

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: 20-965**

**PHARMACOLOGY REVIEW(S)**

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**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:**

**KEY WORDS:** 5-aminolevulinic acid, ALA, photodynamic therapy, actinic keratoses  
**Reviewer Name:** Amy Nostrandt  
**Division Name:** Division of Dermatologic and Dental Drug Products  
HFD# 540  
**Review Completion Date:** 11/19/1999

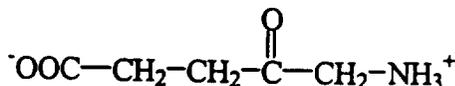
NOV 19 1999

**Review number:** 2  
**IND/NDA number:** NDA 20-965  
**Serial number/date/type of submission:** resubmission (AZ), response to AE letter, received 10/4/1999

**Information to sponsor:** Yes ( ) No (X)  
**Sponsor (or agent):** Guidelines, Inc. for DUSA Pharmaceuticals, Inc.  
**Manufacturer for drug substance:** [redacted]

**Drug:**

**Code Name:** 5-ALA HCl, 5-ALA, ALA  
**Generic Name:** 5-aminolevulinic acid HCl  
**Trade Name:** Levulan® (aminolevulinic acid HCl) Kerastick™ for topical solution 20%  
**Chemical Name:** 5-amino-4-oxopentanoic acid  
**CAS Registry Number:** not included in this submission  
**Molecular Formula/ Molecular Weight/Structure:**  
(5-aminolevulinate)  
C<sub>5</sub>H<sub>9</sub>NO<sub>3</sub>, MW = 131.13



**Relevant INDs/NDAs/DMFs:**

in HFD-150:

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

[redacted]

in HFD-540:

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

for topical photodynamic therapy of actinic keratosis

[redacted]

in HFD-580:

IND [redacted]  
IND [redacted]

Drug Class: photodynamic therapy

Indication: for the treatment of [redacted] actinic keratoses of the face and scalp

Clinical formulation: constituted Levulan® (5-ALA HCl)

Ingredient	"theoretical quantity" mg/ml
Levulan (5-ALA HCl)	[redacted]
Alcohol, USP	
Purified water, USP	
Laureth-4	
Isopropyl alcohol, USP	
Polyethylene glycol	
[redacted]	
Total	

Route of administration: An ampoule containing 5-ALA HCl in powder form is broken to allow admixture with the vehicle to form a 20% solution. The solution is immediately applied topically to the lesion only. After [redacted] hours, the site is irradiated with the applicant's companion device, a blue light source with an emission peak of 417 nm and a bandwidth of [redacted] nm.

Introduction and drug history: The current submission is a resubmission of an approvable NDA. The only changes that impact pharmacology and toxicology are in the label. Those changes are addressed below.

**OVERALL SUMMARY AND EVALUATION:**

**Communication Review:**

**- Labeling Review (NDA):**

1. Under "Carcinogenesis, Mutagenesis, Impairment of Fertility," all references to "5-ALA" were changed to read [redacted] by the applicant. References to the drug substance, as opposed to the drug product, were changed to ALA HCl, as proposed by the chemistry reviewer. For descriptions of genotoxicity studies, which were conducted using a solution of 5-ALA in an acetate buffer, the wording should be changed back to read ALA.

2. The sentence, "PpIX formation was not demonstrated in the in vitro studies" should be moved to a position immediately after the descriptions of those studies and "the" should be changed to "these."

3. Other minor changes, such as more concise wording, case corrections, etc., are made below.

**RECOMMENDATIONS:**

The following sections should be revised as follows:

**Carcinogenesis, Mutagenesis, Impairment to Fertility:** No carcinogenicity testing has been carried out using [redacted] ALA. No evidence of mutagenic effects was seen in four studies conducted with ALA to evaluate this potential. In the *Salmonella-Escherichia coli* mammalian microsome reverse mutation assay (Ames mutagenicity assay), no increases in the number of revertants were observed with any of the tester strains. In the *Salmonella-Escherichia coli* mammalian microsome reverse mutation assay in the presence of solar light radiation (Ames mutagenicity assay with light), [redacted] ALA did not cause an increase in the number of revertants per plate of any of the tester strains in the presence [redacted] or [redacted] absence of [redacted] simulated solar light. In the L5178Y TK mouse lymphoma forward mutation assay, [redacted] ALA was evaluated as negative with and without metabolic activation under the study conditions. PpIX formation was not demonstrated in any of these *in vitro* studies. In the *in vivo* mouse micronucleus assay, [redacted] ALA was considered negative under the study exposure conditions. In contrast, at least one report in the literature has noted genotoxic effects in cultured rat hepatocytes after ALA exposure with PpIX formation. Other studies have documented oxidative DNA damage *in vivo* and *in vitro* as a result of ALA exposure.

No assessment of effects of [redacted] ALA HCl on fertility has been performed in laboratory animals. It is unknown what effects systemic exposure to [redacted] ALA HCl might have on fertility or reproductive function.

**Pregnancy Category C:** Animal reproduction studies have not been conducted with [redacted] ALA HCl. It is also not known whether LEVULAN KERASTICK Topical Solution can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. LEVULAN KERASTICK Topical Solution should be given to a pregnant woman only if clearly needed.

**Nursing Mothers:** The levels of ALA or its metabolites in the milk of subjects treated with LEVULAN KERASTICK Topical Solution have not been measured [redacted]. Because many drugs are excreted in human milk, caution should be exercised when LEVULAN KERASTICK Topical Solution is administered to a nursing woman.

APPEARS THIS WAY  
ON ORIGINAL

[redacted] TS/ 11/19/99  
Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist/Toxicologist

cc:

NDA 20-965

HFD-340

HFD-540

HFD-540/PHARM/Nostrandt

HFD-540/TLPHARM/Jacobs

HFD-540/MO/Okun

HFD-540/CHEM/Hathaway

HFD-540/PMS/Cintron

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Draft date (# of drafts): 11/19/99 (1)

Concurrence Only:

HFD-540/DD/WILKIN

**S** 11/21/99  
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HFD-540/TLPHARM/JACOBS

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ON ORIGINAL

Evaluation of Pharmacology and Toxicology Data  
Division of Dermatologic and Dental Drug Products, HFD-540

MAR - 8 1999

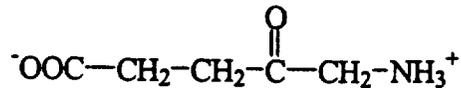
NDA No.: 20-965

Date Submitted: 6/29/98  
Date CDER Received: 7/1/98  
Date Assigned: 7/13/98  
Date Review Completed: 3/4/99

Name of Drug: Levulan (aminolevulinic acid HCl) Kerastick for topical solution 20%

Structure: (5-aminolevulinate)

C<sub>5</sub>H<sub>7</sub>NO<sub>3</sub>  
MW 131.13



Pharmacological Category: photodynamic therapy (PDT)

Sponsor: Guidelines, Inc. for DUSA Pharmaceuticals, Inc.  
10320 USA Today Way 400 Columbus Avenue  
Miramar, FL 33025 Valhalla, NY 10595  
Attn: Samuel D. Swetland 914-747-4300  
954-433-7480

Indication: for the treatment of  actinic keratoses of the face and scalp

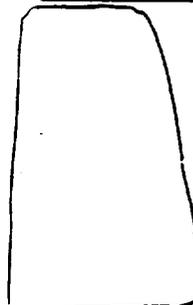
Administration: An ampoule containing 5-ALA in powder form is broken to allow admixture of the vehicle to form a 20% solution. The solution is immediately applied topically to the lesion only. After  hours, the site is irradiated with the applicant's companion device, Blu-U, a blue light source with an emission peak of 417 nm and a bandwidth of

Formulation: constituted Levulan (5-ALA HCl)

Ingredient  
Levulan (5-ALA HCl)  
Alcohol, USP  
Purified water, USP  
Laureth-4  
Isopropyl alcohol, USP  
Polyethylene glycol

"theoretical quantity" mg/ml

Total



## Related IND's/NDA's:

in HFD-150:

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted] DUSA

[redacted]

in HFD-540:

IND [redacted] DUSA

for topical photodynamic therapy of actinic  
keratosis

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

IND [redacted]

in HFD-580:

IND [redacted]

IND [redacted]

**Index of Nonclinical Studies:**

## Nonclinical Toxicology studies

## Acute toxicology studies of 5-ALA:

1. Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride in mice ([redacted] 6703-101)
2. Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride in rats ([redacted] 6703-102)
3. Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride in dogs ([redacted] 6703-103)

## Acute toxicology studies of pyrazine 2,5-dipropionic acid:

4. Acute oral toxicity study of pyrazine 2,5-dipropionic acid in mice ([redacted] 70104863)
5. Acute oral toxicity study of pyrazine 2,5-dipropionic acid in rats ([redacted] 70104862)
6. Acute intraperitoneal toxicity study of pyrazine 2,5-dipropionic acid in mice ([redacted] 70104865)
7. Acute intraperitoneal toxicity study of pyrazine 2,5-dipropionic acid in rats ([redacted] 70104864)

