, 1999 contains Sponsor proposed labeling revisions. Proposed labeling pertaining to pharmacology/toxicology issues is provided in the NDA review. Therefore, these revisions will not be reviewed here.

III. The following pharmacology/toxicology comments were forwarded to the Sponsor on November 29, 1999. The initial comment, Sponsor's response [dated November 29, 1999], and Reviewer's comment are provided below:

1. **Comment** – There appeared to be differences in the exposure to verteporfin based on AUC at comparable doses in the 14-day vs. the 28-day repeat dose toxicity study in rats [Study Nos. 92020 and TX-96010, respectively]. Expressing exposure to total drug as the sum of the individual regioisomers, the exposure based on AUC on Day 1 in the 14-day study was 13.21, 96.3, and 300.9 µg•hr/ml at 2, 10, and 25 mg/kg in male rats. In the 28-day study, the AUC on Day 1 in male rats was 23.2, 147.6 and 441.3 µg•hr/ml at 2, 10, and 25 mg/kg. Comparable differences were observed in females. Please comment on these differences. Please also indicate which values you feel are the most appropriate for calculating multiples of the human exposure based on AUC [e.g. for labeling purposes]. Furthermore, please provide the basis for this determination.
Sponsor's Response – Different methodologies used in these studies may have contributed to the differences. However, no other explanation for the differences in the apparent plasma concentrations was proposed. They recommended use of the more conservative values for extrapolation of exposure from rats to humans.
Reviewer's Comment – This response is adequate.

2. **Comment** - Please indicate what nonclinical data are available which specifically addresses the potential for local toxicity [e.g. irritation, etc] following accidental extravasation of verteporfin. If these data have been submitted, please indicate in which reference[s] this information can be found.
Sponsor's Response – No studies have been conducted which specifically address the potential for local toxicity following accidental extravasation of verteporfin. They submitted a study in rabbits that assessed the potential for irritation of an intra-arterial injection. This study [reviewed below] indicated that there was no irritation at doses up to 0.6 mg/kg. The Sponsor also stated that "the relatively high local verteporfin concentrations achieved after local extravasation are expected to result in extreme local photosensitivity".
Reviewer's Comment – In general, this response is adequate. However, the irritation potential of unactivated drug has not been adequately addressed in nonclinical studies.

3. **Comment** – The following comments refer to the formulation used in the nonclinical studies.

a. **Comment** - Please indicate which nonclinical studies were conducted with test article that was prepared by the thin film manufacturing process and which were conducted with test article prepared by the presomal manufacturing process.
Sponsor's Response – With the exception of study TX 98008 [Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation with a Confirmatory Assay], all nonclinical studies were conducted with test article prepared by the thin film manufacturing process.
Reviewer's Comment - This response is adequate.

b. **Comment** – In Study No. 315.11 [Reference 344], there is a statement on pg. 10, that the test article "was prepared up to two days prior to use and was maintained at room temperature". In addition, you indicate that the test article was determined to be stable under these conditions. If you have already submitted the data that support this statement, please indicate where it can be found in the submission. Otherwise, please provide these data.
Sponsor's Response – Data to support the stability of reconstituted verteporfin was provided in this submission.
Reviewer's Comment – The response is adequate [This conclusion is based on discussions with the reviewing chemist for this NDA, Allan Fenselau].

c. **Comment** – In several pivotal studies [e.g. Study Nos. TX-96009 (Ref. 340), TX-98003 (Ref. 347), and TX-96010 (Ref. 317)], you indicate that formulations were prepared weekly and refrigerated. You indicated that you have determined that refrigerated reconstituted test article is stable for up to 10 days. If you have already submitted the data that support the stability of verteporfin under the experimental conditions [e.g. reconstituted and refrigerated for up to a week] in these nonclinical studies, please indicate where it can be found in the submission. Otherwise, please provide these data.
Sponsor's Response – Data to support the stability of reconstituted verteporfin was provided in this submission. These data indicate that the liposomal BPD-MA stored at 5° C was "good" [<2% hydrolysis] for 1 month following reconstitution and for 2 weeks when diluted. Aggregates formed when reconstituted material was stored frozen.
Reviewer's Comment – The response is adequate [This conclusion is based on discussions with the reviewing chemist for this NDA, Allan Fenselau].

