Test Material/ Group Designation	Dose*				N		Species/Strain
	mg/kg	ml/kg	Route	# days dosed	M	F	
Group 1 –10% lactose	0	12.5/5***	iv	14	4	4	Beagle dogs
Group 2 - BPD-MA	0.5	0.25					
Group 3 - BPD-MA	5	2.5					App. 7-8 months at study start
Group 4 - BPD-MA	25/10**	12.5/5**					M = 10.7-12 kg; F = 8.3-10.9 kg

*A saline flush was used both before and after dose administration. Animals were maintained in lighting of <20 foot candles

**Dogs were dosed at 25 mg/kg for 2 days but due to what the Sponsor felt was intolerance, the dose was decreased to 10 mg/kg/day.

***Dose volume was adjusted to 5 ml/kg on Days 2-16. At the higher volume and dose, the vehicle dogs developed generalized erythema. This was not observed at the 5 ml/kg volume.

Parameters Evaluated	Time Point(s)*
Clinical examination Mortality/moribundity Clinical observations Physical examination	 BID 2 hours before, during [days 1 and 2], and 2 and 4 hours after dosing prior to dosing, Days 3 and 13
Body Weight	Days –11, -4, -1, 6 and 13
Food Consumption	Daily beginning Day –11
ECG	Days –12 and 10
Ophthalmology [indirect ophthalmoscopy] – included modifications of lighting, conducted by a board certified veterinary ophthalmologist	Days –7 and 9
Hematology [jugular vein, fasting] – RBC count and morphology, MCV, MCH, MCHC, Hct, Hb, platelet count, reticulocyte count, WBC and differential Coagulation parameters – APTT, PT	Days –8, 8, and 14
Serum Chemistry [jugular vein, fasting] – A/G ratio, ALT, alb., SAP, AST, BUN, Ca, Cl, chol., creat., glob., gluc., P, K, Na, total bili., TP, triglyc.	Days –8, 8, and 14
Urinalysis [fresh sample] – appearance, bili., blood/Hb, color, gluc., ket, nitrite, pH, prot., SpG, urobil., vol., sediment exam	Days –7, 1, and 9
Gross Pathology	At sacrifice
Organ Weights – adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, thymus, thyroid and parathyroids	At sacrifice
Histopathology - adrenals, aorta, bone [distal femur], brain [three levels], cecum, colon, duodenum, epididymides, esophagus, eyes, gall bladder, heart, ileum, injection site, jejunum, kidneys, liver, lungs, lymph nodes [submandibular, mesenteric], macroscopic lesions, mammary gland, marrow [rib], ovaries, pancreas, peripheral nerve, pituitary, prostate, rectum, salivary glands [mandibular], seminal vesicles, skeletal muscle, skin, spinal cord [3 levels], spleen, stomach, testes, thymus, thyroid and parathyroids, tongue, tonsil, trachea, ureters, urethra, urinary bladder, uterus, vagina	At sacrifice
Toxicokinetics [N=4/sex/group] – calculated based on the log-linear trapezoidal rule, HPLC with UV detector	Days 0 and 14 – pre-dose [Day 14 only], immediately after dosing, 0.5, 1, 2, 4, 8, 12, and 24 hours post dose

*Dosing was started on Day 0

Results

Mortality – No treatment-related deaths

Clinical Observations/Physical Examinations

- Erythema was observed in the control animals on Day 1. Decreasing the volume of vehicle from 12.5 ml/kg to 5 ml/kg on Day 2 eliminated this sign.
- On Day 1, the high dose dogs were administered 25 mg/kg. This was associated with the following signs: [1] pale gums in 2/4 for each sex; [2] hypoactivity in 3/4 and 4/4 in males and females, respectively; and [3] red urine in 4/4 and 2/4 males and females, respectively. On Day 2, the Sponsor decreased the dose to 10 mg/kg because they felt that these signs were

"indicative of intolerance". Hypoactivity was observed in 1/4 males and females on Day 2 but not after Day 2. The red urine and pale gums were observed through Day 4 although the frequency decreased. Mucosal membrane pallor was observed in 1/4 and 2/4 males and females, respectively, on Days 0-4. [**Reviewer's Comment:** In Study 93031, dogs received 10 mg/kg/day X 3 days without developing any clinical signs.]

- Dark green stool, due to presence of dark green test article, was observed in all animals at ≥5 mg/kg by the end of the first week.
- Other signs including soft stool, emesis, and neck swelling [1 mid dose male] were considered incidental.

Body Weight and Food Consumption – There was a decrease in mean body weight gain in males and females in the high dose group. The weight gain in males from Day –1 to 13 was 285.5 g and – 35.3 g in the control and high dose males, respectively and –32.2 g and –145.5 g in the control and high dose females, respectively. These changes represent <1-3% of total body weight. There was a decrease in food consumption primarily in the high dose dogs that correlated with the decrease in body weight gain, e.g. those animals demonstrating the greatest decrease in body weight gain generally demonstrated the greatest decrease in food consumption. The maximum decrease in food consumption was observed during the first few days of dosing when high dose animals were exhibiting clinical signs.

ECG [Report prepared by A. W. Beardow, BVM&S, MRCVS, ACVIM] – There were no treatment-related effects.

Ophthalmic Examination [Report prepared by Lionel Rubin, VMD] - There were no treatment-related effects.

Hematology

- **RBC Indices** – A number of statistically significant perturbations in RBC parameters were observed in the high dose males and females compared to control animals on both Days 8 and 14. These changes included a decrease in RBC numbers, Hb, and Hct and an increase in reticulocytes, normoblasts, platelets, and MCV. A decrease in MCHC and an increase in MCH [males only] of <10% was also noted. No effects were observed at ≤5 mg/kg. The values for control and high dose animals are provided in the table below.

Parameter	Dose [mg/kg]			
	0		25/10	
	M	F	M	F
Hematocrit [%]				
Day 8	40.9 ± 1.1	42.8 ± 1.5	26.9 ± 3.4	30.7 ± 1.8
Day 14	40.5 ± 0.7	42.1 ±2.0	30.7 ± 1.8	33.7 ± 3.8
Hemoglobin [g/dl]				
Day 8	14.2 ± 0.4	14.9 ± 0.7	8.8 ±1.3	10.1 ±1.5
Day 14	13.9 ±0.4	14.5 ± 0.8	10.5 ± 0.6	10.9 ±1.4
RBC [E^6/mm^3]*				
Day 8	6.2 ± 0.2	6.4 ±0.3	3.6 ±0.6	4.2 ±0.9
Day 14	6.1 ±0.2	6.3 ± 0.4	4.1 ±0.3	4.6 ± 0.7
Reticulocytes [%]				
Day 8	0.8 ±0.2	0.7 ±0.2	11.2 ±3.1	10.0 ±6.3
Day 14	0.5 ±0.2	0.7 ±0.1	7.1 ±1.7	6.3 ± 2.2
Normoblasts [per 100 WBC]				
Day 8	0	0.3 ± 0.5	2.3 ± 2.1	3.3 ± 2.9
Day 14	0	0.3 ± 0.5	0.3 ± 0.5	2.3 ±1.5
MCV [μ^3]				
Day 8	65.9 ± 1.6	67.2 ± 2.0	75.4 ±2.9	73.4 ± 5.7
Day 14	66.2 ± 1.4	67.3 ±1.9	74.1 ± 1.1	73.2 ± 3.9
Platelets [E^3 X mm^3]*				
Day 8	280.0 ± 50.3	250.0 ± 26.3	812.5 ± 126.9	616.5 ± 218.3
Day 14	255.0 ±26.5	242.5 ± 18.9	780.0 ± 137.6	702.5 ± 235.3

*Number of cells/mm^3

The following changes in RBC morphology were observed in the majority of animals in the high dose at both time points: increase in poikilocytosis, target cells, polychromasia, spherocytes, macrocytes, and anisocytosis. With the exception of moderate anisocytosis, the severity of the other morphological changes was graded slight/few.

- **WBC Indices** – WBC counts were elevated by approximately 40 and 60% in the low and high dose males, respectively on Day 8. The increase in the low dose could be attributed to a single animal and the relationship to drug-treatment is questionable since comparable changes were not observed in the mid dose animals. All males at the high dose demonstrated an increase in WBC counts. WBC counts increased by 124% and 40% in high dose females on Days 8 and 14, respectively. The WBC counts in the mid dose female group were greater than controls at all time points [e.g. including Day-8] making interpretation difficult. The increase in both males and females was secondary to an increase in PMNs. These data are outlined in the table below.

Parameter	Dose [mg/kg]			
	0		25/10	
	M	F	M	F
WBC [E^3/mm^3]*				
Day 8	10.6 ± 0.6	7.9 ± 0.8	16.9 ± 4.1	17.9 ± 5.5
Day 14	11.5 ± 1.9	7.7 ± 0.8	9.3 ± 1.7	10.4 ± 2.6
Neutrophils [E^3/mm^3]*				
Day 8	6.1 ± 0.4	4.5 ± 0.9	13.1 ± 3.5	13.9 ± 5.2
Day 14	7.9 ±1.1	4.4 ± 1.2	5.3 ± 1.1	7.0 ±2.4

*Number of cells/mm^3

- **Coagulation Parameters- There were no treatment-related effects.**

Serum Chemistry

- K^+ - There was a statistically significant increase in K^+ for both high dose males and females on Days 8 and 14 when compared to controls.
- **Hepatic indices** –There was a statistically significant increase in bilirubin for both high dose males and females on Days 8 and 14 when compared to controls. There was an increase in AST and SAP for both high dose males and females when compared to controls which reached statistical significance on Day 8 but not Day 14. The increase in females can be attributed to a single female exhibiting a large magnitude of change. There was an increase in cholesterol for both high dose males and females when compared to controls which reached statistical significance in females on Day 8 only. ALT was not affected.
- **Renal indices** - There was a 30% increase in BUN in high dose males on Day 8 compared to controls. The relationship to treatment is not known. However, since there was not a concomitant increase in creatinine, the change in BUN is unlikely to reflect renal changes.
- **Glucose** – There was approximately a 10-15% decrease in glucose in high dose males and females compared to control animals. The relationship to treatment is not known. However, this decrease is considered not to be toxicologically significant due to the small magnitude of change.

The data for changes in potassium, bilirubin, AST, SAP, and cholesterol are outlined in the table below.

Parameter	Dose [mg/kg]			
	0		25/10	
	M	F	M	F
Potassium [mEq/L]				
Day 8	4.6 ± 0.2	4.5 ± 0.1	5.3 ± 0.1	5.1 ± 0.3
Day 14	4.6 ± 0.4	4.6 ±0.0	5.4 ± 0.1	5.1 ± 0.2
Bilirubin [mg/dl]				
Day 8	0.1 ± 0.0	0.1 ±0.0	0.3 ±0.1	0.6 ±0.4
Day 14	0.1 ± 0.0	0.1 ±0.0	0.2 ±0.1	0.4 ± 0.3
Cholesterol [mg/dl]				
Day 8	166.3 ± 17.3	168.5 ± 16.8	221.5 ± 55.7	226.8 ± 22.0
Day 14	165.3 ± 14.2	176.0 ± 25.3	182.8 ± 36.4	200.8 ± 23.8
Alk Phos [IU/L]				
Day 8	101.3 ± 6.6	104.5 ± 16.2	153.0 ± 37.1	275.3 ± 302.7
Day 14	95.0 ± 5.0	98.0 ± 14.0	134.0 ± 34.8	257.0 ± 282.2
AST [IU/L]				
Day 8	27.3 ± 5.9	27.3 ± 2.1	44.0 ± 4.5	41.8 ± 2.5
Day 14	26.0 ± 5.6	25.5 ± 2.1	29.5± 2.4	30.3 ± 1.0

Urinalysis - The findings observed on Days 1-3 [unscheduled urine sampling] were consistent with hemoglobinuria. By Day 9, there were no treatment-related effects with the exception of an increase in bilirubin in the high dose males. Urine samples were obtained from only 2 high dose females on Day 9. The data for Days 1-3 are presented in the table below.

Parameter	Dose [mg/kg]			
	0		25/10	
	M	F	M	F
RBC/HPF	+1 [2/4]	+1 [1/3]	+2 [4/4]	1/6 [3/3]
Color	yellow	Yellow	dark cherry	dark cherry
Dipstick				
Blood	trace - +1 [2/4]	trace [1/3]	3 [2/2]*	3 [2/2]*
Protein	trace - +1 [4/4]	trace [3/3]	182.8 ± 36.4	200.8 ± 23.8

*unable to interpret in the other animals in the group

Gross Necropsy – Red mediastinal lymph nodes were observed in 1/8, 2/8, and 3/8 animals at 0.5, 5, and 25/10 mg/kg. The relationship to drug treatment is not known.

Organ Weights – No spleen weights were obtained. Changes in the weight of other organs were sporadic and generally <15%. No treatment-related pattern was observed in either absolute or relative weights.

Histopathology – The primary target organs appeared to be the liver, kidneys, spleen, and bone marrow with changes observed in both sexes at 10/25 mg/kg. The incidence and severity of the lesions are indicated in the table below.

	Males				Females			
Group:	1	2	3	4	1	2	3	4
Dose (mg/kg/day):	0	0.5	5	25→10[a]	0	0.5	5	25→10[a]
Number of animals:	4	4	4	4	4	4	4	4
LIVER								
-Brown pigment, globuler, Kupffer cells	0	0	0	4	0	0	0	3
(mean severity)	-	-	-	2.5	-	-	-	3.0
-Infiltrate, neutrophils	0	0	0	3	0	0	0	3
(mean severity)	-	-	-	1.7	-	-	-	2.0
KIDNEYS								
-Nephritis, interstitial	1	0	0	2	0	0	0	0
(mean severity)	1.0	-	-	2.5	-	-	-	-
-Basophilia, tubular	0	0	1	3	1	0	0	2
(mean severity)	-	-	1.0	2.3	1.0	-	-	1.5
-Pigment, tubule granular	0	0	0	2	0	0	0	1
(mean severity)	-	-	-	2.0	-	-	-	2.0
SPLEEN								
-Hematopoiesis	4	4	4	4	2	4	4	4
(mean severity)	1.0	1.0	1.0	3.5	1.0	1.0	1.5	3.3
BONE MARROW, RIB								
-Erythropoiesis, increased	0	0	0	1	0	0	0	2
(mean severity)	-	-	-	2.0	-	-	-	2.0
MEDIASTINAL LYMPH NODE[b]								
-Hemorrhage/ congestion	0	0	1	3	0	1	1	0
(mean severity)	-	-	3.0	2.7	-	3.0	3.0	-

[a] This group was given 25 mg/kg/day for the first two days of dosing.
[b] Only mediastinal nodes with gross findings were examined microscopically.

Note: Severity grades- 1-minimal, 2-slight, 3-moderate, 4-marked, and 5-massive.

In the liver, there was perivascular neutrophilic infiltration in the portal areas. Based on Perl's Iron stain and Periodic Acid Schiff [PAS] stain, the pigment was positive for iron [e.g. hemosiderin] and also PAS positive [possible lipofuscin]. Mononuclear infiltration tended to increase in severity and/or incidence in both high dose males and females. The overall incidence in control animals was 5/8 [1] and in high dose animals was 7/8 [1.8]. There was a similar trend in the mid dose animals with an incidence of 6/8 [1.5].

The pigment in the kidney was generally associated with interstitial nephritis and/or tubular basophilia. As in the liver, the pigment was identified as iron positive.

In the spleen, in addition to the hematopoiesis, there was also an increase in the incidence [severity] of congestion. Congestion was not observed in any control dogs but was seen in 2 [2.5], 1[3], 1[3] males at 0.5, 5, and 10/25 mg/kg and 1 [3] and 2 [3] females at 5 and 25/10 mg/kg.

There was an increase in the incidence of lung granulomas in females but not in males. The incidence in females was 1/4, 2/4, 3/4 and 4/4 animals at 0, 0.5, 5, and 25/10 mg/kg, respectively. The overall incidence was 3/8 control animals and 6/8 high dose animals. The relationship to treatment is not known, but a similar increase of low magnitude was observed in the rats treated for 28 days.

The incidence and severity of injection site reactions were comparable across all treatment groups.