## Genotoxicity studies:

8. Salmonella - Escherichia coli/mammalian-microsome revers. mutation assay with a confirmatory assay ([redacted] 18554-0-409R)

9. Salmonella – Escherichia coli reverse mutation assay in the presence of solar light radiation with a confirmatory assay ( ) 18554-1-409RSL)
  10. L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay ( ) 18554-0-431R)
  11. In vivo mouse micronucleus assay ( ) 18554-0-4550ECD)
- Special toxicity studies:
12. Acute subcutaneous toxicity study of 5-aminolevulinic acid (ALA) in rats ( ) 21202228)
  13. Acute dermal toxicity study of 5-aminolevulinic acid (ALA) formulations in rabbits (solution) ( ) 6703-100)
  14. Acute dermal toxicity study of 5-aminolevulinic acid (ALA) formulations in rabbits (cream) ( ) 21202229)
  15. Intravesical stability and absorption of 5-aminolevulinic acid in beagle dogs ( ) 6703-105)
  16. Acute intravesical toxicity study with 5-aminolevulinic acid hydrochloride in dogs ( ) 6703-104)
  17. Assessment of an intravesical dose administration procedure in female dogs ( ) 6703-107)
  18. Acute intravesical toxicity and toxicokinetic study with 5-aminolevulinic acid hydrochloride in dogs ( ) 6703-106)

#### Pharmacokinetic studies

1. Pharmacology and pharmacokinetics of 5-aminolevulinic acid in beagle dogs after oral and intravenous administration.

#### Nonclinical Pharmacology studies

### INTRODUCTION

Aminolevulinic acid (5-ALA,  $\delta$ -ALA, or ALA) is an endogenous precursor in the heme synthetic pathway. It is enzymatically converted to protoporphyrin IX (PpIX), which is photoactive. The absorption peaks for PpIX are at 405 (largest), 505, 540, 575, and 630 nm. Administration of exogenous ALA bypasses the feedback inhibition of ALA synthesis by the presence of excess heme. According to the sponsor, ALA and PpIX are selectively accumulated in epithelial tissues. It has been reported in the literature that PpIX accumulates in some tumor tissues that lack the enzyme ferrochelatase, which catalyzes the formation of heme from PpIX.

The applicant's product consists of 5-ALA in powder form in an ampoule, which is broken to allow admixture of the vehicle to form a 20% solution. Packaging includes an applicator tip, which is used to apply material to the lesion only. After ( ) hours, the site is irradiated with the applicant's companion device, Blu-U, a blue light source with an emission peak of 417 nm and a bandwidth of ( ) nm.

A number of references from the scientific literature were supplied by the applicant to supplement their nonclinical information. These include review articles related to the clinical presentation and pathogenesis of porphyrias in human patients. Additionally there are research articles describing studies in animals to elucidate the mechanisms by which the classical signs of porphyria are developed.

A number of the articles dealt with experimental models of neurotoxicity associated with porphyria. In a number of in vitro preparations, ALA was shown to impair neuromuscular signal transmission, to inhibit neurotransmitter release at the motor endplate, and to affect membrane ion conductance. ALA inhibition of Na-K-ATPase (with no effect on Mg-ATPase until high concentrations are reached) has been demonstrated and shown to be reversible. Effects on neuronal membrane electrical resistance appear to be similar to those produced by GABA. In fact, competition by ALA has been demonstrated for GABA receptors in CNS at ALA concentrations that are consistent with those thought to exist in the CNS of porphyric patients. Several researchers speculate that ALA may be a GABA receptor agonist. Low concentrations of ALA displaced GABA at specific GABA binding sites; it was concluded that ALA may be specific for presynaptic GABA receptors. High ALA concentrations have been shown to stimulate GABA release and inhibit GABA uptake. Some studies have shown similar effects on glutamic acid. The effects demonstrated in vitro appear to be predominantly prejunctional and occur at concentrations that might be reasonably expected in patients with porphyria.

In vitro studies in liver tissue demonstrated inhibition of liver tryptophan pyrrolase activity by ALA, which was thought to possibly lead to alterations in tryptophan and serotonin levels. Other studies of effects on transmembrane and mitochondrial membrane potentials in liver showed calcium-dependent oxidative damage to the mitochondria at 50-100  $\mu$ M ALA. Those concentrations were estimated to occur in the livers of porphyric patients. Lipid peroxidation demonstrated in vitro resulted in increased permeability in liposomes and may represent the potential for altered membrane permeability in vivo.

In vivo, ALA has been found in the cerebrospinal fluid of porphyria patients and can cross the blood-brain barrier. In a study of motor activity in mice injected ip with 0.76 mmol/kg ALA, marked depression of spontaneous activity relative to controls was seen in the first five minutes after injection. By 30 minutes after injection, activity was significantly increased relative to controls and persisted to at least 90 minutes after injection. In rats treated with 40 mg/kg ALA every other day for 2 weeks, there were indicators of oxidative stress in brain.

ALA in alkaline solution condenses to form pyrazine 2,5- dipropionic acid (PDPA). One article provided by the applicant suggests that alternative forms and autocondensation products may serve as the active toxin in porphyrias. Another study determined optimal conditions to minimize condensation of ALA for preparation of a product for clinical use. Those optimal conditions were: 0.18 M (3%) ALA, pH 5, 4°C. The authors speculated that it also might be important to control urine pH in patients treated with ALA.

ALA clearance is by glomerular filtration with limited tubular reabsorption. One reference stated that, in overwhelming neuropathy cases, the maximum plasma concentrations of ALA are 9-12  $\mu$ mol/L.

According to the applicant, the clinical trials submitted to this NDA demonstrate 80-90% complete response after a single use and 90-100% complete response rate after the second use. The human dose is estimated to be 7-15 mg Levulan per patient, or approximately 2-4% of the estimated 358 mg ALA synthesized daily by the human body.

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**NONCLINICAL TOXICOLOGY STUDIES**

Acute toxicology studies of 5-ALA:

1. Study title: Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride  
in mice

Study number: [redacted] 6703-101

Performing organization: [redacted]

Drug lot and batch: lot # L2260-401-007

Date of study: 9/4/96 – 9/24/96

GLP compliance: yes

**Study design:**

Dosing: single intravenous bolus into lateral tail vein on day 1

Dose groups: 10 ml/kg of vehicle, 30, 100, or 300 mg/kg ALA (90, 300, 900 mg/m<sup>2</sup>, or human equivalent doses of 2.5, 8, or 25 mg/kg)

Formulation: 5-ALA in 0.2 M sodium acetate buffer; final pH was approximately 5.

Test animals: CrI:CD-1<sup>®</sup>(ICR)BR VAF/Plus<sup>®</sup> mice, 10 /sex/group, 6 weeks old at the initiation of treatment, 20-29 g. Animals were observed for clinical signs at 1, 2, and 4 hours post-dose on the day of treatment and at least once daily thereafter. The mice were kept under low lighting for the first two days after treatment. Body weight and feed consumption were recorded weekly. Blood samples were collected on day 16 prior to sacrifice; samples from 5/sex /group were used for hematological evaluation, and samples from 5/sex/group were used for abbreviated clinical chemistry evaluation (glucose, BUN, total protein, albumin, globulin, ALT).

**Findings:**

Deaths: none

Clinical signs: No signs were observed that were related to treatment. No significant effect was noted in body weight, body weight gains, or food consumption.

Clinical chemistry and hematology: No effects were seen on hematological or examined serum chemistry parameters.

Pathological examination: All animals were examined macroscopically, and macroscopic lesions identified at necropsy were examined microscopically. No effect of the test material was noted.

The NOEL was determined to be 300 mg/kg (900 mg/m<sup>2</sup>, HED = 25 mg/kg).

2. Study title: Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride  
in rats

Study number: [redacted] 6703-102

Performing organization: [redacted]

Drug lot and batch: lot # L2260-401-007

Date of study: 9/9/96 – 9/25/96

GLP compliance: yes

**Study design:**

Dosing: single intravenous bolus of 10 ml/kg into lateral tail vein on day 1

Dose groups: vehicle, 30, 100, or 300 mg/kg (0, 180, 600, 1800 mg/m<sup>2</sup>, or human equivalent doses of 0, 5, 16, or 50 mg/kg)

Formulation: 5-ALA in 0.2 M sodium acetate buffer; final pH was approximately 5.

**Test animals:** Cri:CD<sup>®</sup>(SD)BR VAF/Plus<sup>®</sup> rats, 6 weeks of age (150-209 g) at the beginning of treatment, 5 /sex/group. The rats were observed for clinical signs at 1, 2, and 4 hours post-dose on the day of treatment and at least once daily thereafter. Animals were kept under low lighting for the first 2 days after treatment. Body weight and feed consumption were recorded weekly. Blood and urine samples were collected on days 3 and 17. Animals were sacrificed on day 17, followed by gross necropsy and histopathological examination of macroscopic lesions.

**Findings:**

**Deaths:** One male at 100 mg/kg died after blood sample collection on day 3. There was no evidence that this was related to treatment.

**Clinical signs:** No clinical signs were noted; no test material-related differences were noted in body weight, body weight gains, or food consumption.

**Clinical chemistry and hematology:** No test material-related differences were noted in results of hematology, serum chemistry, or urinalysis.