IV. The following pharmacology/toxicology comments were forwarded to the Sponsor on October 20, 1999. The initial comment, Sponsor's response [dated November 30, 1999], and Reviewer's comment are provided below:

1. **Comment** – The following comment is in reference to the 28-day repeat dose toxicity study in rats [TX-96010]. It is indicated on page 12 of the study report that 8, 4, and 3 animals were replaced on Days 1, 6, and 7, respectively. Please provide the numerical identifier for the replaced and replacement animals. Please also indicate the dose group to which each animal was assigned and finally, how many doses the replacement animals received.
Sponsor's Response – The Sponsor provided a table that indicated that on Day 1 there were 1, 2, 1, and 4 animals replaced in Groups 1, 2, 3, and 4, respectively. On Day 3, 1

Group 1 animal was replaced. On Day 6, 3 and 1, Group 3 and 4 animals, respectively, were replaced. On Day 7, 2 animals each from Groups 2 and 4 were replaced. All replacement animals were dosed for the entire 28 days.
Reviewer's Comment – Although it would have been preferred that the number of replacement animals was comparable across the groups, it was felt that this deviation did not significantly impact the integrity of the data obtained.

2. **Comment** – The following comments are in reference to the ocular toxicity studies conducted in cynomolgus monkeys specifically, Studies TX 96008 and 94027.

a. Comment – The grading system for histopathological damage to the retinal/choroidal tissues appears to be weighted based on damage to the outer nuclear layer [ONL]. There is no apparent quantification outlined for differentiation of the severity of the choriocapillaris closure, retinal pigmented epithelial damage, or photoreceptor damage for Grades 2, 3, and 4. The main difference then would appear to be the degree of ONL pyknosis. In addition, medium/large choroidal vessel damage or retinal vessel damage does not appear to be a requisite for classification as Grade 5 retinal/choroid damage. Therefore, in the absence of medium/large choroidal or retinal vessel damage, there does not appear to be a difference in the description of Grade 4 vs. Grade 5 classification. Please provide a rationale for and a clarification of the grading system.
Sponsor's Response – According to the Sponsor, the grading system was designed to "grade the effects on retinal vessels, large choroidal vessels, and the neurosensory retina when PDT was applied to normal retina and choroid". Choriocapillaris occlusion and retinal pigmented epithelial damage were not graded since damage was expected and/or observed in every lesion. In the experience of the primary investigator in these studies, damage to the choriocapillaris is seen at the same doses of light and drug of other PDT agents. The difference in the first 4 grades was based on ONL pyknosis, which served as a marker for photoreceptor damage. Grades 4 and 5 are differentiated on the basis of the absence or presence, respectively, of choroidal or retinal vessels. The Sponsor stated that the "goal [was] to determine the bounds of acceptable dosimetry".
Reviewer's Comment – Based on the Sponsor's response, the goal of this grading system was to establish dosimetry, and consequently it is inadequate as a grading scheme to assess ocular toxicity, especially in a regulatory setting. A complete description of the extent and severity of the damage to the various layers and cell types of the eye should have been provided.

b. Comment – Please provide the identification and qualifications of the individual[s] reading the histopathological slides for these studies and whether the read was blinded or peer reviewed.
Sponsor's Response – "The histopathological slides were reviewed by a team comprised of Dr. Joan Miller (principal investigator), Dr. Thomas Flotte, and Norman Michaud, with additional research fellows over the years working under Dr. Miller's direction." The reads were not blinded.

Reviewer's Comment – A veterinary pathologist did not evaluate the slides. Slides in a given study should have been evaluated by a single individual. In addition, research

fellows would not be considered qualified to conduct a histopathological evaluation. Therefore, the histopathological evaluations conducted for these studies are considered inadequate for regulatory purposes.

Additional Reviewer Comments – The Sponsor also provided 2 published papers that had not been submitted in the NDA package. It is not clear if these literature citations are based on data presented in the NDA package. Both of these manuscripts were based on data generated in Dr. Miller's laboratory. Based on the considerations delineated above, they will not be reviewed. It is felt that they would not provide any additional safety data.

V. The following pharmacology/toxicology comments were forwarded to the Sponsor on October 20, 1999. The initial comment, Sponsor's response [dated November 30, 1999], and Reviewer's comment are provided below:

1. **Comment** – Please provide the range of the historical control data for tne incidence of total resorptions in rabbits from the laboratory in which Study TX-93001 was conducted. The data should be within 5 years of the year in which the study was conducted [e.g. 1993].
   Sponsor's Response – The historical control data were provided for total litter resorptions. There were no total litter resorptions in 9/11 studies and 1 female with litter resorptions in 2/11 studies. The range was 0 – 6.3%. Although the incidence in the mid-dose was within this range [1/18; 5.6%], the high dose was not [2/18; 11.1%]. The Sponsor also states that there was maternal toxicity at the high dose.
   Reviewer's Comment - The incidence of total litter resorption is outside of the historical control range. However, the relationship to treatment is not known. For this application, Age-Related Macular Degeneration, there is minimal concern for human risk with respect to this finding. This is due to [1] the age of the intended population and [2] the clinical usage of 1 injection q3-4 months vs. the repeat dosing regimen used in the nonclinical study.