Toxicokinetics – Based on AUC, exposure to both regioisomer, CL 315,585 and CL 315, 555, was roughly dose proportional. C_{max} was less than dose proportional, which was attributed to differences in rate of dose administration in the low [bolus, <1 min.] vs. mid and high [rapid infusion, >1 min.] dose groups. Exposure to CL 315,585 was approximately 2X that of CL 315,555 which was a function of slower clearance rather than differences in volume of distribution. Values on Day 1 were similar to those obtained on Day 14 indicating that there was essentially no accumulation. There were no apparent gender differences in the toxicokinetics of the drug. The table below delineates the toxicokinetic data for both regioisomers.

Mean (CV%) Toxicokinetic Parameters for CL 315,555 and CL 315,585 (Regioisomers of BPD-MA) in Dogs Given Daily IV doses of BPD-MA for 2 Weeks

BPD-MA Dose (mg/kg)	Study Day	Cmax (µg/mL)	Tmax (hr)	AUC(0-24) (µg*hr/mL)	AUCo-inf (µg*hr/mL)	TOTAL CLEARANCE (mL/min/kg)	Vss (L/kg)	T½ (hr)
CL 315,555								
0.5	0	3.1 (16)	0.0	2.0 (16)	2.0 (16)	2.2 (16)	0.26 (20)	1.8 (21)
0.5	14	2.8 (19)	0.0	2.0 (14)	2.0 (14)	2.7 (12)	0.38 (11)	2.1 (13)
5	0	20.2 (21)	0.07 (36)	19.4 (17)	19.4 (17)	2.2 (20)	0.31 (11)	1.9 (10)
5	14	17.0 (14)	0.07 (12)	18.2 (19)	18.3 (20)	2.4 (21)	0.36 (21)	2.9 (69)
25	0	38.3 (17)	0.34 (12)	88.6 (12)	88.8 (12)	2.4 (11)	0.48 (11)	2.8 (11)
10	14	26.3 (24)	0.12 (17)	36.8 (13)	37.4 (13)	2.3 (12)	0.44 (20)	4.9 (64)
CL 315,585								
0.5	0	3.7 (15)	0.0	3.9 (15)	3.9 (15)	1.1 (14)	0.28 (15)	3.9 (11)
0.5	14	3.3 (14)	0.0	3.8 (16)	3.8 (16)	1.2 (20)	0.31 (10)	3.5 (11)
5	0	26.7 (21)	0.07 (33)	42.5 (23)	43.4 (24)	1.0 (25)	0.30 (17)	4.3 (8)
5	14	23.5 (12)	0.07 (12)	39.0 (21)	40.4 (23)	1.1 (23)	0.35 (13)	5.5 (13)
25	0	71.5 (13)	0.34 (12)	218 (11)	224 (12)	0.9 (12)	0.33 (8)	4.4 (11)
10	14	37.4 (22)	0.12 (17)	84.8 (14)	87.9 (14)	1.0 (12)	0.33 (9)	5.5 (13)

Cmax values reported are the observed values.
CL(t) and Vss values corrected for contribution (~50%) of each regioisomer
Note – Since the dosing solution concentration and rate of dosing were kept constant across dose groups, the duration of dosing increased as dose increased.
AUC(0-24)= In order to compare exposure in each animal over a 24 hr dosing interval, the conc.-time profile was extended for some animals. In these cases, a comparison of AUC (0-t) and AUC (0-24) revealed little (<5%) differences. For details, consult the study notebook.

Reviewer's Comments – Study Design and Data Presentation – In general, the data presentation and study design were adequate.

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

1. Doses of 0.5 and 5 mg/kg/day administered intravenously daily for 2 weeks were well tolerated by beagle dogs without any toxicologically significant effects. **Reviewer's Comment-** The Reviewer concurs.

2. A dose of 25/10 mg/kg/day resulted in hemolysis and secondary clinical pathology and histopathological changes. These changes indicated liver and kidney toxicity. **Reviewer's Comment** – The Reviewer concurs.

Additional Reviewer's Comments-

1. The Sponsor suggested that the erythrophagocytosis and/or congestion in the mediastinal lymph nodes were not treatment related since no other lymph nodes were affected and there was not a dose-dependent response in severity. This lesion was not observed in any control animals and a treatment-related effect can not be totally ruled out. The significance of this lesion is not known.

2. There was an increase in the incidence of lung granulomas in female.

IV. *In Vitro* Studies

a. Direct hepatotoxicity of BPD verteporfin [BPD-MA] in human liver slices cultured *in vitro* [Ref. 336]

Study Identification: TX-94015

Site: [redacted]

Study Dates: Dec. 1993 – Mar. 1994

Formulation and Lot No. – liposomal BPD-MA- Lot No. not provided

Vehicle – 5% dextrose

Certificate Analysis: No (X)

Final Report: June 24, 1994

GLP and QA Statements Signed: No (X)

Objective: "To investigate whether BPD-MA had any direct effect on liver metabolism and cell integrity at both clinically relevant and excessive doses"

Study Design - Human liver slices [N=6] were incubated with BPD-MA at 0 [5% dextrose], 0.5, 1.5, 4.5, and 45 μg/ml. At 3, 6, 12, 24, and 48 hours samples were collected and the following endpoints were evaluated: [1] cellular ATP concentration; [2] cellular K^+ concentration; [3] protein synthesis; and [4] histopathology. The drug concentration selection was justified as follows. [1] 1.5 μg/ml is comparable to the maximum plasma levels obtained within 1 hour post human dosing of 0.5 mg/kg [Study BPD-001; range of 1.5-2.4 μg/ml]. [2] 0.5 μg/ml is comparable to the plasma concentration at 3-hour post drug administration. [3] 4.5 and 45 μg/ml represent approximately 2-3 and 25X the C_{max} for a human dose of 0.5 mg/ml.

Results – The table below delineates the individual results. Values represent change from control.

Donor [Death/medications]	ATP Concentration	K^+ Concentration	Protein Synthesis
43-yr-old M - [head injury – ethanol abuse, dilantin]	↓46-60% @ 4.5 μg/ml; 12-48 hrs. ↓27-71% @ 45 μg/ml; 6-48 hrs.	↓13% @ 4.5 and 45 μg/ml; 48 hrs.	↓24-45% @ 1.5-45 μg/ml; 48 hrs.
43-yr-old F - [intracranial bleed – diffuse hepatic intracellular microvesicular fat]	↓20% @ 1.5 μg/ml; 48 hrs. ↓28-30% @ 4.5 μg/ml; 24-48 hrs. ↓35-42% @ 45 μg/ml; 24-48 hrs.	↓19% @ 4.5 μg/ml; 48 hrs. ↓18% @ 45 μg/ml; 48 hrs.	↓20% @ 1.5 μg/ml; 48 hrs. ↓30% @ 4.5 μg/ml; 48 hrs. ↓21-40% @ 45 μg/ml; 12-48 hrs.
53-yr-old F - [head injury]	↓20% @ 45 μg/ml; 48 hrs.	↓14-18% @ 4.5 and 45 μg/ml; 48 hrs.	↓25% @ 45 μg/ml; 48 hrs.
6-yr-old F – [developmental disorder and seizures - phenobarbital]	No change	No change	↓24% @ 4.5 μg/ml; 48 hrs. ↓32-68% @ 45 μg/ml; 24-48 hrs.

Donor [Death/medications]	ATP Concentration	K^+ Concentration	Protein Synthesis
2-yr-old F –[drowning]	No change	↓25% @ 45 µg/ml; 48 hrs.	↓28% @ 4.5 µg/ml; 48 hrs. ↓38-50% @ 45 µg/ml; 6-48 hrs.
54-yr-old F – [Cantizen SR]	↓21-35% @ 4.5 µg/ml; 12-48 hrs. ↓26-39% @ 45 µg/ml; 12-48 hrs.	↓13% @ 45 µg/ml; 48 hrs.	↓19% @ 1.5 µg/ml; 48 hrs. ↓31% @ 4.5 µg/ml; 48 hrs ↓31-34% @ 45 µg/ml; 24-48 hrs.

No histopathological changes were observed at any concentration or time point.

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

1. "There is evidence of some direct action of BPD-MA on cultured liver tissue, particularly at late time points and high drug doses." **Reviewer's Comment** – The Reviewer concurs.

2. "Comparison with published data on a compound [valproic acid] known to have hepatotoxic properties leads to the conclusion that at clinically relevant doses, the effects are minimal." **Reviewer's Comment** – The robustness of this type of comparison is dependent on the data being generated from the same study. In addition, the experimental conditions under which valproic acid was evaluated is not known.

V. **Literature Reviewed:**

a. Ref. 315: Kobayashi, T., et. al. [1985]. **Lysis of erythrocytes by phosphatidylcholine containing polyunsaturated Fatty Acid.** *J. Biochem.* 93:675-680.

This manuscript describes a study in which human RBCs were incubated with egg yolk phosphatidylcholine. The results indicated that at 37°C and a concentration of 60 µM, 50% of the human RBCs were lysed. When human RBCs were preincubated with 0.5 µM of albumin, RBC hemolysis was inhibited by 50%. The authors also demonstrated hemolysis of erythrocytes following incubation with phosphatidylcholine from other animals indicating that this effect is species-independent: [1] human – 260 minutes incubation; [2] rabbit – 125 minutes incubation; [3] rat – 90 minutes incubation; and [4] mouse – 60 minutes incubation. The authors also cited literature that suggested that cholesterol was removed from RBCs membranes when the RBCs were incubated with egg yolk phosphatidylcholine liposomes.

b. Ref. 320: Discussion related to the need to conduct a 28-day repeated dose toxicity study in nonrodents with verteporfin™ [Liposomal benzoporphyrin derivative monoacid: BPD-MA); prepared by CanTox Inc., Mississauga, Ontario

This reference provided a rationale for not conducting a 28-day repeat dose study in dogs concluding that "such a study would not contribute any substantially different information from which to characterize the toxicity profile of the drug under conditions of its intended use in the treatment of AMD". This conclusion was based on the following considerations. [1] Verteporfin will be administered at a maximum of 4X/year. "This regimen does not easily fit into the standard framework of the ICH guidance" [e.g. M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals]. However, for Phase I, II, and III trials in the U.S., a 2-4 week repeat dose study in rodents and a 2 week repeat dose study in nonrodents is adequate to support a trial lasting up to 2 weeks. [2] The elimination $t_{1/2}$ is approximately ≤7 hours and there is no indication that there is any cumulative systemic toxicity. Nor is it considered likely that drug will accumulate over 3 months between treatments. [3] The mechanism of action [e.g.

local generation of singlet oxygen following photoirradiation] and the adverse events in humans primarily due to local effects [e.g. tissue exposed to light] "argues against systemic toxicity of therapeutic doses". [4] The animal models based on PK data appear adequate. [5] Significant exposure was obtained in the 28-day exposure in rats and the systemic toxicity observed was largely reversible following a 28-day recovery. [6] Although there were quantitative changes observed in the 28-day vs. the 14-day repeat dose toxicity study in rats, there were no qualitative differences. [7] Comparable toxicities were observed in the rat and the dog. In general, the Reviewer agrees. The hepatic and renal toxicity was more pronounced in the dog after a 14-day exposure compared to the rat. However, it is anticipated that quantitative, but not qualitative, differences in toxicities would be observed. The Reviewer concurs that a 28-day repeat dose toxicity in a nonrodent is not warranted.

Summary of Toxicology - **Ocular Toxicity** - The Sponsor conducted studies or provided literature which described the ocular toxicity of verteporfin only, irradiation only, and/or verteporfin plus irradiation in cynomolgus monkeys, dogs, and rabbits. These results should be interpreted keeping in mind the following concerns. The studies in the rabbits were provided 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. In addition, there are differences in the retinal vasculature of rabbits compared to both human and nonhuman primates. Localization of BPD-MA in corneal neovascularization was evaluated. The normal cornea, unlike the retina, is an avascular structure. 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.] In the monkey studies, the N was small generally ranging from 1-2 animals, although there were multiple lesions per eye. In general, there was only 1 lesion evaluated under a given test article and light dose regimen. This is essentially an N of 1, which is inadequate for a pivotal study. The studies were not conducted in compliance with GLP according to 21 CFR 58. **Based on these considerations, these monkey studies are considered inadequate for regulatory purposes. Despite these limitations, the data suggest 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 is 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 under the following treatment regimens/experimental designs: [1] exposure of the retina to irradiation only in cynomolgus monkeys (N =1 monkey/time point; 698 nm; 600 mW/cm^2, 100 J/cm^2; 167 seconds) and in rabbits (692 nm; 100 mW/cm^2; up to 100 J/cm^2); **[2] verteporfin only in Cynomolgus monkeys maintained at <20 light foot candles; and [3] verteporfin only (10 and 20 mg/kg) + 6-hour exposure to sunlight (1200 J/cm^2) 24-96 hours following drug administration in Beagle**

dogs. In the dog study, in which dogs were administered BPD-MA and then exposed to sunlight 24 hours later, although no retinal lesions were observed, 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.

The Sponsor conducted studies or provided literature which described the ocular effects [both efficacy and safety] of verteporfin + irradiation in both cynomolgus monkeys and rabbits. These studies used fundus photography, fluorescein angiography, and histopathology to evaluate the effects of treatment on both normal choroid and retina as well as on experimentally induced choroidal neovascularization [CNV]. Both single dose and repeat dose studies were conducted in the monkey. The single dose study in the monkey evaluated the effect of varying [1] dose of verteporfin (0.25-1 mg/kg); [2] time of irradiance (5-120 minutes post drug administration); [3] light intensity (irradiance of 150-1500 mW/cm^2); and [4] fluence (50-600 J/cm^2).

The efficacy of CNV closure following a single treatment in the monkey [e.g. 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. In the animals with laser-induced CNV evaluated at 4 weeks post irradiation, although it would appear that the lesions were resolving, 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 of the normal retina/choroid resulted in a number of lesions in both the monkey and the rabbit. 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. No effect or minimal residual retinal/choroidal damage was observed at 7 weeks post PDT [0.375 mg/kg; 150 J/cm^2; 600 mW/cm^2]. 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 3 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 [q1week X 3 weeks] of verteporfin at approximately 0.47, 0.96, and 1.4 mg/kg followed in 20 minutes by irradiation of either the fovea or the optic nerve head resulted in the development of several lesions. The severity of these lesions was dose-dependent. Fundus photography 24-hours post irradition revealed retinal whitening in all test article groups only after the first treatment. Retinal edema was observed after the second and

third treatments at doses ≥0.96 mg/kg and was severe in the high dose animals. The edema tended to persist for at least 1 week. Fluorescein angiography revealed closure of the choriocapillaris at all doses. Histopathology revealed damage to the retina, choroid, and optic nerve. At 0.47 mg/kg, there was minimal choriocapillaris damage, mild RPE and outer photoreceptor damage, and mild optic nerve atrophy. At doses ≥0.96 mg/kg, histopathological findings included choriocapillaris closure, severe RPE and outer sensory retina damage, and severe vascular closure and hemorrhage within the optic nerve. Lesions in the fovea treated group largely resolved in three weeks.

Systemic Toxicity – The Sponsor conducted GLP acute and repeat dose toxicity studies in rats and dogs. These studies were conducted with and without photoactivation. The study design for the single dose rat studies was as follows: [1] verteporfin at 0.2 –20 mg/kg + photoactivation 30 minutes after drug administration [app. 690 nm; 71 mW/cm^2; 25-100 J/cm^2] and [2] verteporfin at 10-100 mg/kg [5 doses q2 hrs] without photoactivation. One single dose dog study was conducted with dogs [N=1] dosed at 0.1-20 mg/kg of verteporfin and 50-100 J/cm^2. One group of dogs was administered 20 mg/kg of verteporfin only. [**Note:** The Sponsor indicated that the dogs administered 10 and 20 mg/kg of verteporfin became stressed and did not receive the full 100 J/cm^2 dose of irradiation. These animals received between 3-15 J/cm^2.] An intermittent dose study [animals dosed with verteporfin q72 hr X 4 and skin irradiated at 50 J/cm^2 3 hours after dosing] was conducted in both rats and dogs. Doses in the rat and dog were 0.5, 1, and 2 mg/kg and 0.1, 0.5, and 0.7, respectively. There was a saline and a liposomal solution without BPD-MA control group in both the rat and dog study. A dose escalation study was also conducted in the dog [0.2 – 10 mg/kg X 3 days without light activation]. Two-week repeat dose studies without photoactivation were conducted in the rat [2 studies] and dog [1 study]. Doses in the rat and dog were 2, 10 and 25 mg/kg or 25 mg/kg BID and 0.5, 5, and 10/25 mg/kg, respectively. Both studies included a liposomal and a 5% dextrose or 10% lactose control groups. [**Note:** In the dog study, the dose was reduced from 25 to 10 mg/kg/day on Day 2. The Sponsor felt the dogs were exhibiting signs of intolerance at 25 mg/kg including pale gums, hypoactivity, and red urine.] A 28-day repeat dose toxicity study was conducted in rats only at doses of 2, 10, and 25 mg/kg/day. A group of animals administered 5% dextrose and the high dose were maintained through a 28-day recovery period. 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, the NOAEL for local effects was 0.5 mg/kg and 0.1 + 50 J/cm^2 in the rat and dog, respectively.

Hematopoietic Effects - RBC Parameters - Decreases in RBC indices [RBC, Hb, Hct] was observed in multiple studies. The magnitude of the decreases 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. 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.]. Clinical pathology was not conducted in the single dose studies in rats. Three hours after irradiation in the single dose dog study, RBC indices in males and

females were decreased by approximately 25% at 20 mg/kg + photoactivation. RBC indices also tended to be decreased by <12% in males females at doses of 0.5-10 mg/kg + irradiation and at 20 mg/kg without irradiation. However, the relationship to treatment was less clear at these doses since comparisons are made to a liposomal control. In addition both test article and light dose varied. Compared to the liposomal control, RBC indices were normal by Day 14. In the intermittent study in rats but not dogs similar changes in RBC indices [e.g. a 6-15% dose-responsive decrease in RBC count at ≥0.5 mg/kg were also observed on Day 13. 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 count in the rat at 10 mg/kg, there was a 2X increase in percent reticulocyte suggesting a perturbation at this dose. See below.] In rats administered verteporfin for 28 days [≥10 mg/kg/day in males and ≥2 mg/kg/day in females], RBC count, 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, with the difference reaching statistical significance for some indices.

There were a number of other findings described that were secondary to the effect of BPD-MA and the liposomal control on RBCs. In general, the magnitude of 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 an **increase in absolute and relative spleen weights** in rats ranging from 40-90% in the intermittent study at 25 mg/kg, >100% in the 14 days study at 25 mg/kg, and 25% and 160% in the 28 day study in males at ≥10 mg/kg/day and in female at 25 mg/kg/day. Although returning towards baseline values, spleen weights were still increased after the 28-day recovery period in rats. [3] There was also an **increase in bilirubin** of approximately 2X at ≥1mg/kg in the intermittent rat study, a 2-6X increase in the 14 day rat and dog study 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, and spherocytes. [5] **Percent reticulocytes** were also significantly increased by 2X and 6-10X at 10 and 25 mg/kg in the 2-week rat study, 10-15X at 25/10 mg/kg/day in the 14-day dog study, and 5-7X and 15-40X in the 28-day rat study at 10 and 25 mg/kg/day, respectively. [6] **Histopathological changes** included an increase in the severity of extramedullary hematopoiesis in the spleen and/or liver and bone marrow erythroid hyperplasia. The 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 days of 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 suggest 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. [**Note:** Hematology was not conducted in the single dose rat studies.] There was considerable variability in the WBC counts in the single dose dog study. There tended to be an increase in absolute neutrophil count [approximately 15-

200%] in all treatment groups with or without photoactivation. These values were also elevated compared to baseline values, were observed on both Days 1 and 14, and tended to be greater in males than in females. This increase was associated with a decrease in lymphocyte count in several groups [males at ≥ 2 mg/kg and females at ≥ 10 mg/kg + photoactivation.] In the intermittent dose study in rats, but not dogs, the WBC increase was dose-responsive [1.3-2.3X; at ≥0.5 mg/kg in males and ≥1.0 mg/kg in females] and characterized by an increase in neutrophil and lymphocyte counts [≥1.0 mg/kg]. There was an increase in WBC count in males and females [35% and 120%, respectively] at 25 mg/kg in the 2-week study. There was a 60% and 40-124% increase in WBC counts for male and female dogs dosed with verteporfin at 10/25 mg/kg/day. The increase in WBC counts in males and female rats administered verteporfin for 28 days was approximately 1.5-2X and 3-4X control values at 10 and 25 mg/kg/day, respectively. After a 28-day recovery, the WBC counts were comparable to concurrent controls. 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. The following alterations in hepatic enzymes were observed. [1] In the single dose dog study, there was a 25-100% increase in **AST** in all males and females 3 hours following irradiation. Values had returned to baseline by Day 14 and there were no concomitant changes in either ALT or SAP. Clinical pathology was not conducted in the single dose studies in rats. [2] In male and female dogs dosed with 10/25 mg/kg/day for 2 weeks, there was approximately a 60% increase in **AST** on Day 8, but not Day 14. In these dogs, there was a concomitant increase in **SAP** of approximately 60-100% on Days 8 and 14. [3] In female rats dosed with 25 and 50 mg/kg/day for 2 weeks, there was a 27% and up to 37% increase in **ALT** and in **SAP**. There was no indication of hepatotoxicity in the intermittent study in dogs or rats. Histopathological changes were observed in the liver only 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.

The Sponsor conducted *in vitro* studies in human liver slices to evaluate the potential of direct hepatotoxicity induced by verteporfin. These studies demonstrated decreases [generally ≤40%] in cellular ATP and K^+ concentration and in protein synthesis at various concentrations of and incubation periods with verteporfin. In general, these effects were observed at higher concentrations and longer incubation periods.

The *in vitro* data suggest that BPD-MA may induce biochemical changes associated with toxicity. The histopathological changes observed in both the rat and dog are treatment-related. The fact that the pigment is positive for iron would indicate that these changes, 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 in liver toxicity. However, this was generally 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 any study was minimal. [1] In the intermittent rat study, there was approximately a 40% increase in creatinine and creatinine kinase at 2 mg/kg. [2] In

male rats administered verteporfin at 25 mg/kg/day or liposomal control for 2 weeks, there was approximately a 20% increase in creatinine and in BUN. In rats administered 50 mg/kg/day for 2 weeks, there was a 12% increase in BUN but no increase in creatinine. [3] There was a 30% increase in BUN without a concomitant increase in creatinine on Day 8 but not Day 14 in male dogs administered 10/25 mg/kg/day for 2 weeks. [4] No changes in either BUN or creatinine were observed in the 28-day rat study. Changes in **urinalysis** were uncommon. [1] Hemoglobinuria was identified on Days 1-3, in the 2-week dog study in dogs administered 25 mg/kg. On Day 9, there was also an increase in bilirubin in the urine at 25/10 mg/kg/day. [2] Urinalysis in the 28-day rat study showed 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. 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 histopathological changes observed in both the rat and dog are treatment-related. The fact that the pigment is 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. In addition, the increases in BUN and creatinine were not consistent, generally did not occur concomitantly, and the increase in any study was minimal. 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.

Additional Findings – Mortality - In the acute dose rat study, 3/5 animals died 24 hours following administration of 20 mg/kg of verteporfin + 100 J/cm^2 light dose. In rats administered 25 mg/kg/day BID or liposomal solution for 14 days, 3/9 females died following blood collection on Day 15. Mortality was also observed in 2 rats at 10 and 25 mg/kg/day each on Day 28 following blood collection. Death was attributed to treatment site edema [following irradiation] and blood loss [sampling for PK data].

- Skin Phototoxicity - A pilot study was conducted in which beagle dogs [N=1/sex/group] were administered 20 mg/kg and exposed to sunlight 24, 48, 72, and 96 hours later. The most severe phototoxicity occurred in animals exposed to sunlight 24 hours post-dosing. The female in this group was euthanized prematurely in moribund treatment-related condition. The male dog exposed to sunlight at 24 hours exhibited well-defined erythema and alopecia of the forelimbs that persisted until study termination [Day 14]. Animals exposed to sunlight at ≥48 hours post drug administration, exhibited phototoxicity for 3-4 days including slight to moderate erythema of the shaved forelimb and perinostril area. Animals were normal for the remainder of the study period.

- Pulmonary Findings- In the single dose rat studies, there was an increase in the incidence of interstitial pneumonia from 0/5 control rats to 4/5 and 2/5 rats at 10 mg/kg without or with photoactivation. In addition, there was an increase in the incidence of mononuclear infiltration in the lungs from 1/5 control rats to 4/5 rats at 10 mg/kg ± photoactivation in treated animals. The severity for both lesions was scored as Grade 2. The incidence of lung granuloma in the 14-week rat study was comparable across all groups. The

incidence of lung granuloma in the 28-day rat study was increased from 5/10 and 4/10 control males and females, respectively, to 7/10 and 8/10 males and female rats at 25 mg/kg/day. The incidence of lung granuloma was increased in females, but not males, in the 14-day study with an incidence of 1/4, 2/4, 3/4, and 4/4 dogs at 0, 0.5, 5, and 25/10 mg/kg/day. The relationship to treatment is not known.

- **Electrolytes** – In male and female rats administered 25 mg/kg/day of verteporfin for 2 weeks, there was a 20% decrease in serum K^+. In male and female dogs, there was a statistically significant increase [approximately 10-15%] in K^+ at 10/25 mg/kg/day.

Toxicokinetics – Exposure, based both on AUC and C_{max}, in the dog was roughly dose-proportional at doses of 0.5 to 10 mg/kg. In the rat, exposure to the regioisomer, 315,555, appeared to be roughly dose proportional but slightly greater than dose proportional for 315,585 for doses of 0.5 to 25 mg/kg. Consequently, exposure to BPD-MA was slightly greater than dose proportional. In both the rat and the dog, exposure, based on AUC, to CL 315,585 was approximately two to four times greater than for CL 315,555. This difference appeared to be due differences in clearance. In dogs, there was no apparent difference in exposure to either regioisomer. In rats, there was no apparent gender difference in exposure to CL 315,555. However, exposure to CL 315,585, and consequently to BPD-MA, was approximately 30-45% greater in males than in females. $T_{1/2}$ of CL 315,555 was reported as approximately 2-5 hour in dogs, 5-9 hours in male rats, and 6-14 hours in female rats. $T_{1/2}$ of CL 315,585 was reported as approximately 4-6 hour in dogs, 4-6 hours in male rats, and 3-4 hours in female rats. 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 –$AUC_{0\text{-}24}$ [µg•hr/ml]			CL 315,585 –$AUC_{0\text{-}24}$ [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, based on AUC, to BPD-MA 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 $BPD\text{-}MA_C$ and $BPD\text{-}MA_D$ and the AUC values for BPD-MA exposure provided by the Sponsor in the 28-day rat study. These are delineated in the table below. [Note: The Sponsor was 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 approximately 8X and 6 X 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 $BPD\text{-}MA_C$ and $BPD\text{-}A_D$. The AUC for $BPD\text{-}MA_C$ 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•hr/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 represents approximately 14X and 11X in male and female rats, respectively, 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.

Carcinogenicity: Studies were not conducted.

Reproductive Toxicology:

I. Segment I – Fertility Studies – Male and Female

A. Rat

a. An intravenous fertility study of benzoporphyrin derivative monoacid (BPD-MA) in the rat [Ref. 340]

Study Identification: TX-96009

Site:

Study Dates [in-life]: January 6 - March 6, 1998

Formulation and Lot No. – liposomal BPD-MA- TC0715; reconstituted with sterile water for injection; diluted with 5% dextrose; dosing solutions were prepared weekly and maintained at 4° C; weekly samples were collected [apparently on the day of preparation] and analyzed

Vehicle – 5% Dextrose

Certificate Analysis: Yes (X)

Final Report: Dec. 22, 1998

GLP and QA Statements Signed: Yes (X)

Objective: "To investigate the effects of intravenously administered liposomal Benzoporphyrin Derivative Monoacid (BPD-MA) (verteporfin) on the reproduction and fertility of rats of the F_0 generation and on the early *in utero* development of the F_1 generation."

Study Design

Test Material/ Group Designation	Dose*				N***		Species/Strain
	mg/kg	ml/kg	Route	# days dosed**	M	F	
Group 1 –5% Dextrose	0	5	iv		22	22	Sprague-Dawley-CD (Crl:CD®(SD)BR)
Group 2 - BPD-MA	1						
Group 3 - BPD-MA	3						F – app. 63-65 days; M – app. 82-88 days
Group 4 - BPD-MA	10						M = 371-454 g; F = 179-264 g – at start

*The doses selected were based on results from the 28-day rat study [TX-96010] that used doses up to 25 mg/kg. Based on this study, it was anticipated that 10 mg/kg would result in definitive toxicity; see Reviewer's Comments below.

** Males were dosed 28 days prior to mating and during mating until necropsy [app. 60 days]; females were dosed 14 prior to mating and during mating until Gestation Day 7 [at least 22 days]; females were sacrificed on Gestation Day 13; light level was maintained at <20 foot candles

*** "due to difficulties encountered with performing injections on the first day of dosing, more males were replaced at the start of their dosing period than females, whose dosing treatment started 2 week later"; replacement animals were from spares from the same batch ordered for the study; the Sponsor states that all animals were dosed over the complete study period.

Parameters Evaluated	Time Point(s)
Clinical examination	
Mortality/moribundity	BID
Clinical examination	Weekly
Body Weight	
Males, Females until mating	2X/week
Mated females	Daily
Food Consumption	
Male and females until pairing	Weekly
Mated females	Gestation Day 0-3, 3-7, 7-10, and 10-13
Hematology [abdominal aorta] – RBC count and morphology, MCV, MCH, MCHC, Hct, Hb, platelet count, reticulocyte count, WBC and differential	At sacrifice
Estrous Cycles [vaginal lavage]	10 days prior to mating, females not mating after 8 consecutive days until they were sperm positive
Sperm Analysis – count, motility, morphology	At sacrifice
Gross Pathology	At sacrifice
Organ Weights – epididymis, ovaries, prostate, seminal vesicles, testes	At sacrifice
Histopathology - testes	At sacrifice
Fertility Indices – mating index; fertility index; conception rate; days to mating no. of corpora lutea, implantation sites, live and dead fetuses, no. of resorptions; percent pre and post-implantation losses	Uterine indices assessed on Gestation Day 13

Results

Parental Findings

Mortality – There were no unscheduled deaths throughout the study period.

Clinical Signs – In general, the signs observed were related to the hair coat [e.g. thinning] and to the skin and tail. Although the treated animals tended to exhibit a slightly higher overall incidence of these signs, the incidence was ≤3/22 animals and a relationship to treatment was not established.

Body Weight and Food Consumption

- Males - There was an 11-25% decrease in food consumption in all treated males compared to the control values for Day 1-8. However, body weight and body weight gain were comparable across all groups for this period.
- Females – There was an 83% decrease in weight gain in the high dose females for Days 1-4. This was due primarily to weight loss of approximately 30 g in 2 females. There was no concomitant change in mean food consumption for Days 1-8.

Hematology

- RBC Indices – There were statistically significant decreases in RBC counts in males [-7%] and females [-5%] at 10 mg/kg/day but no concomitant decrease in either Hb or Hct. MCV, MCH, MCHC, and RDW% were also increased [statistically significant at the high dose]. The magnitude of the changes was <10-15%. The magnitude of change was greater in the males than females, which probably reflects differences in the dosing regimen. In males only, there was a 20% and 150% increase in platelet and percent reticulocytes. The percent reticulocytes in the VH and high dose males was 0.4 ± 0.29% vs. 1± 0.63%, respectively. There was an increase in the severity of polychromasia and anisocytosis in high dose males [mean of 1.9 for each] compared to controls [mean of 1 for each]. RBC morphology was comparable across female groups.
- WBC Indices - There was approximately a 40% increase in WBC and absolute lymphocyte counts and a 30% increase in absolute PMN counts in the high dose males compared to control animals. The change in WBC count and lymphocyte count was statistically significant. Mean monocyte, eosinophil, and basophil counts were also increased. With the exception of basophils the individual values in the high dose group were comparable to the control values. Change in these parameters in females was <15% compared to control and were considered to reflect differences in the dosing regimen.

Gross Pathology – Lesions either occurred at a comparable incidence and severity across all groups, and/or the lesions were observed in ≤2 animals/group.

Organ Weights -

- Males – There were no treatment-related changes in the weights of the reproductive organs.
- Females – There was approximately a 15-30% increase in the absolute and relative weight of the right but not the left ovary. A relationship to treatment is considered unlikely.

Histopathology

- Males – The following lesions – failure of sperm release; degeneration of round or elongated spermatids and 2° spermatocytes; and degeneration of testicular seminiferous tubules – occurred at a comparable incidence and severity across all treatment groups.
- Females – No tissues were evaluated histopathologically

Reproductive/Fertility Assessment

- **Estrous Cycle** – There were no treatment-related effects on estrous cyclicity..
- **Sperm Analysis** - There were no treatment-related effects on sperm motility, counts, and morphology. Control motility was 64%. The incidence of spermatic morphological abnormalities was comparable across dose groups.

Fertility Indices

- **Parental Performance** - All pairs appeared to have mated within 14 days of cohabitation in all groups except at the high dose. Of the pairs that had mated within 14 days, the majority had mated within 4 days. In the high dose group, there were 7 pairs that appeared not to have mated by 14 days. Partners were switched among these pairs. Following this switch, 2 pairs mated within 3 days, 2 pairs did not mate, and mating went undetected in 3 pairs. The relationship to treatment is not known. The 5 animals, which did not mate or in which mating went undetected, were euthanized on Day 35. However, the various fertility indices were comparable across treatment groups: [1] mean time to mating of 3.2 ± 3.45 vs. 3.8 ± 4.88 days; [2] mating index of 100% vs. 90.9 %; [2] fertility index of 90.9 % vs. 86.4%; and [3] conception rate of 90.9% vs. 95.5% for the control group and high dose groups, respectively.

- **Uterine Findings** – The mean number of corpora lutea, implantation sites, live and dead embryos, early resorptions, and percent of pre- and post-implantation losses were comparable across all groups.

Reviewer's Comments – Study Design and Data Presentation

The toxicity observed in males and in females was slight. ICH Guideline S5A; Detection of Toxicity to Reproduction for Medicinal Products indicates that "some minimal toxicity is expected to be induced in the high-dose dams". However, based on the findings in this study, it is felt that a dose of 25 mg/kg/day [the maximum based on solubility constraints] would have resulted in more definitive toxicity without significantly compromising the animals. There were differences between the RBC effects in this study and those observed in the 28-day repeat dose toxicity study in rats [TX-96010] on which dose selection was based. In the fertility study, the decrease in RBC count at 10 mg/kg/day was 7% and 5% in males and females, respectively. In the 28-day repeat dose toxicity study, the decrease in RBC counts at 10 mg/kg/day was 13% and 25% in males and females, respectively. In addition, the reticulocytosis was greater in the 28-day repeat dose toxicity than in the fertility study. The factors accounting for this difference between the two studies is not known. There were differences in the duration of dosing in the males as well as the timing of blood collection with respect to the last dose in females. This may have contributed to the apparent disparity. However, the study is adequate to support the intended clinical application for the treatment of ARMD based on the following considerations. [1] The clinical usage will be 1 injection q3-4 months vs. the repeat dosing regimen used in the nonclinical study. [2] The 10-mg/kg/day 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.]

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

1. The NOAEL for parental toxicity was 1 mg/kg/day for both males and females. **Reviewer's Comment** - The Sponsor is basing the NOAEL on a statistically significant increase in MCHC of 2% in the mid dose females and approximately a 25% decrease in food consumption in the mid dose males. The Reviewer believes that a NOAEL of 3 mg/kg is more appropriate. The Reviewer agrees that the hematopoietic system is a target organ for toxicity. However, a decrease of 2% in the MCHC in the females at 3 mg/kg without changes in other RBC indices more likely represents biological variability rather than a test article effect. In the males, there was not a concomitant decrease in body weight gain associated with the decrease in food consumption at 3 mg/kg, and the relationship to treatment is not known.

2. The NOAEL for reproductive toxicity parameters assessed in this study [fertility, early embryonic development] was 10 mg/kg/day. **Reviewer's Comment** – In general, the Reviewer concurs.

II. Segment II – Developmental Studies

A. Rat

a. An intravenous range-finding developmental toxicity study of CL 318,952 [Benzoporphyrin derivative monoacid. A photosensitizer for photodynamic therapy] in rats [Ref. 344]

Study Identification: 3151.11

Site:

Study Dates [in-life]: July 15 – August 7, 1993

Formulation and Lot No. –liposomal BPD-MA- H92-902-017- reconstituted with sterile water for injection; diluted with 5% dextrose; reconstituted up to 48 hours prior to dosing and maintained at room temperature [**Note:** The Sponsor has provided stability at room temperature for only up to 11 hours]; analysis indicated

Vehicle – 13.3% w/v lactose

Certificate Analysis: Yes (X)

Final Report: February 14, 1994

GLP and QA Statements Signed: Yes (X)

Objective: "To provide information concerning the potential toxic effects of CL 318,952 ... when administered intravenously to pregnant rats for the purpose of selecting dosage levels for a subsequent developmental toxicity study in rats"

Dr. Oluwadare M. Adeyemo previously reviewed this for IND Submission pp. 16-17. Additional comments by the current Reviewer [in italics] are provided below.

Doses used in this study were 2, 10, and 25 mg/kg/day

1. Clarification of study design – Sex, body weight, and external anomalies in the fetuses were not assessed.

2. Results

- *There were no apparent treatment-related effects on number of corpora lutea, implantation sites, viable fetuses, pre and post implantation losses, and mean gravid uterus weights.*
- *WBC counts on Day 16 were increased by 83, 47, 46, and 150% at doses of 0, 2, 10, and 25 mg/kg/day compared to the respective baseline values. Compared to the concurrent control, the high dose females exhibited a 45% increase in WBC counts. The WBC counts in high dose females on Day 20 were comparable to baseline values, but 27% lower than concurrent controls. The Sponsor suggests that the increase in WBC count is possibly due to the close proximity of the blood collection site to the drug injection site. A treatment-related increase in WBC counts has been observed in rats in repeat dose studies. On Days 16 and 20, RBC counts were decreased by 7% and approximately 25-30% at 10 and 25 mg/kg/day, respectively, compared to concurrent controls. The reticulocyte counts on Day 16 were 6.08 ± 1.5%, 9.70 ± 3.0 % and 38.92 ± 6.5% at 0, 10, and 25 mg/kg/day, respectively. On Day 20, the reticulocyte counts were 3.18 ± 0.7%, 4.27 ± 0.9%, and 11.80 ±3.0% 0, 10, and 25 mg/kg/day, respectively.*

b. An intravenous developmental toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, A photosensitizer for photodynamic therapy) in rats [Ref. 341]

Study Identification: TX-93002

Site: [redacted]

Study Dates [in-life]: January 25 – February 17, 1994

Formulation and Lot No. –liposomal BPD-MA- E93-120-0877- reconstituted with sterile water for injection; diluted with 5% dextrose; the dosing solution was prepared up to 72 hours prior to administration and stored in the refrigerator; dosing solutions were collected on the first, middle and last days of drug administration and analyzed by HPLC; [redacted]

Vehicle – 10% lactose

Certificate Analysis: Yes (X) [redacted]

Final Report: September 30, 1994

GLP and QA Statements Signed: Yes (X)

Objective: "To detect and evaluate the potential embryotoxic, fetotoxic or teratogenic effects of BPD-MA, when administered intravenously to pregnant rats during the period of major organogenesis"

Study Design

Test Material/ Group Designation	Dose*				N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed	F	
Group 1 –10% Lactose	0	12.5	iv	Gestation Days 6-15	25	Sprague-Dawley-CD (Crl:CD® BR [redacted]
Group 2 - BPD-MA	2	1.0				
Group 3 - BPD-MA	10	5.0				F – app. 85 days – at study start
Group 4 - BPD-MA	25	12.5				F = 233-311 g – at study start

*2.5 ml/min; animals were maintained at light levels of <20 foot candles; dose selection based on results of the dose range-finding study [3151.11] which demonstrated toxicity at the 25 mg/kg/day dose

Parameters Evaluated	Time Point(s)
Clinical observations	
Mortality/moribundity	BID
Clinical observations	0.5-1 hour post-dosing, daily
Body Weight and Body Weight Gain	
Body weight	Gestation Days 0, 6, 9, 12, 16, and 20
Body weight gain	Gestation Days 0-6, 6-9, 9-12, 12-16, 16-20, 6-16, 0-20
Food Consumption	Gestation Day 0-6, 6-9, 9-12, 12-16, 16-20, 6-16, 0-20 [measured daily]
Gross Pathology	Gestation Day 20
Cesarean Parameters - no. of corpora lutea, implantation sites, live and dead fetuses, early and late resorptions; pre and post-implantation losses, fetal sex and weight	Gestation Day 20
Fetal Observations – external, skeletal* and visceral* examination	Gestation Day 20

*Approximately 50% of the fetuses underwent visceral examination and 50% underwent skeletal examination

Results

Maternal Findings

Survival and Pregnancy Rates – With the exception of 1 VH female, all animals survived to scheduled necropsy. Pregnancy rates were 24/24 [100%], 23/25 [92%], 22/25 [88%], and 23/25 [92%] at 0, 2, 10, and 25 mg/kg/day.

Clinical Signs- There was a dose-dependent greenish discoloration of the tail and/or the injection site that was attributed to the color of the test article. Red vaginal or brown mucoid vaginal discharge [pre-dose and/or post-dose] was observed on 1-2 occasions on Days 13-15 in 2, 2, 4, and 7 females at 0, 2, 10, and 25 mg/kg/day. However, in the independent expert report [Ref. 343. See below], it was stated that this is an expected event identified as a "placental sign". The other signs were observed at a comparable frequency across all groups or occurred sporadically. The relationship to treatment is not known.

Body Weight, Weight Gain, and Food Consumption – There was no difference in body weight and body weight gain based on group means between treated and control animals. There were 4 control animals and 8 high dose animals [6 gravid/2 nongravid] which lost weight during Days 6-9. There was a statistically significant decrease in food consumption in the high dose group on Days 6-9 and 9-12. The magnitude of the decrease was <10%. In general, the animals in the high dose group that lost weight had a concomitant mild decrease in food consumption. The Sponsor suggested that since the decrease was minor, "the decreased food consumption was not considered to be biologically meaningful".

Necropsy - One high dose female was found to have an enlarged spleen and an enlarged placenta and green fluid uterine content. The relationship to treatment is not known, although treatment-related enlarged spleen has been observed in the repeat dose toxicity studies. The other necropsy findings occurred at a comparable frequency across all treatment groups.

Fetal Findings

Cesarean Results- The number of corpora lutea, implantation sites, viable fetuses, early and late resorptions, and mean fetal body weights were comparable across all treatment groups. The biological significance of the statistically significant increase [12%] in the number of female fetuses observed in the high dose group compared to the concurrent controls is not known.

Fetal Morphology – Malformations – No malformations were observed in the control animals. There was a statistically significant increase in the incidence of anophthalmia and/or microphthalmia in the high dose group compared to control animals. Although there was only 1 fetus in the mid-dose group exhibiting this malformation, it was considered treatment-related based on findings at the high dose. The total number of malformations for fetuses and litter was increased [statistically significant for the litters] compared to controls. The table below delineates the incidence of malformations observed.

	FETUSES				LITTERS			
GROUP:	1	2	3	4	1	2	3	4
LEVEL (MG/KG/DAY):	0	2	10	25	0	2	10	25
NUMBER EXAMINED EXTERNALLY	363	368	341	375	24	23	22	23
ANOPHTHALMIA AND/OR MICROPHTHALMIA	0	0	1	5	0	0	1	4*
ABSENT TAIL	0	0	0	1	0	0	0	1
ECTROMELIA	0	1	0	0	0	1	0	0
OMPHALOCELE	0	1	0	0	0	1	0	0
NUMBER EXAMINED VISCERALLY	185	181	172	186	23	23	22	23
SITUS INVERSUS	0	0	0	1	0	0	0	1
LUNG AGENESIS	0	0	0	1	0	0	0	1
HEART AND/OR GREAT VESSEL ANOMALY	0	0	0	1	0	0	0	1
RETROESOPHAGEAL RIGHT-SIDED AORTIC ARCH	0	0	0	1	0	0	0	1
NUMBER EXAMINED SKELETALLY	178	187	169	189	24	23	22	23
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0	1	1	1	0	1	1	1
COSTAL CARTILAGE ANOMALY	0	0	1	1	0	0	1	1
RIB ANOMALY	0	1	0	0	0	1	0	0
STERNEBRA(E) MALALIGNED (SEVERE)	0	1	0	0	0	1	0	0
STERNOSCHISIS	0	1	0	0	0	1	0	0
LIMB BONE ANOMALY	0	1	0	0	0	1	0	0
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS	0	1	1	5	0	1	1	4*
NUMBER WITH SOFT TISSUE MALFORMATIONS	0	0	0	2	0	0	0	2
NUMBER WITH SKELETAL MALFORMATIONS	0	3	1	1	0	3	1	1
TOTAL NUMBER WITH MALFORMATIONS	0	3	2	7	0	3	2	6*

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05

The situs inversus, lung agenesis, and heart and/or great vessel anomaly, anophthalmia and/or microphthalmia occurred in a single fetus. The fetus with the absent tail also exhibited anophthalmia and/or microphthalmia. The ectromelia and omphalocele occurred in the same fetus. Sternoschisis and vertebral anomaly occurred in the same fetus. Vertebral anomaly and costal cartilage anomaly occurred in the same fetus at 10 and 25 mg/kg/day.

Variations- There was a statistically significant increase in the incidence of bent ribs: 0/0 [fetus/litter] vs. 23/11 [fetus/litter] for control vs. high dose animals, respectively. The fetus and litter incidence of the other variations [e.g. distended ureter, 14th rudimentary rib, malaligned sternebrae, and reduced skull ossification] was, in general, comparable across all groups. There was also an increase in fetal alterations at 25 mg/kg/day.

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

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

1. There were no treatment-related effects on clinical observations, body weight and body weight gain, food consumption, maternal gross findings, and cesarean section data. However, based on results in the dose range finding study [Study 3151.11], the maternal NOAEL was considered to be 2 mg/kg/day. **Reviewer's Comment** - Making the assumption that there was no difference in the response between the range finding and the definitive studies, the NOAEL was based on a 7% decrease in RBC count and approximately a 60% increase in the percentage of reticulocytes. However, the NOAEL for a given study should not be based on results from another study. Based on the endpoints evaluated in this study, therefore, the maternal NOAEL was ≥25 mg/kg/day.

2. An increase in the incidence of anophthalmia and/or microphthalmia, external malformations, total malformations, and bent ribs were observed at 25 mg/kg/day. Based on these findings, the anophthalmia and/or microphthalmia, observed in a single fetus from a dam administered 10 mg/kg/day, was considered to be treatment-related. The NOAEL for

developmental toxicity was 2 mg/kg/day. **Reviewer's Comment**-The Reviewer concurs. The historical controls provided by the testing laboratory indicated that the mean fetal incidence for anophthalmia and/or microphthalmia was 6 out of 6321 fetuses or 0.09% [range of 0-0.6%] and the mean litter incidence was 5 out of 430 or 1.2% [range of 0-4.3%]. The mean fetal and litter incidence at 25 mg/kg/day was 1.3% and 17.4%, respectively. The mean fetal incidence at 10 mg/kg/day was within historical control ranges [0.3%] and the mean litter incidence 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.

3. The malformations and the variations seen in the high dose group appear to be secondary to drug-induced maternal toxicity. **Reviewer's Comment** – As noted, under Comment #1, the Sponsor is setting the NOAEL for this study on results from the range-finding developmental study [3151.11]. However, the NOAEL for a given study should not be based on results from another study. 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. Study A9301 Placental transfer of BPD-MA (CL 318,592) after a single intravenous dose to pregnant rats indicates that <1% of a dose, based on radioactivity, of BPD-MA administered to the dam crosses the placenta and is distributed to fetal tissue. This would support the Sponsor's argument that the malformations were secondary to maternal factors. The Sponsor also states that microphthalmia and/or anophthalmia and wavy ribs have been associated with maternal toxicity. However, a direct treatment-related effect on the fetus can not be ruled out.

B. Rabbits

a. **An intravenous range-finding developmental toxicity study of CL 318,952 [Benzoporphyrin derivative monoacid, A photosensitizer for photodynamic therapy] in rabbits [Ref. 346]**

Study Identification: 3151.3
Site: [redacted]
Study Dates [in-life]: September 20 – October 19, 1993
Formulation and Lot No. – liposomal BPD-MA- H92-902-017- reconstituted with sterile water for injection; diluted with 5% dextrose; reconstituted within 96 hours of dosing and maintained refrigerated [1-4° C]; samples collected on the first and last days of administration and analyzed [redacted]
Vehicle – 13.3% lactose
Certificate Analysis: Yes (X)
Final Report: Mar. 29, 1994
GLP and QA Statements Signed: Yes (X)
Objective: "To provide information concerning the potential toxic effects of CL 318,952 ... when administered intravenously to pregnant rabbits for the purpose of selecting dosage levels for a subsequent developmental toxicity study in rats"

Dr. Oluwadare M. Adeyemo previously reviewed this study for IND [redacted] Submission [redacted] pp. 17-20. [redacted] Additional comments by the current Reviewer [in italics] are provided below.

Doses used in this study were 2, 10, and 25 mg/kg/day

1. *Clarification of Study Design*
   - *Organ weights were not obtained in the dams*
   - *Number of early and late resorptions were determined*

- *Fetal sex, body weight, and external anomalies were not assessed*

2. *Clarification of Results*
   - *Two females at 25 mg/kg/day died on Gestation Days 8 and 9 and the remainder were sacrificed prematurely*
   - *RBC counts were decreased in females at 10 mg/kg/day by 40% and 20% on Gestation Days 19 and 29, respectively. There was approximately a 75% decrease in RBC counts on Day 9 in the rabbits administered 25 mg/kg [N=4]. Hematocrit and hemoglobin were both decreased at 10 mg/kg/day by approximately 20% and 5% on Gestation Days 19 and 29, respectively. Reticulocyte percentages were increased compared to control by approximately 200% and 100% on Days 19 and 29, respectively.*
   - *There was a decrease in mean implantation sites, viable fetuses and an increase in pre-implantation losses at 2 mg/kg/day. This was attributed to the low pregnancy rate [50%]. The lack of a dose-dependent response suggests an effect unrelated to treatment. There were no changes in number of corpora lutea, implantation sites, pre and post implantation losses, early and late resorptions, viable/dead fetuses, and gravid uterus weight at 10 mg/kg/day.*

b. <u>An intravenous developmental toxicity study of CL 318,952 (Benzoporphyrin derivative monoacid, A photosensitizer for photodynamic therapy) in rabbits [Ref. 342]</u>

Study Identification: TX-93001
Site: [redacted]
Study Dates [in-life]: November 19 – December 20, 1993
Formulation and Lot No. – liposomal BPD-MA- G93-120-0893- reconstituted with sterile water for injection; diluted with 5% dextrose; prepared up to 3 days prior to use and stored in the refrigerator; samples were collected on the first, middle and last days of drug administration and analyzed [redacted]
Vehicle – 10% w/v lactose
Certificate Analysis: Yes (X) [redacted]
Final Report: Sept. 1, 1994
GLP and QA Statements Signed: Yes (X)
Objective: "To detect and evaluate the potential embryotoxic or teratogenic effects of BPD-MA...when administered intravenously to pregnant rabbits during the period of major organogenesis"

Study Design -

Test Material/ Group Designation	Dose*				N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed	F	
Group 1 –10% Lactose	0	5.0	iv	Gestation Days 6-18	20	New Zealand White Rabbits [redacted]
Group 2 - BPD-MA	1	0.5				
Group 3 - BPD-MA	3	1.5				F – app. 7 mos. – at study start
Group 4 - BPD-MA	10	5.0				F = 3.2-4.4 kg – at study start

*6.5 ml/min; Animals were maintained in lighting <20 foot candles; animals were artificially inseminated and then administered 0.1 ml of human chorionic gonadotropin iv; animals were sacrificed on Gestation Day 29

Parameters Evaluated	Time Point(s)
Clinical observations	
Mortality/moribundity	BID
Clinical observations	0.5-1 hour post-dosing, daily
Body Weight and Body Weight Gain	
Body weight	Gestation Days 0, 6, 9, 12, 15, 19, 24, and 29
Body weight gain [absolute and corrected for gravid uterus weights]	Gestation Days 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29, and 0-29
Food Consumption	Gestation Day 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29, and 0-29
Gross Necropsy	Gestation Day 29, unscheduled euthanasia
Cesarean Parameters – uterine weight, live and dead fetuses, no. of corpora lutea, implantation sites, early and late resorptions, pre and post-implantation losses,	Gestation Day 29
Fetal Observations – external, skeletal and visceral examination, fetal sex and weight	Gestation Day 29

Results

Maternal Findings

Survival and Pregnancy Rates – Two rabbits in the 1 mg/kg/day group were euthanized after aborting on Gestation Days 23 and 24. There were no other premature decedents. The pregnancy rate at scheduled necropsy for the 0, 1, 3, and 10 mg/kg dose groups was 17/20 [85%], 17/18 [95%], 18/20 [90%], and 18/20 [90%]. However, due to total resorptions in 1 and 2 females at 3 and 10 mg/kg/day, there were 17 litters evaluated at 0, 1, and 3 mg/kg and 16 evaluated at 10 mg/kg.

Clinical Signs - The only treatment-related sign was a dose-dependent greenish discoloration of the pinna that was attributed to the color of the test article. The other signs were observed at a comparable frequency across all groups or occurred sporadically.

Body Weight, Body Weight Gain, Food Consumption- Does at the high dose exhibited a mean weight loss [-44 g] Gestation Days 6-9. Control does exhibited a mean weight gain [17 g] for the same period. For Gestation Days 9-12, the high dose had a 75% reduction in weight gain compared to the control animals [37 vs. 9 grams]. This translated into only a 2-3% difference in body weights on Days 9 and 12. This was associated with a slight decrease in food consumption on GD 6-12 of approximately 15%. Food consumption was decreased [approximately 10-15%] in the high dose GD 12-24 without a concomitant reduction in body weight gain. The weight loss in the low dose does on GD 24-29 is considered not to be treatment related due to a lack of a dose response.

Necropsy - The necropsy findings were observed at a comparable frequency across all groups or occurred sporadically.

Fetal Findings

Cesarean Findings – There were no statistically significant differences between treated and control groups for any parameters evaluated. Two does at 1 mg/kg/day aborted on GD 23 and 24. Total resorptions occurred in 1 and 2 does at 3 and 10 mg/kg/day, respectively. The expert report [Ref. 343] suggests that these resorptions are not treatment-related because [1] "the incidences are within the ranges observed historically at the testing facility and literature [MARTA]" and [2] "the presence of an apparent increase in resorbed litters...reflects the number of litters in these two dosage groups with only one or two implants, a spontaneous event

[Feussner, et. al., 1993] that occurred before treatment was initiated". While the number of animals with total resorptions is low, the percent incidence of females with total resorptions at 3 and 10 mg/kg/day [5.6% and 11.1%] exceeded the mean percentage reported in the historical control data [8/387; 2.1%]. The Sponsor did not provide the range for the incidence of total resorptions, which may have encompassed the incidence observed in this study. The incidence in this study is within the historical control range reported by MARTA [for AI: number ranges from 0-2 animals, mean of 0.61 ± 0.68: % resorbed ranges from 0-13%, mean of 2.91 ± 3.76%]. The second doe that underwent total resorption had 7 implants and not just 1-2 implants. Treatment began on GD 6, coinciding with onset of implantation. Therefore, a treatment-related effect on implantation can not be ruled out. There was, however, only a minimal increase in pre-implantation loss: mean of 2.1 ± 2.5 and 3.6 ± 4.0 at 0 and 10 mg/kg/day, respectively. There were minimal increases in the incidence of early resorptions [mean of 0.4 ± 0.6 vs. 0.8 ± 1.7 in the control vs. the high dose group]. However, the incidence of post-implantation loss was comparable in the control and 10 mg/kg/day groups. In addition, the incidence of post-implantation losses at 10 mg/kg/day is within the historical control data provided by the Sponsor [Appendix N; Sponsor does not indicate the dates during which these data were collected.] The increase in resorptions at 3 and 10 mg/kg/day resulted in a decrease in mean gravid uterine weights compared to controls. A treatment-related effect, with respect to total resorptions, pre-implantation losses, and early resorptions, can not be totally ruled, but based on the considerations outlined above, it is considered unlikely.

Fetal Morphology – There were no treatment-related malformations. All malformations occurred sporadically. These are listed in the table below.

	FETUSES				LITTERS			
GROUP:	1	2	3	4	1	2	3	4
LEVEL (MG/KG/DAY):	0	1	3	10	0	1	3	10
NUMBER EXAMINED EXTERNALLY	121	129	122	109	17	17	17	16
FLEXED PAW	0	3	0	2	0	1	0	2
CLEFT PALATE	0	0	0	1	0	0	0	1
MICROGLOSSIA	0	0	0	1	0	0	0	1
SPINA BIFIDA	0	0	1	0	0	0	1	0
OMPHALOCELE	0	0	0	1	0	0	0	1
NUMBER EXAMINED VISCERALLY	121	129	122	109	17	17	17	16
HYDROCEPHALY	0	3	0	1	0	1	0	1
IRIS BOMBE	0	1	0	0	0	1	0	0
HEART AND/OR GREAT VESSEL ANOMALY	0	1	0	0	0	1	0	0
NUMBER EXAMINED SKELETALLY	121	129	122	109	17	17	17	16
RIB ANOMALY	0	0	0	1	0	0	0	1
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1	2	2	0	0	2	1	0	0
STERNEBRA(E) MALALIGNED (SEVERE)	0	1	1	0	0	1	1	0
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0	0	0	1	0	0	0	1
COSTAL CARTILAGE ANOMALY	0	0	1	0	0	0	1	0
SKULL ANOMALY	0	1	1	0	0	1	1	0
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS	0	3	1	2	0	1	1	2
NUMBER WITH SOFT TISSUE MALFORMATIONS	0	5	0	1	0	3	0	1
NUMBER WITH SKELETAL MALFORMATIONS	2	3	3	2	2	2	3	2
TOTAL NUMBER WITH MALFORMATIONS	2	7	4	4	2	4	3	4

A single fetus in the high dose group exhibited all of the listed external malformations.

Fetal variations occurred either sporadically or at a comparable incidence across all groups and were generally within the historical data range provided by the Sponsor [Appendix N].

Reviewer's Comments – Study Design and Data Presentation – Study design and data presentation were adequate. The dose was selected based on excessive maternal toxicity at 25

mg/kg/day. A larger dose spread would have been preferred but it is felt that a maximum dose of 10 mg/kg/day was appropriate.

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

1. The NOAEL for maternal toxicity based on a transient weight loss and decreased food consumption was 3 mg/kg/day. **Reviewer's Comment** - The Reviewer concurs. Although there was only an overall 2-3% decrease in body weight in the high dose females compared to controls, on GD 6-9, high dose does exhibited a mean body weight loss while control does exhibited weight gain. Weight gain was also decreased by 75% in the high dose female GD 9-12 compared to the controls. This would suggest that the high dose was appropriate.

2. There was no evidence of teratogenicity or embryofetal toxicity up to a dose of 10 mg/kg/day. **Reviewer's Comment** – The Reviewer concurs with the following caveat. The relationship of the total resorptions [3 and 10 mg/kg/day], and the pre-implantation losses and early resorptions [10 mg/kg/day] to treatment is not known.

C. Literature

a. Ref. 343: Expert report on intravenous developmental toxicity of CL 318,952 [Benzoporphyrin derivative monoacid, A photosensitizer for photodynamic Therapy] in rats and rabbits; TX-94026; Prepared by Mildred S. Christian, Ph.D., ATS; Dec. 9, 1994. – The following are conclusions of the independent expert:

RAT STUDY

1. The maternal NOAEL, based on hematology, was 2 mg/kg/day.
2. The developmental NOAEL based on an increased incidence of fetal alterations [anophthalmia/microphthalmia, bent/wavy ribs] was 10 mg/kg/day. The anophthalmia/ microphthalmia, which was observed in a single fetus at 10 mg/kg/day, was considered unrelated to treatment since the litter incidence is within the historical incidences reported by MARTA.
3. The developmental toxicities observed "commonly occur at maternally toxic doses".
4. The placental transfer study in the rat indicated that <1% of the administered dose crosses the placenta. The fetal alterations, therefore, are considered to be secondary [e.g. indirect] rather than direct drug effects.
5. Verteporfin was not selectively toxic to the rat fetus.
6. There were no adverse effects on embryofetal viability or growth.

4. **Reviewer's Comment** – In general the Reviewer concurs with the following exceptions. [1] The NOAEL of 2 mg/kg for maternal toxicity was not based on the current study but was extrapolated from the dose-range finding study. [2] The incidence of anophthalmia/ microphthalmia at 10 mg/kg/day was within in historical controls reported by MARTA. The historical controls provided by laboratory indicated that the mean fetal incidence for anophthalmia and/or microphthalmia was 6 out of 6321 fetuses or 0.09% [range of 0-0.6%] and the mean litter incidence was 5 out of 430 or 1.2% [range of 0-4.3%]. The mean fetal and litter incidence at 25 mg/kg/day was 1.3% and 17.4%, respectively. The mean fetal incidence at 10 mg/kg/day was within historical control ranges [0.3%] and the mean litter incidence 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.

RABBIT STUDY

1. The maternal NOAEL, based on transient decrease in body weight gain and food consumption, was 3 mg/kg/day.

2. The developmental NOAEL was 10 mg/kg/day.
3. Verteporfin was "not embryotoxic, fetotoxic or teratogenic to rabbit conceptuses at either of two maternally toxic doses".

Reviewer's Comment – In general, the Reviewer concurs with the following exception. A complete evaluation of the reproductive effect at 25 mg/kg could not be performed since rabbits were euthanized on Gestation Day 9. Therefore the #3 statement should be that no effects on the fetuses was observed at the single maternally toxic dose.

A discussion of the expert report's conclusion with respect to the relationship of the complete resorptions to treatment is provided above and will not be repeated here.

III. Segment III – Pre and Postnatal Reproductive Toxicology

A. Rat

a. An intravenous pre and postnatal study of benzoporphyrin derivative monoacid [BPD-MA] in the rat [Ref. 347]

Study Identification: TX-98003
Site: [redacted]
Study Dates [in-life]: March 2 – July 20, 1998
Formulation and Lot No. – liposomal BPD-MA- TC0175- reconstituted with sterile water for injection; diluted with 5% dextrose; the dosing solution was prepared weekly and stored at 4° C
Vehicle – 5% dextrose
Certificate Analysis: Yes (X) [redacted]
Final Report: March 29, 1999
GLP and QA Statements Signed: Yes (X)
Objective: "To investigate the effects of Benzoporphyrin Derivative Monoacid (BPD-MA)[verteporfin] upon gestation, parturition and lactation ... in the dams and the development of the pups and their survival, physical development, behavior and reproductive performance"

Study Design

Test Material/ Group Designation	Dose*				N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed	F	
Group 1 –5% Dextrose	0	5	iv	Gestation Day 6 - Post Partum Days 21-23 or until euthanasia in preterminal rats	24	Sprague-Dawley [Crl:CD®(SD)BR]
Group 2 - BPD-MA	1					[redacted]
Group 3 - BPD-MA	3					F – app. 71-75 days – at study start
Group 4 - BPD-MA	10					F = 212-266 g – at study start

*2.5 ml/min; dose selected based on 28-day repeat dose study in rats [TX-96010] in which mild dose-related anemia, erythroid hyperplasia, increased WBC counts, and spleen enlargement were observed at ≥2 mg/kg/day and pigment accumulation was noted in the renal cortical epithelium and hepatic cells at ≥10 mg/kg/day; lighting was maintained at <20 foot candles

Parameters Evaluated*	Time Point(s)
Clinical observations	
F_0	
Mortality/moribundity and observations	BID
Physical examination	Gestation Days 0, 3, 6, 9, 12, 15, 18 and 20; Post partum Days 0,4, 7 ,14, 17 and 21
F_1 pups	Daily during lactation
F_1 adult generation	
Mortality/moribundity and observations	BID
Physical examination	Weekly
F_2 pups	Daily during lactation
Body Weight	
F_0	Gestation Days 0, 3, 6, 9, 12, 15, 18 and 20; Post partum Days 0, 4, 7, 14, 17 and 21
F_1 pups	Birth, Post partum Days 4, 7, 14, and 21
F_1 adult generation	Weekly premating; Gestation Days 0, 3, 6, 9, 12, 15, 18 and 20; Post partum Days 0 and 4
F_2 pups	Post partum Days 0 and 4
Food Consumption	
F_0	Gestation Day 0-3, 3-6, 6-9, 9-12, 12-15, 15-18
Clinical Pathology	
F_0 – Hematology [abdominal aorta] – all females [not TK group] – RBC count and morphology, WBC count and differential, MCV, MCH, MCHC, and RDW, Hct, Hb, MPV, platelet count, reticulocyte count	Necropsy
Toxicokinetics [Groups 2-4; N=4]**	
F_0 [orbital sinus; isoflurane]	Gestation Days 6,17, and 20; Lactation Days 4 and 21 [1, 2, 4, and 24 hours post dosing]
Observations at Parturition	
F_0 and F_1	
time of parturition onset and completion, dystocias, behavior immediately post partum, F_1 generation [live and dead pups, sex, external examination***, pup weight]	From Gestation Day 20 [TID]
Gross Pathology –	
F_0 and F_1 adult generation [males and females****]	Death/Sacrifice females; 3 weeks post mating for F_1 males
F_1 pups	
any pup found dead, any pup sacrificed Post partum Days 8-21	Death/Sacrifice
Physical Development	
F_1 pups	
Pinna unfolding	From Post partum Day 1 until Day 4 or all pups had a positive response
Tooth eruption	From Post partum Day 7
Eye opening	From Post partum Day 12
F_1 adult generation	
Vaginal opening	From Post partum Day 26 until development
Preputial separation	From Post partum Day 34 until development

Parameters Evaluated*	Time Point(s)
Behavioral Development	
F_1 pups	
Righting reflex	From Post partum Day 2 until Day 4 or all pups had a positive response
Negative geotaxis test	From Post partum Day 8 until all pups had a positive response
Auricular startle response	From Post partum Day 12 until all pups had a positive response
F_1 adult generation	
Visual Function – pupillary closure, visual placing	Post partum Day 21
Motor activity – figure 8 mazes	Post partum Days 35 and 60 [±1-2]
Auditory startle habituation	Post partum Day 55 [±2]
'E' water maze	Post partum Days 64 and 68

* Pups were culled on Post partum Day 4 to give a litter size, if possible, of 8 [4 females and 4 males]; pups were weaned and 1 male and 1 female from each litter randomly selected to comprise the F_1 adult generation; F_1 generation pups were paired for mating at 85 days of age; F_2 pups were euthanized on Post partum Days 4-6 and not examined further

**Group 1 rats [N=2] were sampled at 2 and 24 hours post dosing; samples were not analyzed in any of the treatment groups

***Externally malformed pups [F_1 pups] were euthanized and underwent external examination [dissecting microscope]

****F_1 females which failed to mate were euthanized Days 24-28 after the mating period; number of implantation sites were recorded; F_0 and F_1 females which mated but did not litter were euthanized on Days 26-28 after mating and the reproductive tract was thoroughly examined; F_0 dams were euthanized Post partum Days 21-23; any F_0 or F_1 female whose litter died prior to weaning were euthanized and implantation sites recorded

Results

Mortality

- F_0 – All animals survived to scheduled sacrifice.
- F_1 Pups – In general, the viability index [no. of live pups Postpartum Day 4 (precull)/no. of live pups on postpartum Day 0]; survival index [no. of live pups Postpartum Day 7 and 14/no. of live pups on postpartum Day 0 (postcull)]; and lactation index [no. of live pups Postpartum Day 21/no. of live pups on postpartum Day 0 (postcull)] were comparable across all groups. The slightly lower survival [Day 14] and lactation indices in the 1 mg/kg/day group compared to controls was attributed to the death of 7 pups from a single litter which drowned in a wet bin on post partum Day 11.
- F_1 adults – One mid-dose male was euthanized due to an ulcerated mass at the base of the ear.
- F_2 pups – There were no treatment-related effects.

Clinical Observations

- F_0 – There was 1 high dose female that exhibited tail sloughing. This finding is potentially related to treatment. All other findings were considered incidental, generally occurring at a comparable frequency across all treatment groups or in ≤2 animals/group.
- F_1 pups – There were 2 litters at the high dose in which there were 3 fetuses/litter that were thin and/or had empty stomachs [Postpartum Days 0-1]. The relationship to treatment is not known. Generally, the incidence of the findings was comparable across all groups and generally at <1-2% in all groups.
- F_1 adults - There were no treatment-related effects.
- F_2 pups – Findings [e.g. skin lesions, abnormal hindlimb flexure, severed tail, missing hindlimb] occurred in ≤2 animals/group, did not demonstrate a dose-dependent relationship, and were considered unrelated to treatment.

Body Weight, Body Weight Gain, and Food Consumption

- F_0 – There were nonsignificant decreases in body weight gain [approximately 60%] on Lactation Days 4-7 at 10 mg/kg/day. The decrease was primarily due to a single high dose female that lost 46 grams. However, 1 control vs. 6 high dose females lost weight. There was a statistically significant increase [approximately 50%] in body weight gain Lactation Days 7-14 at 3 and 10 mg/kg/day. The Sponsor suggests that these effects were due to biological variation. A relationship to treatment can not be ruled out.
- F_1 pups – The mean total pup, mean female, and mean male body weights were comparable across treatment groups. Decreased weights throughout the postpartum period were observed in several pups in the two litters in which empty stomachs and thinness were noted. [See Clinical Observations]. These decreases did not significantly impact group or litter means. The relationship to treatment is not known.
- F_1 adults – There were no changes that were considered to be treatment-related.
- F_2 pups – Mean total pup, mean female, and mean male body weights were comparable across all treatment groups on Post partum Days 0 and 4.

Hematology

- F_0 – There were changes in a number of the RBC indices [↑MCV, MCH, MCHC, and % reticulocytes; and ↓ RDW, MPV, and RBCs], some of which reached statistical significance at doses ranging from 1 – 10 mg/kg/day. At 1 and 3 mg/kg/day, changes in MCV, MCH, and/or MCHC were increased by ≤ 3%. There was no change in RBC count, RDW%, or % reticulocytes at these doses. At 10 mg/kg/day, there was a 3-4% decrease in RBC count and RDW% and a 3-6% increase in MCV, MCH, and MCHC. Percent reticulocytes increased only in the high dose group from 1.5 ± 1.05% in the controls to 2.0 ± 1.6%. The hematopoietic system is a target. However, relationship of the changes observed at 1 and 3 mg/kg/day to drug administration are questionable since there was no change in either RBC count or % reticulocytes. It is possible that these changes reflect biological variation. However, the changes observed at 10 mg/kg/day, which are minimal, are considered related to treatment related since RBC counts, % reticulocytes, MCV, MCHC, MCH, and RDW% are affected.

Maternal Performance

- F_0 – Generally, the pregnancy rate; live birth index [no. of live pups at birth/no. of implantation sites]; gestation index [no. of rats with live litters/no. of pregnant rats]; fertility index; conception rate; gestation length; parturition duration; implant number; number of live/dead and/or malformed pups; and sex ratios were comparable across all treatment groups. The statistically significant increase in dead pups in the control group compared to the 3 and 10 mg/kg/day groups was attributed to cannibalization of pups at birth by one dam.
- F_1 adults - The pregnancy rate; live birth index; mating index; mean day to mating; gestation index; fertility index; conception rate; gestation length; parturition duration; implant number; number of live/dead and/or malformed pups; and sex ratios were comparable across all treatment groups.

Physical, Reflexological, and Behavioral Development

- F_1 pups – There were no treatment-related effects on pinna detachment, righting reflex, negative geotaxis, tooth eruption, auricular startle, and eye opening.
- F_1 adults – There were no treatment-related effects on visual placing and pupillary closure; mean day of vaginal opening or preputial separation; motor activity; and water maze. The Sponsor indicated that for auditory startle habituation there was a statistically significant difference in the males from high dose dams compared to the control values. However, they

maintain that since there was not a dose-dependent response and the mean value for both sexes was comparable across all groups, the difference was due to inter-group variation.

Gross Pathology

- F_0 – Findings, with the exception of renal dilatation [1 in control and 3 mg/kg/day groups and 3 in high dose group], occurred in ≤1 rat/group. All findings were considered incidental.
- F_1 pups – Major malformations were observed in 1 pup each from 3/23 litters at 1 mg/kg/day. Since no malformations were observed at 3 or 10 mg/kg/day, these malformations were considered unrelated to treatment. Two males and females from a single litter from the high dose group dams had stomachs devoid of digesta [see F_1 pups – Clinical Observations and Body Weight]. Other findings occurred in 1-2 pups/group [<4%], did not demonstrate a dose-dependent response, and/or occurred at a comparable frequency across treatment groups, and were considered incidental.
- F_1 adults – No findings were considered to be related to treatment.
- F_2 pups – Major malformations were observed in 1 pup from the control group and 2 pups [2 litters] from the low dose group. There were no findings that were considered treatment-related.

Reviewer's Comments - Study Design and Data Presentation - The Sponsor states that the maximum dose was based on toxicity observed in the 28-day repeat dose study in rats [TX-96010]. The toxicity that was observed at the maximum dose in the current study was slight. This suggests that a higher dose could have been used [e.g. 25 mg/kg/day] without compromising the integrity of the data. However, it is felt that this study is adequate to support the intended clinical application for the treatment of ARMD based on the following considerations. [1] The clinical usage will be 1 injection q3-4 months vs. the repeat dosing regimen used in the nonclinical study. [2] The 10-mg/kg/day dose in female rats represents approximately 40 times 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.]

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

1. The NOAEL for the F_0 maternal toxicity was 1 mg/kg/day based on changes in RBC indices. The data also suggest that the bone marrow was undergoing a normal response. Reviewer's Comment – The change in MCH, MCHC, and RDW% at 3 mg/kg/day was ≤3%. There was no change in RBC count or % reticulocytes. Although the hematopoietic system is a target organ, it is felt that these changes may reflect biological variability and are of questionable relationship to treatment. However, the changes observed at 10 mg/kg/day, which were minimal, are considered related to treatment related since RBC counts, % reticulocytes, MCV, MCHC, MCH, and RDW% were affected. Therefore, a NOAEL of 3 mg/kg/day is considered more appropriate.

2. "There was no effect on reproductive performance during gestation, parturition or lactation and no effect on the survival, physical development, behavior and reproduction performance of the F_1 generation or on the survival and development of the F_2 generation pups." **Reviewer's Comment** - The Reviewer concurs.

Additional Reviewer Comments - The tail sloughing, observed in a single high dose female, might potentially be related to treatment with BPD-MA.

Summary of Reproduction Toxicology - Fertility Studies in Male and Female Rats – There were no adverse effects on male or female fertility at doses up to 10 mg/kg/day. The systemic toxicity at this dose was minimal. It is felt that a dose of 25 mg/kg/day [the maximum possible based on solubility constraints] would have resulted in more definitive toxicity without significantly compromising the animals. However, this study is adequate to support the intended clinical application for the treatment of ARMD based on the following considerations. [1] The clinical usage will be 1 injection q3-4 months vs. the repeat dosing regimen used in the nonclinical study. [2] The 10-mg/kg/day dose in male and female rats represents approximately 60 and 40 times 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.]

Developmental Toxicity Studies in Rats and Rabbits – The NOAEL for maternal toxicity of 2 mg/kg/day was not identified in the definitive rat developmental toxicity study [Study TX-93002] but was extrapolated from the range-finding study [3151.11]. The NOAEL was based on changes in RBC indices. The NOAEL for maternal toxicity in the definitive rabbit developmental toxicity study [TX-93001] was 3 mg/kg/day based on a transient weight loss and/or decrease in body weight gain, and a decrease in food consumption GD 6-12. Although there was only an overall 2-3% decrease in body weight in the high dose females compared to controls, on GD 6-9, high dose does exhibited a mean body weight loss while control does exhibited weight gain. Weight gain was also decreased by 75% in the high dose female GD 9-12 compared to the controls. This would suggest that the high dose was appropriate. Based on the degree of change in the RBC parameters, 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 6 out of 6321 fetuses or 0.09% [range of 0-0.6%] and the mean litter incidence was 5 out of 430 or 1.2% [range of 0-4.3%]. The mean fetal and litter incidence at 25 mg/kg/day was 1.3% and 17.4%, respectively. 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 may be related to maternal toxicity. The NOAEL was determined by the Sponsor based on results from the range-finding developmental study [3151.11]. As noted earlier, this is inappropriate. 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 is supported by results of the placental transfer study [A9301] which indicates that <1% of the total radioactive dose crosses the placenta and is 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. [**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.] 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. Two females at 1 mg/kg/day aborted and 1 and 2 females at 3 and 10 mg/kg/day underwent total litter resorption. While the number of animals with total resorptions was low, the percent incidence of females with total resorptions at 3 and 10 mg/kg/day [5.6% and 11.1%] exceeded the mean percentage reported in the historical control data [8/387; 2.1%]. The Sponsor

did not provide a range for the incidence of total resorptions, which may have encompassed the incidence observed in this study. The incidence was within the historical control range reported in the database compiled by MARTA. There was a minimal increase in pre-implantation loss and early resorptions. The incidence of post-implantation loss was comparable in the control and 10 mg/kg/day groups and was within the historical control data provided by the Sponsor [Appendix N]. A treatment-related effect, with respect to total resorptions, pre-implantation losses, and early resorptions, can not be totally ruled. Based on the considerations outlined, it is considered unlikely. The 10 mg/kg/day dose represents approximately 20 times the human dose of 6 mg/m^2 based on surface area.

Pre and Postnatal Development - The Sponsor stated that the NOAEL for F_0 maternal toxicity was 1 mg/kg/day based on minimal changes in RBC indices. The change in MCH, MCHC, and RDW% at 3 mg/kg/day was ≤3%. There was no change in RBC count or % reticulocytes. Although the hematopoietic system is a target organ, it is felt that these changes may reflect biological variability and are of questionable relationship to treatment. Therefore, a NOAEL of 3 mg/kg/day is considered more appropriate.

Major malformations were only observed in control and low dose pups. Gross pathology findings in all 3 generations were considered not to be treatment-related.

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. [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.]

Genotoxicity:

a. Ames/Salmonella-E. coli plate incorporation assay on benzoporphyrin derivative [BPD-MA; CL 315,555/315,585] with and without metabolic activation and with and without light irradiation [Ref. 348]

Study Identification: 90077

Site: [redacted]

Study Dates: October 23 – December 5, 1990

Formulation and Lot No. – BPD-MA- Batch No. PC 1094- reconstituted with DMSO and used within 2 hours of reconstitution

Vehicle – DMSO

Certificate Analysis: Yes (X)

Final Report: May 10, 1991

GLP and QA Statements Signed: Yes (X)

Objective: To evaluate the potential of BPD-MA to induce mutations in the Ames Assay with or without metabolic activation and with or without irradiation.

Study Design – The dose-range finding study indicated that BPD-MA was not toxic to Salmonella strains TA1538, TA100 and *E. coli* strain WP2 uvrA at concentrations of 46.1 - 4610 µg/plate ± S9 with or without irradiation. The selected dose of irradiation resulted in an increase in revertants in TA100 from approximately 70-80 to 921 revertants. This exposure also resulted in ≥2X increase in revertants in TA 1535, TA98, and TA 100, but not in TA1537, TA1538, and WP2 uvrA. BPD-MA was insoluble at ≥46 µg/plate. Based on the results of the dose-range finding study, Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 and *E. coli*

strain WP2 uvrA were incubated for 48 hours at 15.4, 46.1, 154, 461, 921 and 1540 µg/plate of BPD-MA in DMSO ± S9. Plates were evaluated with and without light irradiation. Uncovered plates [under a laminar flow hood] were irradiated at 1.04-1.34 J/cm^2. The irradiation source was 8 unshielded internal 30-watt GE cool white fluorescent bulbs. Plates were exposed for approximately 30 minutes at a distance of 75 cm. Irradiance was measured with a spectroradiometer. The positive controls without S9 or irradiation were Na azide, 9-aminoacridine, 2-nitrofluorene, and ENNG. The positive control with S9 and no irradiation was 2-anthramine. No positive control [e.g. a known photogenotoxicant] was included for the irradiated plates. This assay was repeated under the same conditions. In a repeat confirmatory assay, Salmonella strain TA1538 and *E. coli* strain WP2 uvrA were incubated for 48 hours at 0.154, 0.461, 1.54, 4.61, 15.4, 46.1, 154, 461, and 921 µg/plate. uvrA was incubated ± S9 and TA1538 without S9. Plates were evaluated following light irradiation as indicated above. An assay was considered positive by the Sponsor if there was a dose-dependent increase in the number of revertants and there was at least a 2X increase in ≥1 dose group when compared to the solvent control.

Results - The test article was not soluble at concentrations ≥46.1 µg/plate. BPD-MA was not toxic to any of the tester strains at any of the concentrations and under any of the conditions tested. Light irradiation only resulted in ≥2X increase in revertants in TA1535, TA98, and TA100, but not in TA1537, TA1538, and WP2 uvrA. There was no increase in the frequency of revertants at any concentration of test article when compared to the concurrent solvent control under the following test conditions: [1] dark conditions ± S9 activation; [2] light irradiation + S9; [3] light irradiation without S9 activation for the Salmonella strains. There was a statistically significant 2X increase in revertants for WP2 uvrA at 15.4 and 46.1 µg/plate following light irradiation without S9. The Sponsor states that these increases were apparently not dose dependent. However, it is not possible to determine how the insolubility of BPD-MA at ≥46.1 µg/plate impacted exposure. The results are outlined in the table below.

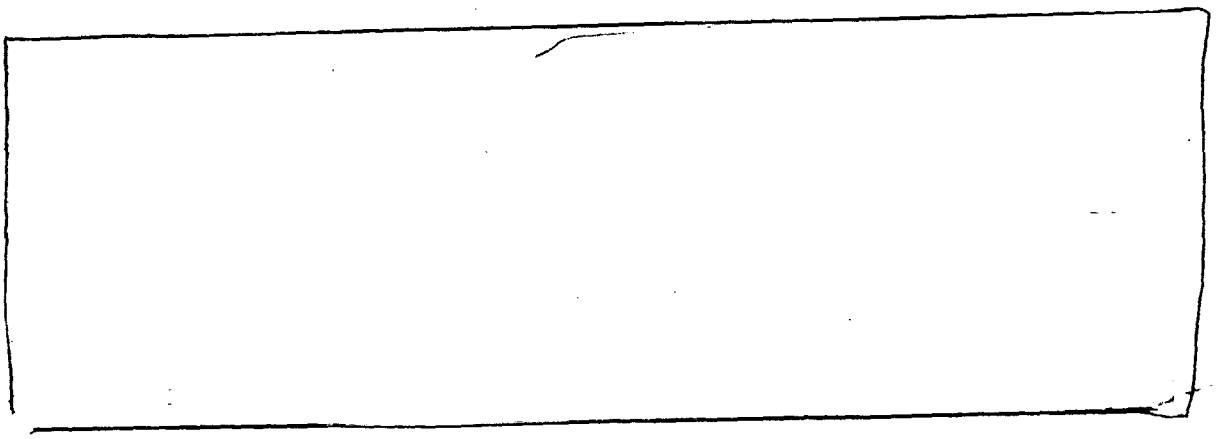
CONTROLS

		AVERAGE REVERTANTS/PLATE					
NEGATIVE CONTROLS	S-9	TA1535	TA1537	TA1538	TA98	TA100	UVR A
UNTREATED	(-)	31 (7)	8 (3)	5 (2)	42 (10)	950 (89)	8 (2)
UNTREATED	(+)	38 (6)	11 (4)	8 (1)	48 (7)	1166 (1041)	13 (5)
DMSO (100 UL)	(-)	32 (8)	8 (2)	7 (3)	52 (19)	859 (205)	7 (3)
DMSO (100 UL)	(+)	34 (14)	11 (2)	9 (2)	58 (17)	1033 (168)	14 (6)

TEST ARTICLE: BPD-MA

		AVERAGE REVERTANTS/PLATE					
DOSE LEVEL UG/PL	S-9	TA1535	TA1537	TA1538	TA98	TA100	UVR A
15.4	(-)	25 (4)	6 (1)	3 (2)	45 (6)	665 (34)	144(6)
46.1	(-)	18 (4)	8 (3)	2 (1)	62 (8)	892 (55)	154(6)
154	(-)	20(1)	5 (1)	2 (1)	51 (8)	650 (128)	13 (6)
461	(-)	25 (2)	8 (2)	3 (2)	54 (13)	618 (26)	10 (1)
921	(-)	20 (2)	7 (3)	5 (2)	45 (9)	524 (85)	12 (3)
1540	(-)	18 (3)	7 (1)	4 (3)	37 (18)	373 (45)	8 (4)
15.4	(+)	20 (3)	7 (2)	5 (2)	49 (8)	588 (61)	14 (2)
46.1	(+)	24 (7)	9 (3)	5 (3)	64 (12)	644 (65)	19 (2)
154	(+)	28 (4)	8 (5)	8 (1)	60 (11)	760 (43)	18 (3)
461	(+)	34 (2)	7 (2)	7 (1)	55 (12)	671 (118)	12 (4)
921	(+)	22 (2)	7 (7)	5 (2)	46 (12)	556 (74)	12 (6)
1540	(+)	19 (2)	7 (2)	8 (3)	25 (3)	348 (30)	11 (5)

Data Reported As: Mean (Standard Deviation)
+ = Positive Response (2X Solvent)
Test Article precipitate at ≥ 50.0 ug/plate



Reviewer's Comments – Study Design and Data Presentation

1. The Sponsor did not adequately describe the emission spectra of the irradiation source. Use of fluorescent lighting resulted in the elimination of light frequency within the UV spectrum. Fluorescent lighting generally includes wavelengths that activate BPD-MA. The amount of light dose at this wavelength is not known. However, selection of the irradiation dose was appropriate since it resulted in minimal genotoxicity in TA100, TA153, and TA98.

2. According to ICH Guideline S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, "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. It should be noted that these studies were conducted prior to issuance of these guidelines. However, 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.

3. A photogenotoxicant, which is known to be positive in this assay, should have been included as a positive control.

Sponsor's Conclusions and Reviewer's Comments –

1. Under the conditions tested, BPD-MA was negative in the Ames/Salmonella-E. coli plate incorporation assay. The increase in revertants observed in WP2 uvrA and TA1538 were slight, sporadic, and were not consistently reproducible. **Reviewer's comment** – The Reviewer concurs with the following caveat. There was concern with respect to [1] lack of a positive control to insure that the assay could detect mutagenic potential of a photoactive drug under the conditions tested; [2] characterization of the light source, and [3] selection of drug concentrations.

b. Test for induction of unscheduled DNA synthesis in rat primary hepatocyte cultures by benzoporphyrin derivative monoacid [BPD-MA; CL 315,555/315,585] with and without light irradiation [Ref. 349]

Study Identification: 90078

Site:

Study Dates: November 5, 1990 – March 5, 1991

Formulation and Lot No. –BPD-MA- Batch No. PC 1094- reconstituted with DMSO: no statement as to when reconstituted in relation to test set up;
Vehicle – DMSO
Certificate Analysis: Yes (X)[redacted]
Final Report: May 9, 1991
GLP and QA Statements Signed: Yes (X)
Objective: To evaluate "the potential [of BPD-MA] to cause unscheduled DNA synthesis in primary rat hepatocytes using ^{3}H-thymidine incorporation with and without light irradiation".

Study Design — Doses were selected for the definitive study based on a dose-range finding study. The Sponsor selected a test article concentration of 0.01 µg/ml, which was non-toxic without light irradiation, and irradiation doses that resulted in a relative cell survival [RCS] ranging from approximately 100 – 40%. Primary rat hepatocytes, obtained from male Sprague-Dawley rats[redacted] were incubated with 0.01 µg/ml of BPD-MA for 2 hours. ^{3}H-thymidine was added to the treated cultures for the assessment of unscheduled DNA synthesis [UDS]. Plates were then irradiated with 0, 1000, 2000, 3000, 4000, and 5000 J/m^2. The irradiation source was 4 Sylvania F30T12 fluorescent bulbs. Cultures were irradiated through the plastic of the plate. The distance of the source from the plate was 17 cm and a light intensity of 5.33 mW/m^2 was employed. Irradiation was measured using a radiometer/ photometer. The UDS plates were incubated for an additional 18 hours following irradiation. A parallel cytotoxicity evaluation, using Trypan blue exclusion, was also conducted 18 hours following light irradiation. Only "healthy" cells were scored. The scoring of grain counts [e.g. ^{3}H-thymidine incorporation] was blinded.

An assay was considered positive if: [1] there was a statistically significant dose response and ≥1 concentration[s] exhibited a significant increase in UDS over concurrent solvent control; or [2] there was a statistically significant increase in UDS in ≥2 successive concentrations. An assay was considered equivocal if: [1] there was a statistically significant dose response without any dose demonstrating a significant increase in UDS compared to controls; or [2] there was no statistically significant dose response but significant increase in UDS at one of the doses.

Results- The RCS at 0.01 µg/ml + 3000, 4000, and 5000 J/m^2 was 100, 84.4, and 66.7%, respectively. The maximum irradiation dose alone did not induce any cytotoxicity. Therefore, these irradiations, as well as 0.01 µg/ml + 0 J/m^2, were scored. The average net nuclear grain count and the % nuclei with ≥5 net nuclear grains were comparable across all groups with the exception of the positive control.

Reviewer's Comments – Study Design and Data Presentation

1. Concerns with respect to selection and characterization of the irradiation source are the same as those discussed for the Ames assay. In addition, it would have been preferred if at least one irradiation source had induced some degree of either cytotoxicity or genotoxicity. The maximum irradiation dose alone and drug alone did not result in cytotoxicity. However, combining light irradiation and drug did result in significant toxicity. This suggests that the irradiation source is activating intracellular drug.

2. A known photogenotoxicant, whieh is positive in this assay, should have been included as a positive control.

3. A single dose without irradiation is not adequate to assess the potential of BPD-MA to induce UDS under dark conditions. Ideally, the experimental design should include both a single dose of BPD-MA with multiple light doses of light as well a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA. A study conducted with a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA would have been preferred to a single dose of BPD-MA with multiple light doses of light.

Sponsor's Conclusions and Reviewers Comments –

1. Under the conditions tested, BPD-MA was negative in the UDS assay with and without light irradiation. **Reviewer's comment** – The Reviewer concurs that under these conditions, BPD-MA was negative with light irradiation but with the following caveat. There was concern with respect to [1] lack of a positive to control to insure that the assay could detect mutagenic potential of a photoactive drug under the conditions tested; [2] characterization of the light source, and [3] selection of irradiation doses [e.g. no genotoxicity or cytotoxicity at the following irradiation only]. As indicated above, a single dose without irradiation is not adequate to assess the potential of BPD-MA to induce UDS under dark conditions.

c. Test for induction of gene mutation at the HGPRT locus in cultured Chinese Hamster Ovary [CHO] cells by benzoporphyrin derivative monoacid [BPD-MA; CL 315,555/315,585] with and without metabolic activation and with and without light irradiation [Ref. 350]

Study Identification: 90080

Site: [redacted]

Study Dates: October 8, 1990 – February 15, 1991

Formulation and Lot No. –BPD-MA- Batch No. PC 1094- reconstituted with DMSO; no statement as to when reconstituted in relation to test set up;

Vehicle – DMSO

Certificate Analysis: Yes (X) [redacted]

Final Report: May 9, 1991

GLP and QA Statements Signed: Yes (X)

Objective: To evaluate " the potential [of BPD-MA] to cause gene mutation at the hypoxanthine guanine phosphoribosyl transferase [HGPRT] locus in cultured Chinese hamster ovary [CHO] cells with and without an exogenous S-9 activation mixture and with and without light irradiation".

Study Design - Test conditions were selected based on a dose range finding study. In this study, BPD-MA resulted in a dose-dependent cytotoxicity without irradiation that was comparable with and without S9 activation up to a concentration of 50 µg/ml. With irradiation, BPD-MA without S9 activation generally demonstrated greater toxicity than BPD-MA with S9 activation. The Sponsor selected a test article concentration of 0.1 µg/ml, which was non-toxic without light irradiation, and irradiation doses that resulted in a relative cloning efficiency [RCE] ranging from approximately 100 – 24%. For the definitive study, CHO cells were incubated for 2 hours with 0. 1 µg/ml of BPD-MA ± S9 followed by light irradiation levels of 0, 105, 210, 315, 420, 525, and 630 J/m^2. The irradiation source was 4 Sylvania F30T12 fluorescent bulbs. Cultures were irradiated through the culture flasks at a distance of 125 cm and a light intensity of 0.35 mW/m^2. Irradiation was measured by a radiometer/photometer. The positive controls, which were not irradiated, were ethyl methanesulfonate [no activation] and 7,12-dimethylbenz[a]anthracene [with activation]. Cell cultures were cloned for cytotoxicity 18-24 following irradiation and allowed to grow for 8-9 days. The average number of colonies per plate and the RCE were calculated. For expression of mutant phenotype, cells were cultured for

another 7-10 days. Following the expression period, cells were cultured in hypoxanthine-free medium for another 7 days. Cloning efficiency and mutant selection were then calculated. The study was repeated because of a high mutant frequency at 630 /m^2 without S9 activation. A confirmatory study was conducted using 0.1 µg/ml without S9 activation + 630 J/m^2.

The assay was considered positive if there was a statistically significant dose response and ≥1 dose[s] had an average mutant frequency >20 mutants/10^6 cells. The assay was considered inconclusive if a dose demonstrated an average mutant frequency >20 mutants/10^6 cells which was statistically significant compared to the concurrent solvent control but there was no statistically significant dose response.

Results – Irradiation only at 630 J/cm^2 did not result in any toxicity. The maximum reduction in RCE was 40%-50% at 0.1 µg/ml + ≥525 J/m^2 and approximately 70% at 0.1 µg/ml + ≥210 J/m^2 with and without S9 activation, respectively. At an irradiation dose of 630 J/cm^2 without activation, the average mutants/10^6 surviving cells was 62. There were 5 and 119 mutants in each replicate. This effect was not repeated in the confirmatory assay. The reason for this difference is not known. The RCE in this assay was 25%. Although, there tended to be an slight increase in the number of mutants/10^6 surviving cells in the plates without activation, the increase was not statistically significant and the average was <20 mutants/10^6 surviving cells. The results from the definitive study without S9 activation are presented below.

Light Intensity. J/sq.m		Ave. No. of C.E. Colonies Per Plate	Standard Deviation	C.E.	Ave. No. of R.M. Colonies	Standard Deviation	Total Mutants Counted	Mutants/10^6 Surviving Cells
DMSO +0	A	226	+/- 23.2	113%	1	+/- 0.5	3	3
	B	112	+/- 14.5	56%	0	+/- 0.4	1	2
	C	NA	NA	NA	NA	NA	NA	NA
	D	130	+/- 7.9	65%	0	+/- 0.0	0	0
DMSO +630B	A	107	+/- 5.1	54%	1	+/- 1.4	7	13
	B	137	+/- 4.7	69%	0	+/- 0.5	2	3
	C	164	+/- 25.3	82%	0	+/- 0.4	1	1
	D	173	+/- 16.9	87%	0	+/- 0.0	0	0
0.1 +0	A	92	+/- 15.4	46%	1	+/- 0.7	4	9
	B	151	+/- 7.4	76%	0	+/- 0.5	2	3
0.1 +105B	A	86	+/- 9.0	43%	1	+/- 1.1	5	12
	B	109	+/- 0.8	55%	1	+/- 0.8	3	6
0.1 +210B	A	135	+/- 10.2	68%	2	+/- 1.0	8	12
	B	98	+/- 13.9	49%	2	+/- 1.4	12	24
0.1 +315B	A	119	+/- 10.1	60%	1	+/- 1.3	5	8
	B	143	+/- 9.5	72%	2	+/- 1.8	10	14
0.1 +420B	A	103	+/- 16.7	52%	0	+/- 0.8	2	4
	B	128	+/- 6.4	64%	2	+/- 1.6	9	14
0.1 +525B	A	95	+/- 7.1	48%	1	+/- 0.8	3	6
	B	109	+/- 8.7	55%	0	+/- 0.0	0	0
0.1 +630B	A	82	+/- 6.9	41%	0	+/- 0.5	2	5
	B	64	+/- 6.2	32%	8	+/- 1.2	38	119
EMS 0.5ul/ml	A	53	+/- 7.9	27%	54	+/- 7.0	270	1019
	B	72	+/- 0.5	36%	50	+/- 6.8	250	694

* C.E. = Cloning Efficiency
**R.M. = Medium with 6-TG
*** EPD-MA concentration was kept constant at 0.1 ug/mL at all treatment conditions

$$\text{Mutants/10}^6 \text{ Survivors} = \frac{100}{\text{C.E.}} \times \frac{\text{Total Mutants Counted per Dose}}{\text{Mutant Plates Counted per Dose}} \times \text{Mutant Plates Seeded per Dose}$$

Reviewer's Comments – Study Design and Data Presentation

1. Concerns with respect to selection and characterization of the irradiation source are the same as those discussed for the UDS assay. [See Comment #1 under Study Design and Data Presentation above]

2. According to ICH Guideline S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, "[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. In addition, in ICH Guideline S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, it is stated that confirmatory assays are not needed if the dose-range finding study assay "is performed with and without metabolic activation, with appropriate positive and negative controls, and with quantification of mutants". This was not done, so no confirmatory study has been conducted. It should be noted that these studies were conducted prior to issuance of these guidelines.

3. A known photogenotoxicant if available, which is positive in this assay, should have been included as a positive control.

4. A single dose without irradiation is not adequate to assess the potential of BPD-MA to induce mutations under dark conditions. Ideally, the experimental design should include both a single dose of BPD-MA with multiple light doses of light as well a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA. A study conducted with a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA would have been preferred to a single dose of BPD-MA with multiple light doses of light.

Sponsor's Conclusions and Reviewer's Comments-

1. Under the conditions tested, BPD-MA was negative in the CHO/HGPRT gene mutation assay with and without light irradiation. **Reviewer's comment** – The Reviewer concurs that under these conditions BPD-MA was negative with light irradiation but with the same caveats as listed for the UDS assay.

d. Test for induction of chromosome aberration in cultured Chinese Hamster Ovary [CHO] cells by benzoporphyrin derivative monoacid [BPD-MA; CL 315,555/315,585] with and without metabolic activation and with and without light irradiation [Ref. 351]

Study Identification: 90081

Site: [redacted]

Study Dates: November 19, 1990 – March 1, 1991

Formulation and Lot No. –BPD-MA- Batch No. PC 1094- reconstituted with DMSO; no statement as to when reconstituted in relation to test set up;

Vehicle – DMSO

Certificate Analysis: Yes (X) [redacted]

Final Report: May 15, 1991

GLP and QA Statements Signed: Yes (X)

Objective: To evaluate " the potential [of BPD-MA] to cause chromosomal aberrations in cultured Chinese hamster ovary [CHO] cells with and without an exogenous S-9 activation mixture and with and without light irradiation".

Study Design - Test conditions were selected based on a two dose range finding studies [relative cell growth (RCG), mitotic index (MI), average growth time (AGT)]. The concentration selected for the cultures without S9 activation was 0.09 μg/ml, which without light irradiation did not result in any change in RCG. With light irradiation at 600 J/m^2, treatment of cultures with 0.09 μg/ml resulted in RCG and RMI of 43% and 24%, respectively. Since the drug concentration [4.6 μg/ml] and irradiation doses used in the S9 activated cultures in the 1st

definitive study resulted in excessive toxicity in the 30-hour harvest time, third range-finding study was conducted. Based on these findings, the Sponsor selected a concentration of BPD-MA of 3.7 μg/ml for the S9 activated cultures. With light irradiation at 600 J/m^2, treatment of cultures with 3.7 μg/ml resulted in RCG and RMI of 25% and 3.8%, respectively. The harvest times reflected approximately 1-1.5X the AGT calculated under the various test conditions. CHO cells were incubated for 2 hours under the conditions listed in the tables below. The irradiation source were 4 Sylvania F30T12 fluorescent bulbs. Cultures were irradiated through the culture flasks at a distance of 125 cm and a light intensity of 0.35 mW/m^2. Irradiation was measured by a radiometer/photometer. The positive controls, which were not irradiated, were triethylenemelamine and cyclophosphamide. After irradiation, cells were incubated in media. Colcemid was added to 2 flasks 2 hours prior to the harvest time for determination of chromosomal aberrations. The other 2 flasks were used to determine RCG. The table below outlines the study design without activation.

Test Article Conc. (ug/mL)	Acti-vation	Light Irradiation Conditions (J/m^2)	(Seconds Exposed)	No. of Cultures Treated	Harvest Times (Hours After Treatment)
Untreated	-	0	0	4	14 only
TEM, 0.75	-	0	0	4	14 only
Solvent	-	600	172	20	14, 20, 24 & 36
0.09	-	0	0	8	14 and 20
0.09	-	120	34	8	14 and 20
0.09	-	240	69	8	14 and 20
0.09	-	360	103	8	24 and 36
0.09	-	480	138	8	24 and 36
0.09	-	600	172	8	24 and 36

["-" indicates that cultures were prepared for these time points]

Cells in the nonactivated system at 0, 120, 240, and 360 J/m^2 were scored "blind". The table below outlines the study design with activation.

Test Article Conc. (ug/mL)	Light Irradiation Conditions (J/m^2)	(Seconds Exposed)	No. of Cultures Treated	Harvest Times (Hours After Treatment)
Untreated	0	0	4	14 only
CP, 50	0	0	4	14 only
Solvent	420	120	16	14, 20 and 30
3.7	0	0	8	14 and 20
3.7	120	34	8	14 and 20
3.7	180	52	8	14 and 20
3.7	240	69	8	14 and 20
3.7	300	86	8	20 and 30
3.7	360	103	8	20 and 30
3.7	420	120	8	20 and 30

Cells in the activated system at 0, 180, 240, and 300 J/m^2 were scored. A total of 300 metaphases [150 per flask] were scored for both activated and nonactivated flasks.

A test was considered positive if there was a statistically significant dose response and a significant increase in chromosomal aberrations in ≥1 dose groups compared to concurrent control or in the absence of significant dose response, ≥2 dose groups exhibited significant increase in chromosomal aberrations.

Results – The table below outlines the cytotoxicity observed in the system without activation.

Test Article Conc. (ug/mL) + Light Intensity (J/m^2)	RCG at 14-Hour Harvest	RCG at 20-Hour Harvest	RCG at 24-Hour Harvest	RCG at 36-Hour Harvest
0.09 + 0	97	88	-	-
0.09 + 120	101	76	-	-
0.09 + 240	84	69	-	-
0.09 + 360	-		71	57
0.09 + 480	-	-	66	49
0.09 + 600	-	-	54	28

The table below outlines the cytotoxicity observed in the system with activation.

Test Article Conc. (ug/mL) + Light Intensity (J/m^2)	RCG at 14-Hour Harvest	RCG at 20-Hour Harvest	RCG at 30-Hour Harvest
3.7 + 0	101	102	-
3.7 + 120	96	92	-
3.7 + 180	95	86	-
3.7 + 240	84	84	-
3.7 + 300	-	74	46
3.7 + 360	-	67	43
3.7 + 420	-	59	33

The percentage of cells with aberrations was ≤5% in all groups, with the exception of the positive control. Although the incidence of mutations was not increased following drug treatment and irradiation in the nonactivated system, there was an increase in the number of chromatid gaps, chromatid breaks, and endoreduplications at various time points and doses. This is suggestive of DNA damage. The results for the control groups and treated groups are provided below.

Test Article Conc. (ug/mL) + J/m^2	No. of Metaphases Scored	Type and Frequency of Aberrations																No. of Aberrations per Cell	% of Cells with Aberrations
		pp	s	s	tb	tb	tf	tf	d	r	qr	tr	Cr	mu	ed	e			
		%	tg	Ieg	tb	Isb	tf	Isf							**				
Untreated + 0 14-hour harvest	300	1.5	1	1	2	1	-	3	-	-	-	-	-	-	-	-		0.02	1.67
DMSO + 600 14-hour harvest	300	3	1	-	1	-	1	4	-	-	-	1	-	-	-	-		0.023	2.33
DMSO + 600 20-hour harvest	300	2	4	-	3	-	-	4	2	-	-	-	-	-	-	1		0.03	3.00
DMSO + 600 24-hour harvest	300	3.5	1	-	1	-	-	2	-	-	-	-	-	-	-	-		0.01	1.0
DMSO + 600 36-hour harvest	300	2	2	-	1	1	-	1	1	-	-	-	-	-	-	-		0.013	1.0
TEM 0.75 + 0 14-hour harvest	100	4	7	3	9	3	3	15	3	-	5	4	-	-	-	-		0.42	35

Test Article Conc. (ug/mL) + J/m^2	No. of Metaphases Scored	Type and Frequency of Aberrations																No. of Aberrations per Cell	% of Cells with Aberrations
		pp	s	s	tb	tb	tf	tf	d	r	qr	tr	Cr	mu	ed	e			
		%	tg	Ieg	tb	Isb	tf	Isf							**				
0.09 + 0 14-hour harvest	300	1	3	-	-	-	-	5	-	1	-	1	-	-	-	-		0.023	2.0
0.09 + 0 20-hour harvest	300	1	3	1	1	-	1	6	1	-	-	-	-	-	-	1		0.03	3.0
0.09 + 120 14-hour harvest	300	3	11	-	-	-	-	6	-	-	-	2	-	-	-	-		0.027	2.33
0.09 + 120 20-hour harvest	300	3	6	3	4	-	-	-	1	-	-	-	-	-	-	2		0.017	1.67
0.09 + 240 14-hour harvest	300	2.5	7	3	7	-	-	1	-	-	-	-	-	-	-	-		0.027	2.33
0.09 + 240 20-hour harvest	300	2.5	5	2	5	1	1	6	2	-	-	-	-	-	-	-		0.053	5.0
0.09 + 360 24-hour harvest	300	3.5	4	1	1	1	-	1	-	-	-	1	-	-	-	5		0.023	2.33
0.09 + 360 36-hour harvest	300	3.5	7	2	1	-	-	4	-	1	-	-	-	-	-	4		0.02	1.67

Reviewer's Comments – Study Design and Data Presentation

1. Concerns with respect to selection and characterization of the irradiation source are the same as those discussed for the UDS assay. [See Comment #1 under Study Design and Data Presentation above].

2. In ICH GuidelineS2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, it indicates that exposure to test article should 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". Finally, it states that the "dose range-finding study should...include quantification of mutants in the cytotoxic range" in order to eliminate the necessity of conducting a confirmatory assay. This was not done, so no confirmatory assay has been conducted. It should be noted that these studies were conducted prior to issuance of these guidelines.

4. A photogenotoxicant if available, which is known to be positive in this assay, should have been included as a positive control.

5. A single dose without irradiation is not adequate to assess the potential of BPD-MA to induce mutations under dark conditions. Ideally, the experimental design should include both a single dose of BPD-MA with multiple light doses of light as well a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA. A study conducted with a single dose of light shown to be slightly genotoxic or cytotoxic with multiple doses of BPD-MA would have been preferred to a single dose of BPD-MA with multiple light doses of light.

Sponsor's Conclusions and Reviewer's Comments-

1. Under the conditions tested, BPD-MA was negative in the CHO/HGPRT gene mutation assay with and without light irradiation. **Reviewer's comment** – The Reviewer concurs that under these conditions BPD-MA was negative with light irradiation but with the same caveats as listed for the UDS assay. There was, however, an increase in the number of chromatid gaps, chromatid breaks, and endoreduplications at various time points and doses suggestive of DNA damage.

e. Test for chemical induction of gene mutation at the HGPRT locus in cultured Chinese Hamster Ovary [CHO] cells by benzoporphyrin derivative monoacid [BPD-MA; CL 315,555/315,585] with and without metabolic activation with a confirmatory assay [Ref. 353]

Study Identification: TX -98008

Site: [redacted]

Study Dates: February 2 – March 23, 1999

Formulation and Lot No. –BPD-MA- Batch No. TC 0992- reconstituted with sterile water and diluted with 5% dextrose; no statement as to when reconstituted in relation to test set up;

Vehicle – DMSO

Certificate Analysis: Yes (X)

Draft Report: (X) April 13, 1999

GLP and QA Statements Signed: No (X) [Since this is a draft report, statements are present but not signed]

Objective: To evaluate " the potential [of BPD-MA] to cause gene mutations at the HGPRT locus in cultured Chinese hamster ovary [CHO] cells with and without an exogenous S-9 activation mixture".

Test Material/ Group Designation	Concentrations		
Solvent control – 5% Dextrose	-		
Positive control with activation – DMBA	0.5 µg/ml		
Positive control without activation – ethyl methanesulfomate	0.5 µl/ml		
Test compound – BPD-MA [µg/ml]	with S-9	without S-9	
	1	1	5*
	5	5	10
	10	10	50
	50	50	100
	100	100	200

CHO-K1-BH4 subclone

*Concentrations for confirmatory assay

Following a 5-hour incubation, cells were washed and seeded for parallel toxicity and mutant expression. For toxicity and mutant expression, cells were subcultured for a total of 8 and 9 days, respectively. After the expression period, the cells from the 2 replicates were plated on cloning medium containing TG for 8 days. Cloning efficiency of the cells at selection time was determined by plating in triplicate cells in the cloning medium for 8 days.

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Parameter Evaluated	Sponsor Criteria for Validity of Assay	Sponsor Criteria for a Positive Result
Toxicity Average number of clones and percent clonable cells [cloning efficiency] Number of TG-resistant mutants per cells seeded and per $1X10^6$ surviving cells	1. No. of mutants/$1X10^6$ surviving cells should not be >25 in solvent control and >100in the positive control. 2.>40% reduction in RCE in at least 1 concentration 3. RCE of ≥65% in solvent control 4. ≥3 scorable test article concentrations	1. A positive dose response and a statistically significant increase in no. of TG-resistant mutants per cells per $1X10^6$ surviving cells or a 2X ↑ in mutants/$1X10^6$ surviving cells* or 2. ≥2 consecutive test doses with significant ↑ in mutants/$1X10^6$ surviving cells

*Historical control data are considered if the 2X increase is due to an unusually low number of mutants in the concurrent control. If no dose group has an average mutant frequency >15 mutants/$1X10^6$ surviving cell, the test article is considered negative.

Results – Osmolality - Solvent control and the highest concentration of test article were less than approximately 325 moms/kg H_2O.

- **Dose Range-Finding Study** – Based on a relative cloning efficiency [RCE = avg. no. of colonies in test plates/avg. no. of colonies in solvent control plates] of 121% -5% and 98%-2% for nonactivated and activated systems, respectively, at concentrations ranging 0.08 – 200 μg/ml, the following concentrations were chosen for the definitive study for systems: 1, 5, 10, 50, and 100 μg/ml. Concentrations ≥ 400 μg/ml were toxic in either the activated or nonactivated system.

- **Definitive Study** – Adequate toxicity was achieved with maximum RCE in the cultures run in parallel with the chromosomal aberration. The RCE was approximately 21% and 50% with and without S9 activation. At 100 μg/ml there were 2 and 37 mutants/10^6 surviving cells [avg. of 20] in the replicates. This was >2X the solvent control. The Sponsor states that the historical control data for solvent control has a maximum value of 33; however, this is for cumulative solvent [H_2O and DMSO – maximum values of 22 and 34, respectively]. Nevertheless, this effect was not reproducible in the confirmatory assay. The results of the definitive study are provided below.