**Pathological examination:** No test material related differences were noted.

The NOEL was determined to be 300 mg/kg (1800 mg/m<sup>2</sup>, HED=50 mg/kg).

3. Study title: Acute intravenous toxicity study with 5-aminolevulinic acid hydrochloride in dogs

Study number: [redacted] 6703-103

Performing organization: [redacted]

Drug lot and batch: lot # L2260-401-007

Date of study: 8/28/96 – 9/16/96

GLP compliance: yes

**Study design:**

**Dosing:** single intravenous bolus of 5 ml/kg into a cephalic vein on day 1

**Dose groups:** vehicle, 10, 30, or 100 mg/kg (200, 600, 2000 mg/m<sup>2</sup>, or human equivalent doses of 5, 15, or 50 mg/kg)

**Formulation:** 5-ALA in 0.2 M sodium acetate buffer; final pH was approximately 5.

**Test animals:** beagles, 3/sex/group, observed for clinical signs at 1 hour post-dose on the day of treatment and at least once daily thereafter. The animals were kept under low lighting for the first 2 days after treatment. Body weights and feed consumption were recorded weekly. Blood and urine samples collected twice before dosing and on days 3 and 19. During week 3, the animals were sacrificed and necropsied. Livers and tissues with macroscopic lesions were examined histologically.

**Findings:**

**Deaths:** none

**Clinical signs:** Excessive salivation was noted during dosing of 16/18 ALA-treated animals and 1/6 vehicle-treated animals. One male at 10 mg/kg and one female at 30 mg/kg vomited within 1 hour of dosing. 14/18 ALA-treated animals were noted to have vomited within first 24 hours, including all high dose animals. No treatment-related effects were seen on body weight or food consumption.

**Clinical chemistry and hematology:** Significant increases were seen in mean AST and mean ALT on day 3 in females at 100 mg/kg (Individual values for the animals in this group were within normal limits with the exception of 1/3 animals for AST and with the exception of 2/3

animals for ALT). Additionally, one male at 30 mg/kg and one male at 100 mg/kg had increases in serum ALT. By day 19, AST values in treated animals were essentially recovered; mean values for mid and high dose females were statistically significantly different from controls, but the differences were not biologically significant. Mean ALT values were not significantly different from controls at day 19, but the individual value for one high dose female was slightly greater than normal.

Pathological examination: No treatment-related changes were seen either on gross examination or on microscopic examination of the liver and gross lesions.

The NOEL was not determined, but findings were limited to salivation and emesis at 10 mg/kg (200 mg/m<sup>2</sup>, HED 5 mg/kg).

Included in the submission were a number of journal articles, including one authored by Kennedy et al. (Food Cosmet. Toxicol. 14:45-47, 1976) in which the subacute toxicity of up to 100 mg/kg (300 mg/m<sup>2</sup>, HED=8 mg/kg) 5-ALA injected ip three times per week for 13 weeks was assessed. No toxic effects were noted. Fertility was reported to be unaffected, and there was no evidence of embryotoxicity or reduced numbers of fetuses. Urinary excretion of ALA was evaluated, and a dose-related increase in that parameter was noted.

Acute toxicology studies of pyrazine 2,5-dipropionic acid:

*Reviewer's comment: None of the four studies conducted included an untreated or vehicle control group.*

4. **Study title:** Acute oral toxicity study of pyrazine 2,5-dipropionic acid in mice

**Study number:** [redacted] 70104863

**Performing organization:** [redacted]

**Drug lot and batch:** lot # RD 85/229

**Date of study:** 4/18/97 - 6/3/97

**GLP compliance:** yes

**Study design:**

Dosing: single dose by gavage of 10 or 15 ml/kg

Dose groups: range-finding phase - 500, 1000, 3000, 5000 mg/kg; definitive study - 5000 mg/kg (15,000 mg/m<sup>2</sup>, HED= 417 mg/kg)

Formulation: pyrazine 2,5-dipropionic acid in 0.5% carboxymethylcellulose (w/v) in distilled water

Test animals: Crl:CD<sup>®</sup>-1(ICR)BR mice, 4-6 weeks of age, 22-29 g; 1/sex/group for the range-finding phase and 5/sex for the definitive study. Animals were observed at 1, 2.5 and 4 hours after test article administration and once daily thereafter. Body weights were determined weekly. Animals in the definitive study were sacrificed and subjected to an abbreviated gross necropsy on day 14.

**Findings:**

Deaths: none

Clinical signs: In the definitive study, soft stools were noted in 4/5 males and 2/5 females on the day of treatment. Another female exhibited hypoactivity and staggered gait on the day of treatment. Body weight gain was seen throughout the study.

Pathological examination: No lesions were noted on gross examination.

The LD<sub>50</sub> was determined to be >5000 mg/kg.

5. Study title: Acute oral toxicity study of pyrazine 2,5-dipropionic acid in rats

Study number: 70104862

Performing organization: [REDACTED]

Drug lot and batch: lot # RD 85/229

Date of study: 4/18/97 - 6/3/97

GLP compliance: yes

Study design:

Dosing: single dose by gavage of 10 or 15 ml/kg

Dose groups: range-finding phase - 500, 1000, 3000, 5000 mg/kg; definitive study - 5000 mg/kg (30,000 mg/m<sup>2</sup>, HED= 833 mg/kg)

Formulation: pyrazine 2,5-dipropionic acid in 0.5% carboxymethylcellulose (w/v) in distilled water

Test animals: Crl:CD<sup>®</sup>(SD)BR rats, 9-13 weeks of age, 220-300 g; 1/sex/group for the range-finding phase and 5/sex for the definitive study. Animals were observed at 1, 2.5 and 4 hours after test article administration and once daily thereafter. Body weights were determined weekly. Animals in the definitive study were sacrificed and subjected to an abbreviated gross necropsy on day 14.

Findings:

Deaths: none

Clinical signs: Red-stained face, soft stool, and dark- or yellow-stained urogenital areas were noted, but all animals were normal in appearance by day 3. Body weight gain was seen in all treated animals throughout the study.

Pathological examination: No lesions were noted on gross examination.

The LD<sub>50</sub> was determined to be >5000 mg/kg.

6. Study title: Acute intraperitoneal toxicity study of pyrazine 2,5-dipropionic acid in mice

Study number: 70104865

Performing organization: [REDACTED]

Drug lot and batch: lot # RD 85/229

Date of study: 4/18/97 - 6/3/97

GLP compliance: yes

Study design:

Dosing: single intraperitoneal injection of 10 ml/kg

Dose groups: range-finding phase - 100, 250, 500, 1000 mg/kg; definitive study - 1000 mg/kg

Formulation: pyrazine 2,5-dipropionic acid in 0.5% carboxymethylcellulose (w/v) in distilled water

Test animals: Crl:CD<sup>®</sup>-1(ICR)BR mice, 4-6 weeks of age, 21-29 g; 1/sex/group for the range-finding phase and 5/sex for the definitive study. Animals were observed at 0.5, 1 and 4 hours after test article administration and once daily thereafter. Body weights were determined weekly. Animals in the definitive study were sacrificed and subjected to an abbreviated gross necropsy on day 14. Gross lesions were collected for possible microscopic examination.

**Findings:**

**Deaths:** none

**Clinical signs:** Hypoactivity was noted in 4/5 males and 5/5 females on the day of treatment. Body weight gains were seen throughout the study.

**Pathological examination:** One gross lesion was seen in one female: the right median and lateral lobes of the liver were adhered with multiple gray fibrous adhesions that were thought to be due to inflammatory reaction to the test material in the peritoneal cavity.

The LD<sub>50</sub> was determined to be >1000 mg/kg.

7. **Study title:** Acute intraperitoneal toxicity study of pyrazine 2,5-dipropionic acid in rats

**Study number:** [redacted] 70104864

**Performing organization:** [redacted]

**Drug lot and batch:** lot # RD 85/229

**Date of study:** 4/18/97 – 6/3/97

**GLP compliance:** yes

**Study design:**

**Dosing:** single intraperitoneal injection of 10 ml/kg

**Dose groups:** range-finding phase – 100, 250, 500, 1000 mg/kg; definitive study – 1000 mg/kg

**Formulation:** pyrazine 2,5-dipropionic acid in 0.5% carboxymethylcellulose (w/v) in distilled water

**Test animals:** Crl:CD®(SD)BR rats, 9-13 weeks of age, 230-300 g; 1/sex/group for the range-finding phase and 5/sex for the definitive study. Animals were observed at 0.5, 1 and 4 hours after test article administration and once daily thereafter. Body weights were determined weekly. Animals in the definitive study were sacrificed and subjected to an abbreviated gross necropsy on day 14. Gross lesions were collected for possible microscopic examination.

**Findings:**

**Deaths:** none

**Clinical signs:** On the day of treatment, signs were observed in 2/5 males and 4/5 females consisting of hypoactivity, staggered gait, red-stained face, soft stool, and wet urogenital area. All animals exhibited body weight gain during the study.

**Pathological examination:** On gross examination, lesions were seen in 2/5 males and 5/5 females, consisting of multiple gray fibrous adhesions between the spleen, stomach serosa, diaphragm, or liver, and between some lobes of the liver. The splenic capsules were described as opaque and some liver lobes were found to be irregularly shaped, possibly because of adhesions to the capsular surface. All findings were thought to be due to inflammatory reaction to the material injected into the peritoneal cavity.

The LD<sub>50</sub> was determined to be >1000 mg/kg.

**Genotoxicity studies:**

8. **Study title:** Salmonella – Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay

**Study number:** [redacted] 18554-0-409R

**Performing organization:** [redacted]

**Drug lot and batch:** Batch # 335  
**Date of study:** 5/21/97 – 6/19/97  
**GLP compliance:** yes

**Study design:**

**Dose groups:** A dose range-finding study was performed using tester strains TA100 and WP2uvrA and 10 doses ranging from [redacted] µg/plate, one plate/dose in the presence and absence of S9. For the initial mutagenicity assay, doses of 33.3, 100, 333, 1000, 3330, and 5000 µg/plate, 3 plates/dose in the presence and absence of S9 were used. For the confirmatory assay, doses of 33.5, 100, 335, 1000, 3350, and 5020 µg/plate in the presence and absence of S9 were used. Each assay included appropriate vehicle and positive controls. The positive control in presence of S9 was 2-aminoanthracene, and the positive control in absence of S9 was 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), ICR-191 (TA1537), or 4-nitroquinoline-N-oxide (WP2uvrA).

**Formulation:** 5-ALA in 0.2 M acetate buffer

**Test strains:** S. typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2uvrA

**Findings:**

Under the conditions of the assay, 5-ALA did not cause a positive increase in the number of revertants per plate in any strain in the presence or absence of S9.

9. **Study title:** Salmonella – Escherichia coli reverse mutation assay in the presence of solar light radiation with a confirmatory assay

**Study number:** [redacted] 18554-1-409RSL

**Performing organization:** [redacted]

**Drug lot and batch:** Batch # 335

**Date of study:** 7/17/99 – 8/5/99

**GLP compliance:** yes

**Study design:**

**Dose groups:** Doses were 3.33, 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg/plate for the initial mutagenicity assay (*Reviewer's comment: Analysis of solutions used for dosing in the initial assay resulted in no detectable 5-ALA in samples of the three lowest concentrations.*) and 100, 333, 1000, 3330, and 5000 µg/plate for the confirmatory assay. Doses of test article and vehicle controls were administered to cultures in the absence of light and in the presence of 2 solar light doses (the first was that which was demonstrated to result in a 2-fold or greater increase in the number of revertants/plate, the second dose was half of the first; 8 and 4 seconds for TA1537, 16 and 8 seconds for TA102 and WP2(pKM101)) and absence of directed solar light radiation. The vehicle for 5-ALA was 0.2 M acetate buffer, pH 5.0, and the positive control was 8-methoxypsoralen in DMSO. Additional positive controls included in non-light exposed groups only were mitomycin C (TA102), ICR-191 (TA1537), and 4-nitroquinoline-N-oxide (WP2(pKM101)).

**Formulation:** 5-ALA in 0.2 M acetate buffer

**Test strains:** S. typhimurium TA102, TA1537; E. coli WP2(pKM101)

*Reviewer's comment: The study report describes in detail the mutations present in these bacterial strains and their sensitivity to various mutagens, but does not describe why these particular strains were more appropriate for this assay than the five strains normally*

recommended for the standard Ames assay. In fact, lack of positive response of TA1537 when exposed to 8-methoxypsoralen and solar radiation when compared to vehicle control-treated TA1537 exposed to solar radiation (This strain is unusually sensitive to UV light) may indicate that this strain is not appropriate for a photogenotoxicity assay. Also, it is not stated whether or not any of these bacteria possess metabolic capability for PpIX production.

**Findings:**

Under the conditions of the assay, there was no demonstration of mutagenic photoproducts of 5-ALA.

*Reviewer's comment: The assay was done only in the absence of S9. Some sort of metabolic activation would have been required to assess the photo-genotoxicity of the photoactive moiety, PpIX. However, at sufficiently high doses, photoactivation of PpIX should result in cytotoxicity. Perhaps a lower dose of 5-ALA and a lower resultant dose of the metabolite, PpIX, in the presence of an appropriate metabolic activating system or low doses of PpIX only would have been non-cytotoxic and more informative.*

10. Study title: L5178Y TK +/- mouse lymphoma forward mutation assay with a confirmatory assay

Study number: [redacted] 18554-0-431R

Performing organization: [redacted]

Drug lot and batch: batch # 335

Date of study: 5/20/97 - 7/9/97

GLP compliance: yes

**Study design:**

Dose groups: The preliminary dose range-finding experiment included 10 treatments ranging from [redacted] µg/ml, and vehicle and untreated controls. In the definitive study, two trials were performed at doses up to 5000 µg/ml. In the both trials, doses were 78.5, 157, 313, 625, 1250, 2500, and 5000 µg/ml. Only the six highest concentrations were evaluated. Cells were incubated for 4 hours in the presence and absence of S9 followed by 2-day expression period. Vehicle and positive controls (methyl methanesulfonate without S9, methylcholanthrene with S9) were included.

Formulation: 5-ALA in 0.2 M sodium acetate buffer, pH 5.0

Test culture: L5178Y TK +/- mouse lymphoma cells

**Findings:**

No evidence of cytotoxicity was seen in the preliminary assay. None of the six evaluated treatments in either trial with or without S9 induced a positive mutagenic response.

11. Study title: In vivo mouse micronucleus assay

Study number: [redacted] 18554-0-4550ECD

Performing organization: [redacted]

Drug lot and batch: batch # 335

Date of study: 5/8-20/97

GLP compliance: yes

**Study design:**

**Dose groups:** For the dose selection study, a single iv injection of 200, 650, 1100, 1550, or 2000 mg/kg (10 ml/kg) was administered followed by observation for 2 days. In the micronucleus assay, doses were 400, 800, and 1600 mg/kg, plus vehicle and positive controls (cyclophosphamide in sterile deionized water). Animals were kept in a darkened room for the duration of the study.

**Formulation:** 5-ALA in 0.2 M sodium acetate buffer, pH 5.0

**Test animals:** Crl:CD-1@ICR) BR mice, approximately 8 weeks of age, 28-35 g. Three males/dose group were used for the dose selection study and were observed for signs of toxicity or mortality. Six males/dose level/harvest timepoint were used for the definitive study. Animals in the two low dose and positive control groups were euthanized at 24 hours after dosing and their bone marrow was harvested. High dose and vehicle control animals were euthanized at 24 and 48 hours for extraction of bone marrow.

**Findings:**

At 0.6 hours after dosing in the range-finding study, some animals at doses of 650 and above were found either gasping, prostrate, or dead. All high dose animals died. Other deaths were attributed to the rate of infusion and were not repeated in replacement animals when the rate of infusion was slowed. Surviving animals all appeared normal at 1.4 hours. The MTD was estimated to be 1600 mg/kg.

In the definitive study, signs of toxicity consisted of gasping and prostration in one male at the high dose at 0.9 hours, and deaths due to rapid infusion in one animal at 800 mg/kg and five animals at 1600 mg/kg. All animals appeared normal at later timepoints with the exception of one high dose animal which exhibited prostration and labored breathing at 46.9 hours. There was no apparent cytotoxicity to bone marrow based on the PCE:NCE ratio and no significant increase in micronuclei in bone marrow polychromatic erythrocytes in test article-treated animals.

In addition to the genotoxicity studies performed by the sponsor, several journal articles were included in the submission that dealt with genotoxicity and carcinogenic potential of 5-ALA. Fiedler et al. (J. Photochem. Photobiol. B: Biol. 33:39-44, 1996) examined the potential of 5-ALA in the absence of light to produce genotoxicity in cultured rat hepatocytes. They reported a significantly increased frequency of chromosomal aberrations and micronuclei at concentrations of 1 µg/ml or greater, with dose-related increases seen in those parameters at concentrations up to 100 µg/ml. At 1000 µg/ml, the numbers of micronuclei decreased, but the numbers of chromosomal aberrations remained the same as at 100 µg/ml. The authors also examined the kinetics of PpIX formation and found that, after induction with 100 or 1000 µg/ml, PpIX concentrations were the same, which would suggest that the chromosomal aberrations seen may have been due to PpIX. Arnold et al. in Food Cosmet. Toxicol. 13:63-68, 1975 found no genetic damage in the dominant lethal mutation test in mice.

Onuki et al. (Biochimica et Biophysica Acta 1225:259-263, 1994) demonstrated that 0.01-3 mM ALA in the presence of 10 µM Fe<sup>2+</sup> caused DNA single strand breaks in plasmid pBR322 in *E. coli*. The addition of superoxide dismutase and catalase, or a metal chelator inhibited the damage. Oxidative DNA damage was noted by Fraga et al. (Carcinogenesis 15:2241-2244, 1994) in the livers of rats treated with 40 mg ALA/kg every other day for 15 days

and in vitro in calf thymus incubated with 0.05-5 mM ALA in the presence of 10  $\mu\text{M}$   $\text{Fe}^{2+}$ . The authors of both studies speculate that these data may be of importance in the explanation of the previously reported correlation between primary liver-cell carcinoma and intermittent acute porphyria.

Ben-Sasson and Davis (Cancer Causes and Control 3:383-387, 1992) hypothesized that unexplained increases in the incidence of acute lymphocytic leukemia in children in the previous two decades may be due in part to exposure to porphyrin-activating light (400 nm) immediately after birth, which may result in superoxide and free radical generation that might in turn induce breaks in DNA. They cite 5- to 10-fold increases in newborn nursery lighting during the same time period. In their model, exposure of infants with transient protoporphyria to protoporphyrin-activating illumination can induce malignant transformation of protoporphyrin-loaded lymphoblasts migrating from the liver to the bone marrow and thymus through the peripheral circulation. In another article (J. Photochem. Photobiol. B: Biol. 20:5-22, 1993), del C. Batlle proposed a model in which an abnormality of heme synthesis, such as that seen in porphyric patients, is involved in the initiating lesion of carcinogenesis through the production of reactive oxygen species. This model suggests that these patients may be at greater risk of developing cancer. Both articles concluded that further work was needed to identify a definitive effect of ALA or porphyrins in the initiation or promotion of cancer.

Special toxicity studies:

12. **Study title:** Acute subcutaneous toxicity study of 5-aminolevulinic acid (ALA) in rats

**Study number:** 21202228

**Performing organization:** [REDACTED]

**Drug lot and batch:** lot # L2260-221-003

**Date of study:** 1/8/93 - 7/26/93

**GLP compliance:** yes

**Study design:**

**Dosing:** single subcutaneous dose, 5 ml/kg, divided so as to inject no more than 0.5 ml per site.

**Dose groups:** for range-finding, 100, 250, 500, 1000 and 2500 mg/kg; for the definitive study, 0, 100, 500, and 1000 mg/kg (0, 600, 3000, and 6000 mg/m<sup>2</sup>; HED=0, 17, 83, 170 mg/kg).

**Formulation:** 5-ALA in sterile water for injection

**Test animals:** CrI:CD<sup>®</sup>BR rats, 233-321 g, 1/sex/dose level for range finding, 5/sex/group for the definitive study. Animals were housed under low lighting for two days after injection and were observed for clinical signs at 0.5, 1, and 4 hours on the day of treatment and daily thereafter. Body weights were determined weekly. On day 14, animals were sacrificed and abbreviated gross necropsies were performed.

**Findings:**

**Deaths:** no spontaneous deaths

**Clinical signs:** In the range-finding experiment, both animals at 2500 mg/kg were euthanized on day 7 due to necrosis at the site of injection. In the definitive study, ALA-treated animals exhibited irritation and or lesions (described as hardening, scab formation, small lesion, hair loss brown-staining, small mass, enlarged fluid filled mass, necrosis, eschar, or exfoliation).

Earlier onset and increased severity was seen with increased dose concentration, and lesions were presumed to be due to the ionic strength and low pH of the solution. No significant differences were seen in mean body weights or body weight gain between control and treated groups.

**Pathological examination:** Gross findings were limited to lesions at the injection site. The dorsal cervical region of animals in the 500 and 1000 mg/kg groups had crusted or abraded areas, and the underlying subcutaneous tissue in some had red or dark red areas or appeared gelatinous. Similar lesions were seen in 6/10 animals at 100 mg/kg.

13. **Study title:** Acute dermal toxicity study of 5-aminolevulinic acid (ALA) formulations in rabbits (solution)

**Study number:** [redacted] 6703-100

**Performing organization:** [redacted]

**Drug lot and batch:** solution vehicle lot # 265, 5-ALA lot # 270

**Date of study:** 4/4/96 - 4/18/96

**GLP compliance:** yes

**Study design:**

**Dosing:** single topical dose to a shaved and abraded area on the back (approximately 180 cm<sup>2</sup>, at least 20% bsa); 0.3 g/cm<sup>2</sup>

**Dose groups:** vehicle, 10%, 20%, and 30% ALA (w/v) solutions; single dose of 2000 mg/kg bw

**Formulation:** Levulan topical solution

**Test animals:** Hra:(NZW)SPF rabbits, approximately 15 weeks of age, 2232-2489 g body weight, 5/sex/group. Areas of application were covered with occlusive dressing for 24 hours during which the rabbits wore Elizabethan collars. Then, the site was washed with water and paper towels. Clean dressings were applied to each site for an additional 24 hours. Animals were housed under low lighting for the first two days after removal of residual material. Clinical observations were made at 1, 2.5, and 4 hours on the day of treatment and twice daily for next 13 days. Body weights were determined weekly. Dermal observations were made under low lights 30 minutes after initial bandage removal; subsequent readings were made on days 3, 7, 10, and 14. Animals were sacrificed and necropsied on day 14. Microscopic examination was limited to the treatment site.

**Findings:**

**Deaths:** none

**Clinical signs:** All animals appeared normal throughout the study. All gained weight with the exception of one female in the high concentration group which exhibited a slight, but not statistically significant body weight loss during the first week. Body weight in that animal recovered before the end of the second week.

Examination for dermal irritation revealed slight to moderate erythema in the vehicle and two lower concentration groups and moderate erythema in all high concentration group animals. Slight edema was noted in all groups and was dose-related in incidence. Slight desquamation was seen in the vehicle group, and slight to moderate desquamation was seen in all ALA-treated groups. Slight coriaceousness was recorded in the high dose group, and slight fissuring was recorded in low and high dose animals. Blanching was observed in one animal in each of the vehicle and high dose groups.

**Pathological examination:** Minimal to slight hyperkeratosis was present in treated skin of most animals, including controls. Evidence of dermal inflammation was seen in treated and

untreated areas of skin of animals from most groups, including controls, and was considered unrelated to treatment.

*Reviewer's comment: This is the only submitted study utilizing the proposed to-be-marketed formulation.*

14. Study title: Acute dermal toxicity study of 5-aminolevulinic acid (ALA) formulations in rabbits (cream)

Study number:  21202229

Performing organization:

Drug lot and batch: lot # L2260-211-003, placebo batch # 104

Date of study: 1/5/93 - 7/26/93

GLP compliance: yes

Study design:

Dosing: single dose to clipped and abraded sites on the back (approximately 10% total body surface area).

Dose groups: 30% 5-ALA (w/w) for pilot study at 2000 mg/kg bw; vehicle, 5%, 20% and 30% 5-ALA (w/w) at 2000 mg/kg bw (0.05-0.06 g/cm<sup>2</sup>) for the definitive study.

Formulation: 5-ALA in M55A, an experimental topical cream vehicle

Test animals: Hra:(NZW)SPF rabbits, 2373-2934 g, 1/sex for the initial pilot study; 5/sex/group for the definitive study. Animals were collared for 24 hours after test material application. Areas of application were covered with occlusive dressing for 24 hours, then the site was washed with water and paper towels. A clean dressing was applied to each site for an additional 24 hours to shield the treatment site from light. Clinical observations were made at 1, 2.5, and 4 hours on the day of treatment and twice daily for the next 14 days. Body weights were determined weekly. Dermal observations were made under low lights 30 minutes after initial bandage removal; subsequent readings were made on days 3, 7, 10, and 14. Animals were sacrificed and necropsied on day 14. Microscopic examination was limited to the skin at the treatment site and an untreated control site.

Findings:

Deaths: none

Clinical signs: Both pilot study animals appeared normal, but exhibited dermal irritation consisting of moderate erythema and edema and slight to moderate atonia at the treatment site. In the definitive study, all animals appeared normal. No effects were seen on body weights, with the exception of one male in the 20% group which lost 440 g during the second week. In the placebo and 5% groups, dermal irritation, consisting of slight erythema, slight atonia, slight to moderate desquamation and slight fissuring, was seen at the treatment site. In the 20% and 30% groups, slight to moderate erythema, edema, atonia, and desquamation, and slight fissuring were seen at treatment sites.

Pathological examination: On gross examination, one control male was noted to have a red focus in treated skin and one mid-dose male was seen to have thickened skin at the treatment site; neither was accompanied by corresponding microscopic lesions. One female in each of the mid- and high-dose groups had an oviduct cyst that was not considered related to treatment.

Microscopically, minimal hyperkeratosis was noted at the treatment site in control and treated animals and was considered to be secondary to abrasion and/or clipping.

15. Study title: Intravesical stability and absorption of 5-aminolevulinic acid in beagle dogs

Study number: [redacted] 6703-105 (This study number is a [redacted] number and is associated with the microscopic pathology portion of the study only.)

Performing organization: [redacted]

Drug lot and batch: batch # 270

Date of study: 7/9/96 - 8/9/96

GLP compliance: no

**Study design:**

Dosing: single intravesical dose, via urinary catheter, of 15 ml after complete emptying of the bladder; catheter flushed with 2.5 ml air.

Formulation: sterile solution of 3% 5-ALA (0.18 M) spiked with 0.02  $\mu\text{Ci/ml}$  inulin (2.27 mCi/g) using 0.2M potassium phosphate buffer, final pH 5.0.

Test animals: Nine male beagles, 9.8-11.2 kg, were used in three-way crossover design with two-week washout periods between treatments. All nine received an intravesical dose of ALA solution after each of a) no prior treatment, b) oral ammonium chloride for urinary acidification, and c) oral sodium bicarbonate for urinary alkalization. The test material was maintained in the bladder for 2 hours. Urine and blood samples were taken at specified intervals. [redacted]

[redacted] was performed for plasma ALA concentrations, urine samples were analyzed for 2,5 dipropionic acid pyrazine (PDPA), and serum chemistries were determined from serum samples. animals were sacrificed 18-24 hours after removal of the third treatment. Animals were subjected to a gross necropsy and histopathological examination of the urinary bladder.

(Reviewer's comment: Systemic availability of PDPA was not assessed.)

**Findings:**

Deaths: none

Clinical signs: none noted

Clinical chemistry and hematology: No biologically significant alterations in serum chemistries were seen.

Pharmacokinetics: (blood samples taken prior to ALA dose and at 30, 60, 90, 120, 125, 130, 140, 150, and 180 minutes after dosing; 0.2 ml urine samples taken pre-dose, 5, 15, 30, 60, 90, and 120 minutes after instillation, plus final bladder wash)

ALA was not appreciably absorbed into plasma from the urine. The plasma  $C_{\text{max}}$  of ALA was 0.135  $\mu\text{g/ml}$  at 53.5 min (0.165  $\mu\text{g/ml}$  at 70 minutes, 0.124  $\mu\text{g/ml}$  at 44 minutes, and 0.121 at 50 minutes, respectively for animals receiving no pre-treatment, ammonium chloride pre-treatment, and sodium bicarbonate pre-treatment). The  $\text{AUC}_{0-180\text{h}}$  values averaged 9.76  $\mu\text{g}\cdot\text{min/ml}$  (12.2, 9.2, and 8.4  $\mu\text{g}\cdot\text{min/ml}$ , respectively for animals receiving no pre-treatment, ammonium chloride pre-treatment, and sodium bicarbonate pre-treatment). Overall, less than 0.6% of the dose was absorbed into plasma.

The urinary  $\text{AUC}_{0-120\text{h}}$  for ALA averaged 1366  $\text{mg}\cdot\text{min/ml}$  overall (1656, 1390, and 1119  $\text{mg}\cdot\text{min/ml}$ , respectively for animals receiving no pre-treatment, ammonium chloride pre-treatment, and sodium bicarbonate pre-treatment). Urine ALA concentrations decreased to 36.6% (42%, 33%, and 36.5%, respectively for animals receiving no pre-treatment, ammonium chloride pre-treatment, and sodium bicarbonate pre-treatment) of the initial concentration. This

decrease correlated positively with the percent decreases in inulin concentration, indicating that dilution in urine accounted for an average of 84% of the decrease.

PDPA (LOQ=  mg/ml undiluted urine) was detected in samples of urine from all animals after pre-treatment with sodium bicarbonate and in some animals after no pretreatment. No PDPA was detected in animals with acidified urine. Urine pH in the sodium bicarbonate treated animals was 7.8. The urinary  $AUC_{0-120h}$  for ALA in sodium bicarbonate pre-treated animals was significantly less than in animals that had no pre-treatment. There was no significant difference for that value between animals that had no pre-treatment and ammonium chloride pre-treated animals. The report states that "these data suggest that degradation of ALA to pyrazine 2,5-dipropionic acid in urine of basic pH might have contributed significantly to the observed decreases in urine ALA concentrations." However, since the urinary  $AUC_{0-120h}$  for PDPA was less than 1% of that of ALA, the investigators concluded that this was not the case. Plasma PDPA concentrations were not evaluated, and the possibility of ALA degradation to PDPA followed by systemic absorption of that degradant was not considered.

**Pathological examination:** Focal urothelial damage was found in 8/9 animals, most often near the trigone area. It was considered minor and most likely a result of catheterization injury. No abnormalities were noted on the bladder surface. Histological findings included minimal to moderate hemorrhage and submucosal edema and minimal to slight acute inflammation in most bladders, usually in the middle and posterior regions, apparently related to physical trauma (catheter).

16. Study title: Acute intravesical toxicity study with 5-aminolevulinic acid hydrochloride in dogs

Study number:  6703-104

Performing organization:

Drug lot and batch: lot # L2260-401-007

Date of study: 9/24/96 - 10/9/96

GLP compliance: yes

Study design:

**Dosing:** single intravesical administration via Foley catheter; the material was allowed to remain in the bladder for approximately two hours.

**Dose groups:** vehicle, 300, 1000, or 3000 mg/animal in a volume of 10 ml (30, 100, 300 mg/ml)

**Formulation:** 5-ALA in 0.2 M potassium phosphate buffer, pH approximately 5.0

**Test animals:** beagles, 6-8 months of age, 9-16 kg, 6 /sex/group. An interim sacrifice on day 2 included 3/sex/group, and the remaining 3/sex/group were included in the terminal sacrifice on day 16. Clinical observations were made two hours post-dose and at least once daily for up to 16 days. Body weight and food consumption were measured weekly. The dose solutions were collected for recording of appearance, pH and volume at two hours after instillation. Samples of blood and urine were collected pre-treatment and on days 2, 3, and 16. Additional blood samples were taken on day 1 pre-dose and at 0.25, 0.5, 1, 2, 3, and 4 hours post-dose for toxicokinetics.

All animals were subjected to a gross necropsy and microscopic examination of the urethra, urinary bladder, and macroscopic lesions.

**Findings:**

**Deaths:** none

**Clinical signs:** Vomiting was observed at 1-4 hours after instillation in 2/6 females at 300 mg, 2/6 males and 6/6 females at 1000 mg, and 5/6 males and 6/6 females at 3000 mg. Repeated (3-7) incidences of vomiting were seen at the two higher doses during the first day. No treatment-related effects were seen on body weight or food consumption.

**Clinical chemistry and hematology:** Transient mild decreases in serum sodium and chloride were seen in females at the high dose. These changes were thought to be secondary to vomiting and had reversed by day 3. Other differences in clinical chemistry and hematology parameters were not biologically significant.

The expelled dose solution from the high dose group had a lower pH than that from controls. Increased urinary sodium excretion was noted in high dose females, but was highly variable and considered incidental.

**Pharmacokinetics:** The AUC values for ALA were variable. There was a trend toward increased AUC with increased dose and higher AUC values in females than in males. Higher AUC values were correlated with incidence of vomiting. Bioavailability was up to 11%.

**Pathological examination:** At the day 2 necropsy, increased incidence and severity of submucosal hemorrhage and edema, ulceration and suppurative inflammation of the bladder and submucosal hemorrhage in the urethra were noted in treated groups. Hemorrhage was evident in all groups, including controls, and was considered to be due to catheterization trauma. The increased incidence and severity of test material effects in treated groups was considered to be direct effects of test article on already traumatized tissue. At the day 16 necropsy, multifocal submucosal hemorrhage and edema were noted in the urinary bladder of one female at the high dose. Urethral inflammation was present in at least one male in each group and in one female in each of the control and mid-dose groups. All other animals had apparently recovered.

Based on the incidence of vomiting, the NOEL was considered to be <300 mg.

17. Study title: Assessment of an intravesical dose administration procedure in female dogs

Study number: 6703-107

Performing organization:

Drug lot and batch: not applicable

Date of study: 5/1-2/97

GLP compliance: no

Study design:

**Dosing:** single intravesical dose of 10 ml of 0.9% sodium chloride for injection.

**Test animals:** 5 female beagles. On day 1, sterile saline was instilled into the urinary bladder of four animals via a latex urethral catheter with a stainless steel wire stylet and allowed to remain in the bladder for approximately 2 hours. The instillate was removed at two hours and evaluated for volume, pH, and appearance. A fifth animal was catheterized, but not dosed because of injury to the bladder caused by the catheterization procedure, and was sacrificed on day 1 and necropsied. The remaining four animals were observed at two hours after removal of the saline and twice daily thereafter. Body weight was assessed weekly. Blood and urine samples were taken before treatment and on day 2. The animals were sacrificed on day 2 and necropsied.

**Findings:**

**Clinical chemistry and hematology:** One of the four treated animals had 3+ urine occult blood on the day after the procedure, which was considered to be due to catheterization trauma.

Pathological examination: Gross and microscopic pathological examination of the urethra and urinary bladder revealed a low level of relatively mild macroscopic and microscopic changes in urethra and urinary bladder. These were judged to be insufficient in severity to interfere with the interpretation of results of studies of intravesical instillation of test material.

18. **Study title:** Acute intravesical toxicity and toxicokinetic study with 5-aminolevulinic acid hydrochloride in dogs

**Study number:** 6703-106

**Performing organization:** [REDACTED]

**Drug lot and batch:** lot # 335 (R17796)

**Date of study:** 6/5-6/97

**GLP compliance:** yes

**Study design:**

Dosing: single intravesical dose of 10 ml

Dose groups: vehicle, 300, 3000 mg 5-ALA

Formulation: 5-ALA in 0.2 M potassium phosphate buffer, pH approximately 5.0

Test animals: beagle dogs, 3 /sex/group. On day 1, the test material was instilled into the urinary bladder via a latex urethral catheter and allowed to remain in the bladder for approximately 2 hours. Animals were observed at the time of removal of the instillate (2 hours) and at 4 and 6 hours after instillation, then twice daily until sacrifice. At 2 hours, the instillate was collected and evaluated for appearance, pH, and volume. Blood and urine samples were taken before treatment and on day 2. Additional blood samples were taken on day 1 pre-dose and at 0.25, 0.5, 1, 2, 3, and 4 hours post-dose for toxicokinetics. Animals were sacrificed and necropsied on day 2. Histological examination of the urethra, urinary bladder, and gross lesions was performed.

**Findings:**

Deaths: none, but two males were replaced on day 1 due to problems with the catheter.

Clinical signs: red discolored urine in one high dose female at 2 hours. When the dose solution was removed, the material recovered from the high dose group was greater in volume and lower in pH than that of controls.

Clinical chemistry and hematology: There were no biologically significant hematological or serum chemistry changes.

At the high dose, mean overnight urine volume was lower and the incidence and severity of hematuria was increased relative to control. This effect was most obvious in females; those animals also had higher urine protein concentrations and more severe lesions on gross pathological examination. Also at the high dose, lower overnight urinary excretion of sodium (not statistically significant), potassium, and chloride (statistically significant in males) were reported. However, the biological significance of the latter findings is unclear, given the high variability of these values in all groups both before and after treatment.

Pharmacokinetics: The AUC's were variable, but increased with dose, ranging from [REDACTED]  $\mu\text{g}\cdot\text{min}/\text{ml}$  at the 300 mg dose and from [REDACTED]  $\mu\text{g}\cdot\text{min}/\text{ml}$  for the 3000 mg dose.

Bioavailability ranged from [REDACTED] for the 300 mg dose and from [REDACTED] for the 3000 mg dose.

**Pathological examination:** Pathological findings in control animals were attributed to trauma during catheterization. Similar lesions were found in treated animals, but were greater in severity and extent in a dose-related manner. It was concluded that lesions resulting from catheterization were exacerbated by the test material.

On gross examination, reddened areas of varying size were seen on the mucosal surfaces in the urinary tract, with a trend toward wider distribution and larger lesions in treated groups. This trend was more apparent in females. Severity of bladder lesions was greater in high dose animals; this finding was consistent with findings on urinalysis.

Microscopic changes in the urethra and bladder consisted of submucosal edema, intraepithelial blood cells (erythrocytes and neutrophils), submucosal hemorrhage, mixed mononuclear cell infiltrate in the submucosa, congestion, acute inflammation, epithelial vacuolization, and epithelial loss. These changes were more common in treated animals and were seemingly more severe in the high dose group.

### NONCLINICAL PHARMACOKINETICS STUDIES

The following was reviewed as amendment # 046 to IND [redacted]

1. Study title: Pharmacology and pharmacokinetics of 5-aminolevulinic acid in beagle dogs after oral and intravenous administration.

Study number: not given

Performing organization: [redacted]

Drug lot and batch: ALA - lot #4430284, diluent - lot #4450284

Date of study: 2/3/94-2/18/94

GLP compliance: not stated

Study design:

**Dosing:** Two treatments, each consisting of a single dose of 128 mg ALA hydrochloride (equivalent to 100 mg ALA) were administered. One treatment was intravenous, via jugular vein catheter; the other was oral, administered by emptying the contents of the drug-containing syringe into the back of the animal's mouth. The average actual dose was 8.28 mg/kg (166 mg/m<sup>2</sup>) iv and 7.29 mg/kg po (146 mg/m<sup>2</sup>). Human pharmacokinetic study subjects will also receive 100 mg ALA (1.43 mg/kg, or 57.8mg/m<sup>2</sup> for a 70 kg human).

**Formulation:** sterile lyophilized ALA hydrochloride diluted in 10 ml sodium acetate (9 mg/ml) with mannitol (12.5 mg/ml) solution, pH 2.4. The final pH was 4.2 after reconstitution.

**Test animals:** 4 male beagle dogs weighing 10.2-12.6 kg, 2/group, crossover design. The test animals were given a single dose either iv or po, followed by a 2-week washout period and a second single dose by the alternative route. The dogs were monitored for systolic/diastolic blood pressure and pulse. Serum chemistries were examined in blood samples drawn before and 12 hours after drug administration. Blood samples were drawn before each dose and at 2, 5, 10, 15, 30, 60, 120, 240, 480, and 720 minutes after dosing for pharmacokinetic determinations.

Samples were analyzed quantitatively for ALA using an [redacted] method with a limit of detection of [redacted] µg/ml plasma. Pre-dose samples from each animal were used to quantitate endogenous ALA, and this value was subtracted from post-dose plasma samples from the same animal.

**Findings:**

**Clinical signs:** Side effects included increased salivation and licking, indicative of nausea, in all 4 animals after iv dose only. The onset was within 30 seconds of dosing and lasted 1-2 minutes.

Since an additional animal administered diluent alone also exhibited this effect, it was thought to be due to the diluent and not to the drug itself. Vomiting was seen after oral (4/4) and iv (3/4) doses, first occurring between 42-200 minutes after dosing. An iv dose of 20 mg also produced vomiting in all animals, but none was seen after dosing with diluent alone. No effect was seen on cardiovascular parameters.

Clinical chemistry: No effects were seen that could be attributed to the drug.

Pharmacokinetic parameters: (mean  $\pm$  SD)

Dosing:	intravenous	oral
$t_{1/2}$ (minutes)	19.9 $\pm$ 2.62	40.7 $\pm$ 22.9
CL (ml/min/kg)	6.79 $\pm$ 1.77	
Vdss (ml/kg)	259 $\pm$ 128	
AUC <sub>0</sub> ( $\mu$ g-min/ml)	1279 $\pm$ 369	453 $\pm$ 180
C <sub>peak</sub> ( $\mu$ g/ml)		6.22 $\pm$ 3.48
t <sub>peak</sub> (minutes after dosing)		28.1 $\pm$ 21.4

The time course of plasma ALA decline was biexponential. Oral bioavailability averaged 41.2 $\pm$ 14.8% (range ). One dog had a much lower AUC after iv dosing and a delayed peak plasma concentration. This was thought to be due to catheter damage and extravasation of part of the dose. One dog lost part of the oral dose (approximately 3 ml) prior to swallowing, resulting in lower plasma ALA concentrations and lower calculated bioavailability (bioavailability in this animal was calculated using 60 mg as the administered dose). Vomiting occurred in all orally dosed animals, resulting in loss of drug and decrease in bioavailability measure, and affecting the time of peak plasma concentrations.

Comparison of pharmacokinetic parameters in human and canine subjects is presented below:

Parameter (mean $\pm$ SD)	human	dog
number subjects	6	4
IV dose (mg/kg)	1.34 $\pm$ 0.06	8.28 $\pm$ 1.05
IV $t_{1/2}$ (hours)	0.83 $\pm$ 0.05	0.33 $\pm$ 0.04
IV CL (ml/min/kg)	1.88 $\pm$ 0.51	6.79 $\pm$ 1.77
IV Vdss (ml/kg)	116 $\pm$ 30.3	259 $\pm$ 128
IV AUC ( $\mu$ g-hr/ml)	12.5 $\pm$ 2.9	21.3 $\pm$ 6.15
PO dose (mg/kg)	1.34 $\pm$ 0.06	7.29 $\pm$ 1.15 *
PO $t_{1/2}$ (hours)	0.7 $\pm$ 0.18	0.68 $\pm$ 0.38
PO C <sub>peak</sub> ( $\mu$ g/ml)	4.65 $\pm$ 0.94	6.22 $\pm$ 3.48

$t_{peak}$ (hours after dosing)	$0.83 \pm 0.2$	$0.47 \pm 0.36$
PO AUC ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	$7.3 \pm 1.25$	$7.55 \pm 3.00$
Relative bioavailability (%)	$60.3 \pm 13.4$	$41.2 \pm 14.8$

\* This dose is probably an overestimate because all four animals vomited after dosing.

### NONCLINICAL PHARMACOLOGY STUDIES

Two studies in experimentally transplanted tumors in rats were performed by [redacted] at the in order to determine the optimum wavelength to be used in photodynamic therapy. R3230AC rodent mammary adenocarcinomas were transplanted in the axillary region of Fischer female rats. When these tumors became palpable (approximately 0.3 cm in diameter, or 0.03 cc in volume), animals were assigned to control or treatment groups. 5-ALA in 0.9% saline was administered via the tail vein at a dose of 300 mg/kg or approximately 30 mg/rat. Three hours later, the lesions were irradiated.

In the red light action spectra study, irradiation was performed with a laser using wavelengths of 515-640 nm at 100 mW/cm<sup>2</sup> for 30 minutes (180 J/cm<sup>2</sup>). (Reviewer's comment: 33/171 animals were removed from the study because of tumors that grew 2-3 fold faster than control tumors. This effect was attributed to infection, and the possibility of a treatment effect was not considered.) The study endpoint was time for tumors to reach 0.3 cc in volume. Tumor growth delay was significant relative to control in animals treated with ALA and irradiated at 625, 630, 635 or 640 nm and was greatest in animals treated with ALA and irradiated at 635 nm.

In the blue light action spectra study, irradiation was performed using an [redacted] system and [redacted] at wavelengths of 415±5 or 415±12 nm at 10-54 J/cm<sup>2</sup>. In an additional group of animals, the skin overlying the tumor was incised to expose the tumor tissue directly to light. In another group, the tumor was implanted subcutaneously in nude mice; three were untreated controls, and three received 300 mg/kg ALA iv followed at 5 hours by irradiation at 415±5 nm at 10 J/cm<sup>2</sup>. The study endpoint was time for tumors to reach 0.3 cc in volume. Tumor growth was significantly delayed in the irradiated treatment group receiving 415±5 nm at 4 mW/cm<sup>2</sup> (10 J/cm<sup>2</sup>) and in the group in which the skin overlying the tumor was reflected to allow direct exposure of the tumor tissue. In general, the blue light treatment regimens were less effective than the most effective red light treatment regimen.

The following studies were the subject of published articles submitted by the applicant:

1. Divaris DXG et al. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminolevulinic acid correlates with localized protoporphyrin IX fluorescence. Am J Pathol 136:891-897, 1990. (applicant's reference #18)

Female CD-1 albino mice were given a single ip injection of 250 mg/kg 5-ALA. Localization of PpIX in mouse skin was demonstrated by intense red fluorescence in the sebaceous glands with weaker fluorescence in epidermis and hair follicles at 3 hours after injection. Vascular and connective tissue elements of the skin did not appear to be affected. Exposure to photoactivating light (for 6 hours immediately after injection, 100 W tungsten lamp, 21 mW/cm<sup>2</sup>) resulted in destruction of the sebaceous glands, moderate damage to the basal cell layer, focal epidermal necrosis, transient acute inflammation, and diffuse reactive changes in the

keratinocytes. Transient secondary edema and inflammation was seen in the dermis. Light exposed skin recovered, except for a persistent reduction in the number of hair follicles.

2. Goff BA et al. Effects of photodynamic therapy with topical application of 5-aminolevulinic acid on normal skin of hairless guinea pigs. *J. Photochem. Photobiol. B: Biol.* 15:239-251, 1992. (applicant's reference #19)

Topical application of 20% ALA in Eucerin® lotion was performed in normal and tape-stripped skin of hairless guinea pigs. In non tape-stripped animals, no PpIX fluorescence was evident. Slight transient erythema was seen at light doses  $>25 \text{ J/cm}^2$ . Histologically, slight edema and inflammation with PMN cell infiltrate were seen immediately after treatment and at 24 hours, but no effects were seen in biopsy specimens taken at 72 hours and later. When ALA was applied to the skin for 5 hours and irradiated with  $400 \text{ J/cm}^2$ , faint PpIX fluorescence could be demonstrated. Irradiation to those animals resulted in erythema that persisted for 48 hours, followed by peeling. The skin appeared normal by 72 hours. Histologically, edema and inflammation of the dermis were evident immediately after treatment and at 24 and 72 hours. At 24 hours, necrosis of the epidermis and superficial hair follicles were also seen. At 72 hours, significant epidermal hyperplasia was evident. By seven days after exposure, mild epidermal hyperplasia was seen, and the dermis appeared normal. In tape-stripped animals, increased PpIX fluorescence with time was seen as long as ALA was present on the skin (3 hours). Signs produced by irradiation of treated sites included erythema, edema, and superficial epidermal necrosis. Full thickness necrosis of the epidermis and superficial appendages were evident at 24 hours, followed by complete repair within 7 days.

### PREVIOUS HUMAN EXPERIENCE

Review articles describing the clinical presentation and pathogenesis of porphyrias in human patients were submitted. The applicant also states in the submission that a review of data from patients with porphyria revealed no evidence of increased risk of cancer or genotoxicity. No mention was made of a similar review of data to indicate relative risk of reproductive or developmental effects.

In a journal article not submitted by the applicant (*Arch. Dermatol.* 134:821-826, 1998), the immediate and long-term effects of photodynamic therapy with ALA on superficial basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) were investigated. Recurrence of BCC after 19 months was 44%, and recurrence of SCC after 8 months was 69%. At 36 months, 50% of BCC patients were "disease-free" as opposed to 8% of SCC patients. Histological examination revealed a significant increase in fibrosis in the dermis, with a sharp border between fibrotic and nonfibrotic tissue. In many cases, that border was located deeper in the dermis than was the original tumor prior to photodynamic therapy.

It is estimated that the amount of ALA topically applied is too low to alter endogenous systemic levels of that substance in human patients, and systemic effects are not likely to be an issue in that circumstance.

### SUMMARY

The acute toxicity of ALA was examined in mice, rats, and dogs. No test article-related effects were seen in either rodent species after single intravenous doses of 300 mg/kg (HED=25

and 50 mg/kg for mice and rats, respectively). In dogs, administered single intravenous doses of 10, 30, or 100 mg/kg (HED=5, 15, 50 mg/kg, respectively) effects included salivation and emesis at all doses and increases in AST and/or ALT at the mid and high doses.

The acute toxicity of the condensation product, pyrazine 2,5-dipropionic acid (PDPA) was studied in mice and rats after oral and ip administration. After a single oral dose of 5000 mg/kg PDPA in 0.5% carboxymethylcellulose to mice (HED= 417 mg/kg) soft stools were seen in most animals, and 1/10 animals exhibited hypoactivity and staggered gait. The only effect seen in rats after the same oral dose (HED=833 mg/kg) appeared to be soft stools. A single intraperitoneal dose of 1000 mg/kg to mice (HED=83 mg/kg) or to rats (HED=167 mg/kg) resulted in hypoactivity in both species on the day of treatment.

The applicant performed a genotoxicity test battery consisting of an Ames test, an Ames test in the presence of solar light radiation, a mouse lymphoma forward mutation assay, and a mouse micronucleus assay. All studies were negative for genotoxicity. However, PpIX formation was not demonstrated, and genotoxicity due to this product may have been overlooked in some of the test systems. A study from the literature included in the submission indicated positive genotoxic results in cultured rat hepatocytes where PpIX formation was documented. Two other articles described oxidative DNA damage in studies of in vitro and in vivo ALA exposure.

In a study of acute subcutaneous toxicity in rats, all doses ( ) mg/kg) produced irritation and related lesions at the treatment site. These were dose-related in onset and severity, and were attributed to the ionic strength and low pH of the solution.

Acute dermal toxicity was evaluated in the only study performed using the to-be-marketed formulation. ALA solution was applied at concentrations of 10, 20 and 30%. Findings were limited to slight to moderate dermal irritation at all concentrations. In an acute dermal study with a cream formulation, slight to moderate dermal irritation in vehicle and ALA-treated groups was seen in a dose-related manner.

Several studies of intravesical administration of ALA to dogs were performed to support use of the drug for another indication. Signs attributable to the test article were limited to vomiting and exacerbation of catheterization trauma. Systemic absorption of ALA was demonstrated during the two hour exposure period and was dose-related. PDPA formation occurred in non-acidified urine, but systemic absorption of that substance not evaluated.

A pharmacokinetic study in dogs was performed to evaluate a single oral or intravenous dose of ALA. The pharmacokinetic parameters are compared to human parameters above. The study did not evaluate plasma kinetics of PpIX or PDPA.

## CONCLUSIONS

From a nonclinical standpoint, the application is approvable, with the below listed recommendations for revision of the label.

## RECOMMENDATIONS

1. The following revisions should be made to the label:

Under "Clinical Pharmacology," the statement, "Following topical application of exogenous ALA, dysplastic, neoplastic and non-malignant inflammatory and hyperproliferative skin lesions have been shown to accumulate photosensitizing concentrations of PpIX" should be amended to

read, "Following topical application of exogenous ALA, some dysplastic, neoplastic and non-malignant inflammatory and hyperproliferative skin lesions have been shown to accumulate photosensitizing concentrations of PpIX."

The section headed "Carcinogenesis, [redacted] should be headed, "Carcinogenesis, Mutagenicity, Impairment to Fertility." The following statement should be added to the end of the first paragraph, in which the applicant's genotoxicity studies and their negative results are described: PpIX formation was not demonstrated in the in vitro studies. In contrast, at least one report in the literature has noted genotoxic effects in cultured rat hepatocytes after ALA exposure with PpIX formation. Other studies have documented oxidative DNA damage in vivo and in vitro as a result of ALA exposure.

A final paragraph should be added to that section as follows: No assessment of effects of [redacted] on fertility has been performed in laboratory animals. It is unknown what effects systemic exposure to ALA might have on fertility or reproductive function.

APPEARS THIS WAY  
ON ORIGINAL

[redacted] /S/

3/8/99

Amy C. Nostrandt, D.V.M., Ph.D.  
Pharmacologist/Toxicologist

- cc:
- NDA
- HFD-340
- HFD-540
- HFD-540/PHARM/Nostrandt
- HFD-540/TLPHARM/Jacobs
- HFD-540/MO/Okun
- HFD-540/CHEM/Hathaway
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