Pharmacology: No new studies

Safety Pharmacology: No new studies

Pharmacokinetics/Toxicokinetics: No new studies

Toxicology: No new studies

Carcinogenicity: No studies

Reproductive Toxicology: No new studies

Genotoxicity: No new studies

Special Toxicology:

A. Irritancy Studies

a. Rabbit

i. Title: An Acute arterial irritancy study of verteporfin for injection in the rabbit [Vol. 1.1; correspondence date of 11-29-99]
Study Identification: TX-99004
Site:
Study Dates: May 14-21, 1999
Formulation and Lot No.: Verteporfin for injection: Lot No. TC0631
Certificate Analysis: Yes (X) The test article had expired by 1 month. The Sponsor indicates that they have data that supports that this test article lot was suitable for testing based on stability data. purity; dose formulations prepared day of dosing [report indicates that analysis of dose solution concentration was provided
Final Report (X) Aug. 18, 1999
GLP and QA Statement Signed: Yes (X)
Objective: "To investigate the potential acute arterial irritancy of Verteporfin for Injection following a single intra-arterial injection in the rabbit"

Test Material/ Group Designation	Dose*				Sex	N	Species/ Strain
	mg/kg	ml/kg	route	# doses			
Group 1 – 5% dextrose	0	0.5	intra-Arterial	1	M	3	New Zealand White Rabbits -
Group 2 - Verteporfin	0.15						
Group 3 - Verteporfin	0.3						App. 5 mos
Group 4 - Verteporfin	0.6						2.6-3.1 kg at start of study

*Animals were house at <20 foot candles during and for up to 24 hours post dosing

Parameter Evaluated	Timing
Clinical observations/mortality	BID
Draize evaluation* –erythema, edema	Predose, 0.5, 2, 4, and 6 hours post dose, then SID through Day 8
Gross Pathology	Day 8

*Histopathology was not conducted since there were no apparent differences between control and test article animals

Results – Clinical Signs/Mortality – There were neither unscheduled deaths nor any clinical signs.

- **Draize Evaluation** – Draize scores were comparable across all groups. Very slight edema was observed sporadically in all groups. Very slight [1] to moderate/severe [3]

erythema was observed in all groups and all animals through the 6-hour observation period. Very slight to mild [2] erythema was observed in all groups and all animals through 4 days. Very slight erythema was sporadically observed Days 5-8.

- **Gross Pathology** – There were no apparent treatment-related effects.

Reviewer's Comment (Study Design and Data Presentation) – These were adequate

Sponsor's Conclusions and Reviewer's Comments – A single intra-arterial injection of Verteporfin for Injection did not elicit any evidence of arterial irritancy. **Reviewer's Comment** – The Reviewer concurs that there was no gross evidence of arterial irritancy since histopathology was not conducted.

Summary of Special Toxicology -

Overall Summary

Recommendations:

a. Internal Comments:

1. The Sponsor's did not conduct a study in which the potential for local toxicity of verteporfin [without photoactivation] following accidental extravasation was evaluated.

2. The grading scheme and the qualifications of individuals evaluating the histopathology slides in the ocular toxicity studies in cynomolgus monkeys [Studies TX 96008 and 94027] were not adequate for regulatory purposes. Therefore, these studies are considered unacceptable for assessing the ocular toxicity of verteporfin in cynomolgus monkeys.

3. The incidence of total litter resorptions in the rabbit developmental study [TX-93001] was outside of the historical control range [0-1/study; 0 – 6.3%] for the laboratory in which the study was conducted. Although the incidence in the mid-dose was within this range [1/18; 5.6%], the high dose was not [2/18; 11.1%]. The relationship to treatment is not known. For this application, Age-Related Macular Degeneration, there is minimal concern for human risk with respect to this finding. This is due to [1] the age of the intended population and [2] the clinical usage of 1 injection q3-4 months vs. the repeat dosing regimen used in the nonclinical study.

b. External Recommendations:

1. The Sponsor will be asked to provide a statement that indicates whether or not there were any changes from the draft report to the final report for study TX-98008 [Test for chemical induction of gene mutation at the HGPRT locus in cultured Chinese Hamster ovary [CHO] cells with and without metabolic activation with a confirmatory assay].

NDA Issues:

Labeling Review:

Investigator's Brochure/Informed Consent Review:

Reviewer's Signature:

/S/

Susan D. Wilson, D.V.M., Ph.D.

3 Feb 00

Date

Team Leader Concurrence:

/S/

Andrea Weir, Ph.D., D.A.B.T.

3 Feb 00

Date

CC: list:

cc:Original
HFD-550:Division Files
HFD-550:DivDir/KMidthun
HFD-550:DepDir;MO/WChambers
HFD-550/Pharm/SDWilson
HFD-550/CSO/LGorski

Appendix:

Draft Date: Jan. 14, 2000

Addendum: