CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 208630
Supporting document/s:	002
Applicant's letter date:	December 6, 2016
CDER stamp date:	December 6, 2016
Product:	Gliolan® (5-aminolevulinic acid hydrochloride)
Indication:	To Facilitate the Real Time Detection and Visualization of Malignant Tissue During Glioma Surgery
Applicant:	NX Development Corp Lexington, KY
Review Division:	Medical Imaging
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1 Executive Summary

1.1 Introduction

5-Aminolevulinic acid (5-ALA) is an endogenous compound (approximately 600 mg/day is synthesized by the body) that serves as a precursor in the heme synthetic pathway where 5-ALA is eventually enzymatically converted to protoporphyrin IX (PpIX). PpIX is photoactive and is fluorescent when exposed to long ultraviolet waves (375-440 nM). Malignant glial cells are infiltrative in nature resulting in indistinct borders between normal and malignant tissue. Malignant glial cells are capable of producing PpIX from 5-ALA and the exogenous administration of 5-ALA leads to selective accumulation of PpIX in tumor cells and epithelial tissues. The Sponsor is seeking the approval for the use of 5-ALA to facilitate the real time detection and visualization of malignant tissue during glioma surgery. 5-ALA has been approved for this indication in the European Union since 2007 and in a number of other countries.

1.2 Brief Discussion of Nonclinical Findings

In a 14-day toxicity study, rats were administered via gavage 0 (saline), 30, 100, and 300 mg/kg/day 5-ALA. Minimal to moderate dose-related bile duct changes were observed in a few mid dose male rats and all high dose male rats. Changes included bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis/inflammation, intraductal bile plugs and peribiliar bile pigment accumulation. These findings were still present in recovery animals. The NOAEL for this study was 30 mg/kg/day.

In a 4-week toxicity study, dogs were administered orally 0, 1, 3, or 10 mg/kg/day 5-ALA. The NOAEL was 3 mg/kg/day due to a combination of increased vomiting, increased ALT and the presence of brown pigment in the liver in high dose animals that was likely due to excessive accumulation of protoporphyrin.

5-ALA was not mutagenic in the Ames assay, the *in vitro* gene mutation assay using V79 mammalian cells, the chromosomal aberration assay using human peripheral lymphocytes, and the mouse micronucleus test. Studies using the standard genotoxicity battery were performed only in the dark or with subdued lighting. There is evidence in the literature to suggest that 5-ALA is genotoxic in the presence of light (likely due to PpIX formation). Measures to protect patients from light exposure following 5-ALA administration are adequately addressed in the labeling.

The Sponsor submitted only one reproductive and developmental toxicity study report. In a rabbit embryo-fetal developmental toxicity study, the NOAEL for maternal toxicity was 50 mg/kg/day and no adverse effects were observed for maternal reproduction function and embryo-fetal development at the highest dose evaluated (150 mg/kg/day) for orally administered 5-ALA. A number of reproductive toxicology studies were reported in the literature. However, these studies were generally non-GLP and/or the studies were generally not adequately designed. The Nonclinical Reviewer recommends that the use of any data from these studies be very specific and limited (i.e. increased embryotoxicity with light exposure).

The main metabolite of 5-ALA, PpIX, is phototoxic when activated using light between 465 and 514 nm. Lethal phototoxicity was observed in mice intravenously administered a single dose of 250 mg/kg or 750 mg/kg 5-ALA followed 4 h later with exposure to UV light (30 J UV-A/cm^2 and 0.3 J UV-B/cm^2). Phototoxicity concerns are adequately addressed in the labeling. Pivotal nonclinical safety studies and the mouse micronucleus assay were performed either in the dark or under subdued light in order to avoid adverse effects due to phototoxicity.

1.3 Recommendations

1.3.1 Approvability

The drug is approvable from a nonclinical perspective.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Proposed by the Sponsor:

8.1 Pregnancy

Risk Summary

There are no (b) (4) data (b) (4) in pregnant women. Animal studies (b) (4) (b) (4) (b) (4) (see (b) (4) Data). (b) (4) (b) (4).

Animal data

(b) (4), the no-observed-adverse-effect level (NOAEL) was 50 mg/kg/day (b) (4) (b) (4) maternal toxicity, (b) (4) (b) (4) the maximum recommended dose, based on AUC comparisons (b) (4) (b) (4) The NOAEL for embryo-fetal developmental toxicity was 150 mg/kg/day (b) (4) (b) (4)

Nonclinical Recommended edits:

Risk Summary
There are no (b) (4) data (b) (4) in pregnant women. (b) (4) animal (b) (4) studies (b) (4) (see (b) (4) Data). (b) (4) (b) (4)

Animal data
(b) (4), the no-observed-adverse-effect level (NOAEL) was 50 mg/kg/day (b) (4) (b) (4) for maternal toxicity, (b) (4) (b) (4) the maximum recommended dose, based on AUC comparisons (b) (4) (b) (4) The NOAEL for embryo-fetal developmental toxicity was 150 mg/kg/day (b) (4) (b) (4)

Proposed by the Sponsor:

8.2 Lactation
Risk summary
(b) (4)

Nonclinical Recommended edits:

None.

Proposed by the Sponsor:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenesis
No carcinogenicity studies have been conducted with (b) (4).
Mutagenesis
(b) (4) ALA HCl was not mutagenic in the (b) (4) assay in the (b) (4) (b) (4) HPRT-V79 mammalian cell mutagenicity test (b) (4) (b) (4)

(b) (4)

Impairment of Fertility
No fertility studies have been conducted with (b) (4)

Nonclinical Recommended edits:

Carcinogenesis
No carcinogenicity studies have been conducted with (b) (4).
Mutagenesis
(b) (4) ALA HCl was not mutagenic in the Ames assay, (b) (4) HPRT-V79 mammalian cell mutagenicity test, the peripheral human lymphocyte chromosomal aberration assay and the *in vivo* mouse micronucleus test when (b) (4) were performed in the dark or studies (b) (4) under subdued lighting.
Impairment of Fertility
No fertility studies have been conducted with (b) (4)

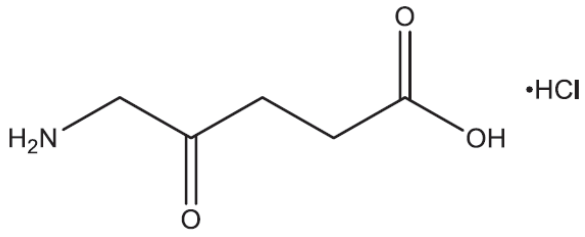
2 Drug Information

2.1 Drug

CAS Registry Number: 5451-09-2 for 5-ALA HCl; 106-60-5 for the free base

Chemical Name: 5-Aminolevulinic acid hydrochloride; 5-amino-4-oxo-pentanoic acid hydrochloride

Molecular Formula/Molecular Weight/Structure



Chemical Formula: $C_5H_{10}ClNO_3$
Molecular Weight: 167.59

Pharmacologic Class: Optical Imaging Agent

2.2 Relevant INDs, NDAs, BLAs and DMFs

None.

2.3 Drug Formulation

Gliolan® is a lyophilized powder contained in a 50 mL glass vial. The composition of the lyophilized powder is 1.5 g 5-ALA HCl (1.17 g 5-ALA). The lyophilized powder is reconstituted with 50 mL of water and 0.666 mL/kg is administered orally to patients.

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities/degrandants of concern.

2.6 Proposed Clinical Population and Dosing Regimen

The recommended dose (20 mg/kg) of 5-ALA HCl is reconstituted in drinking water and the solution is administered orally 3 hours before anesthesia prior to glioma resection surgery. The ready-for-use solution is prepared immediately prior to administration of the medication by dissolving 1.5 g 5-ALA HCl in 50 mL of drinking water. The solution is administered under supervision of a healthcare professional.

3 Studies Submitted

3.1 Studies Reviewed

Safety Pharmacology

Examination of the Influence of MC-506 (5-ALA) on the Spontaneous Mobility in Mice following Intravenous Administration (Study # 10118/1/96)

Examination of the Influence of MC-506 (5-ALA) on the Hexobarbital Sleeping Time in Mice following Intravenous Administration (Study # 10119/1/96)

Examination of the Influence of MC-506 (5-ALA) on Several Cardiovascular Parameters and the Respiration in Anesthetized Beagle Dogs following Intravenous Administration (Study # 10115/1/96)

5-ALA HCl Effect on hERG Tail Currents Recorded from Stably Transfected HEK 293 Cells (Study # A1273)

Examination of the Influence of MC 506 (5-ALA HCL) on the Diuresis and Saluresis in Rats following Intravenous Administration (Study # 10116/1/96)

Examination of MC 506 (5-ALA HCl) for Spasmolytic and Spasmogenic Properties in the Isolated Guinea Pig Ileum (Study # 10117/1/96)

Examination of the Influence of MC 506 (5-ALA HCL) on Intestinal Motility following Intravenous Administration (Charcoal Propulsion Test in the Mouse) (Study # 10120/1/96)

Pharmacokinetics

Pharmacokinetic Study of MC506 (5-ALA) and Determination of Absolute Bioavailability after Single I.V. and P.O. Administration in Beagle Dogs (Study # 13105/00)

ADME Screening ([(b) (4)]3251/2015)

General Toxicology

14-Day Subchronic Toxicity Study of MC-506/1 (5-ALA HCl) by Oral Administration to Sprague Dawley Rats (Study # 11651/98).

14-Day Subchronic Toxicity Study of MC 506 (5-ALA HCl) by Intravenous Administration to Sprague Dawley Rats (Study # 10828/97).

4-Week Repeated Dose Oral Toxicity Study and 4-Week Recovery Study of 5-ALA HCl in Dogs (Study # DM10262).

14-Day Subchronic Toxicity Study of MC 506 (5-ALA HCl) by Intravenous Administration to Beagle Dogs (Study # 11664/98).

Genetic Toxicology

Mutagenicity Study of MC-506 (5-ALA HCl) in the *S. typhimurium* Reverse Mutation Assay (*In Vitro*) (Study # 10112/96).

Mutagenicity Study of MC-506 in Mammalian Cells (V79) in the *In Vitro* Gene Mutation Assay (Study # 10113/96).

In Vitro Assessment of the Clastogenic Activity of MC506 (5-ALA) in Cultured Human Peripheral Lymphocytes (Study # 10114/96).

Micronucleus Test of 5-ALA in Bone Marrow Cells of the NMRI Mouse following Oral Administration (Study # 17551/03).

Reproductive and Developmental Toxicology

Embryo-Fetal Development Study of 5-Aminolevulinic Acid Hydrochloride in Rabbits (Study # DM10264)

Special Toxicology

Phototoxicity Study of MC-506 (5-ALA HCl) by Intravenous Administration to Mice (Study # 10121/96)

3.2 Studies Not Reviewed

Acute Toxicity Study of MC-506/1 (5-ALA HCl) by Intravenous Administration to NMRI Mice (Study # 11654/98) (Note – not reviewed because it was an LD50 study using the intravenous route of administration).

Acute Toxicity Study of MC-506/1 (5-ALA HCl) by Intraperitoneal Administration to NMRI Mice (Study # 10122/1/96) (Note – not reviewed because it was an LD50 study).

Acute Toxicity Study of MC-506/1 (5-ALA HCl) by Intravenous Administration to Sprague Dawley Rats (Study # 11653/98) (Note – not reviewed because it was an LD50 study using the intravenous route of administration).

Acute Toxicity Study of MC-506/1 (5-ALA HCl) by Intraperitoneal Administration to Sprague Dawley Rats (Study # 10123/1/96) (Note – not reviewed because it was an LD50 study).

7-Day Dose-Range-Finding Study of MC 506 (5-ALA HCl) by Intravenous Administration to Sprague Dawley Rats (Study # 10827/97) (Note – not reviewed because using the intravenous route of administration).

7-Day Dose-Range-Finding Study of MC 506 (5-ALA HCl) by Intravenous Administration to Beagle Dogs (Study # 11652/98) (Note – not reviewed because using the intravenous route of administration).

Local Tolerance Test of MC506/1 (5-ALA HCl) in Rabbits after a Single Intravenous, Intraarterial, Paravenous, Subcutaneous, and Intramuscular Administration (Study # 11655/98) (Note – study not relevant to the oral route of administration).

Examination of 5-ALA in a Skin Sensitization Test in Guinea-Pigs According to Magnusson and Kligman (Maximisation Test) (Study # 15393/1/02) (Note – study not relevant to the oral route of administration).

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

Primary pharmacology study reports were not submitted. The Sponsor stated that incubation of a number of tumor cell lines *in vitro* with 5-ALA resulted in the accumulation of PpIX fluorescence in those cells. The Sponsor also stated that systemic administration of 5-ALA resulted in increased PpIX accumulation in several *in vivo* brain tumor models with the location of PpIX fluorescence strongly correlated with tumor. The exact mechanism that leads to increased PpIX accumulation in tumor cells relative to normal cells is unknown.

4.2 Secondary Pharmacology

Secondary Pharmacology study reports were not submitted.

4.3 Safety Pharmacology

Examination of the Influence of MC-506 (5-ALA) on the Spontaneous Mobility in Mice following Intravenous Administration (Study # 10118/1/96)

In a GLP study, spontaneous motility was evaluated using a microprocessor and automated recording of movement in NMRI mice (n = 5/F/group) after intravenous administration of 0, 40, 100, or 250 mg/kg 5-ALA HCl. There were no drug-related effects on spontaneous motility noted in this study.

Examination of the Influence of MC-506 (5-ALA) on the Hexobarbital Sleeping Time in Mice following Intravenous Administration (Study # 10119/1/96)

In this GLP study, hexobarbital sleeping time was evaluated in NMRI mice (n = 5/F/group). Hexobarbital (45 mg/kg i.v.) was administered 5 min after intravenous administration of 0, 40, 100, or 250 mg/kg 5-ALA HCl. Mice were placed on their backs and time until righting reflex was recorded. There were no drug-related effects on hexobarbital sleeping time noted in this study.

Examination of the Influence of MC-506 (5-ALA) on Several Cardiovascular Parameters and the Respiration in Anesthetized Beagle Dogs following Intravenous Administration (Study # 10115/1/96)

In this GLP study, anesthetized dogs (n = 5) were intravenously administered vehicle and then in increasing dose fashion 5, 15, or 45 mg/kg 5-ALA HCl. The following parameters were then evaluated immediately before through 30 min after dosing: peripheral arterial blood pressure, pulmonary arterial blood pressure, wedge pressure, heart rate, cardiac minute output, cardiac stroke volume, systolic left ventricular blood pressure, dp/dt max, central venous pressure and respiration (pH, pO_2, pCO_2, respiratory rate, and volume).

A slight decrease in arterial blood pressure (-9.5% mean systolic and -7.5% mean diastolic) was observed immediately after high dose administration and returned to baseline values by 5 min after dosing. Mean left ventricular pressure (-15%) and dp/dt max (-43%) were decreased immediately after high dose administration and returned to baseline values by 5 min after dosing. The NOEL in this study was 15 mg/kg.

5-ALA HCl Effect on hERG Tail Currents Recorded from Stably Transfected HEK 293 Cells (Study # A1273)

In a GLP study, the effect of 6.0 mM 5-ALA HCl on hERG current was evaluated in stably transfected HEK 293 cells (n = 3 cells) using the whole patch clamp technique. 5-ALA HCl had no effect on hERG current. Cells were effectively blocks when incubated with 100 nM E-4031.

Examination of the influence of MC 506 (5-ALA HCL) on the Diuresis and Saluresis in Rats following Intravenous Administration (Study # 10116/1/96)

In a GLP study, diuresis and saluresis was evaluated in Sprague Dawley rats (n = 10/F/group) after intravenous administration of 0, 40, 100, or 250 mg/kg 5-ALA HCl. There were no drug-related effects on diuresis noted in this study. Urine was collected at 0-1, 1-2 2-3, 3-4, 4-5 and 5-24 h after dosing and volume, sodium, potassium and chloride levels were measured. Cumulative sodium excretion (0-24 h) was increased by approximately 34% and potassium excretion was significantly increased by approximately 3-fold from 0-1 h in the high dose group. The NOEL on saluresis was 100 mg/kg.

Examination of MC 506 (5-ALA HCl) for Spasmolytic and Spasmogenic Properties in the Isolated Guinea Pig Ileum (Study # 10117/1/96)

In this GLP study, Guinea pig ileum was incubated in the presence of 0.5 to 5000 µg/mL 5-ALA HCl. Agonistic effects were evaluated by adding increasing concentrations of 5-ALA to the tissue bath and evaluating the isolated intestinal sections for movement. Antagonistic effects were then evaluated by adding the agonists histamine, barium chloride or acetylcholine to the tissue bath and evaluation if adding increasing concentrations of 5-ALA to this tissue bath inhibited movement. 5-ALA did not produce any agonistic or antagonistic effects in this study.

Examination of the Influence of MC 506 (5-ALA HCL) on Intestinal Motility following Intravenous Administration (Charcoal Propulsion Test in the Mouse) (Study # 10120/1/96)

In this GLP study, intestinal motility was evaluated in NMRI mice (n = 5/F/group). Charcoal (0.3 mL of an aqueous 10% solution) was administered via stomach tube immediately after intravenous administration of 0, 40, 100, or 250 mg/kg 5-ALA HCl. Mice were euthanized 2 h later, the GI tract removed and the distance the charcoal meal had traveled recorded. If any charcoal had visibly entered the cecum, the result was scored as negative for inhibition of motility. If the charcoal remained in the stomach and the cecum contained no charcoal, the result was scored as positive for inhibition of motility. All mice were positive for inhibition of intestinal motility at the 100 and 250 mg/kg dose levels. The EC_{50} as calculated to be 63.2 mg/kg 5-ALA.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetic Study of MC506 (5-ALA) and Determination of Absolute Bioavailability after Single I.V. and P.O. Administration in Beagle Dogs (Study # 13105/00)

In a GLP study, dogs (3 M and 3 F) were administered a single intravenous dose of 20 mg/kg 5-ALA HCl or a single oral dose of 20 mg/kg 5-ALA HCl. Blood samples for plasma drug level determination were collected over a 24 h period for the intravenous route and over a 48 h period for the oral route. There was a 2 week washout period between the treatments.

Emesis was observed in 2 male and 1 female dog after oral administration. Mean PK results for 5-ALA are summarized in study report Table 3.1 below. The absolute bioavailability of orally administered 5-ALA was 86%.

TABLE 3: 1 Overview of arithmetic means and of coefficients of variation of pharmacokinetic parameters derived from concentrations of 5-Aminolevulinic acid hydrochloride in plasma, n = 6

		Treatment A 20 mg/kg i.v.		Treatment B 20 mg/kg p.o.	
		arithmetic	CV (%)	arithmetic	CV (%)
$AUC_{0-\infty}$	[mg h/L]	25.90	19.55	22.29	22.50
C_{max}	[mg/L]	40.90	13.54	14.72	11.29
t_{max}	[h]	0.058 (median)	0.033 – 0.083 (range)	0.625 (median)	0.250 – 1.000 (range)
$t_{½}$	[h]	0.652	9.07	0.623	20.67
MRT	[h]	0.803	11.55	1.334	17.21
CL/f	[L·kg/h]	0.799	20.37	0.940	24.43
V_z/f	[L/kg]	0.751	21.90	0.826	23.21
f_a	[%]			86.2	13.31

ADME Screening ((b) (4) 3251/2015)

5-ALA was evaluated for CYP450 inhibition, apparent permeability and active efflux potential using Caco-2 monolayers MDCK-MDR1 and MDCK-BCRP IC_{50} determination, and for inhibition of uptake transporters using C_{14} labeled 5-ALA.

As summarized in study report Table 7 below, the IC_{50} for 5-ALA induced CYP450 inhibition was >300 µM (the highest concentration evaluated). Thus 5-ALA was not a potent inhibitor of the major human CYP450 isoforms.

Compound ID	Mean IC_{50} (µM)							
	1A2	2B6	2C8	2C9	2C19	2D6	3A (Midazolam)	3A (Testosterone)
5-ALA	>300	>300	>300	>300	>300	>300	>300	>300
Positive control	6.6	3.6	6.2	0.46	22	0.28	0.09	0.17
Negative control	>100	>100	>30	>100	>100	>100	>100	>100

Table 7: IC_{50} (µM) for test and control compounds

Caco-2 cell permeability results are summarized in study report Table 8 below. Poor permeability was observed in both A>B and B>A directions suggesting that 5-ALA is not a substrate for efflux proteins expressed in Caco-2 cells such as P-gp and BCRP.

Compound ID	A>B P_{app} ($\times 10^{-6}$ cm/sec)		B>A P_{app} ($\times 10^{-6}$ cm/sec)		A>B Recovery (%)		B>A Recovery (%)		Mean efflux ratio
	n=1	n=2	n=1	n=2	n=1	n=2	n=1	n=2	
5-ALA	0.82	1.0	0.53	0.61	105	99	92	94	0.62
Digoxin	1.5	1.7	30	26	113	91	112	92	18

Table 8: Permeability in Caco-2 cells for test and control compounds

Mean IC_{50} results for the inhibition of digoxin efflux by 5-ALA or Cyclosporin A (positive control) in MDCK-MDR1 cells are summarized in study report Table 9 below. 5-ALA did not significantly inhibit Cyclosporin A transport by MDR1.

Compound ID	Mean IC_{50} (µM)
5-ALA	>300
Cyclosporin A	0.49

Table 9: IC_{50} values of digoxin transport in the presence of 5-ALA and Cyclosporin A in MDCK-MDR1 cells

Mean IC_{50} results for the inhibition of prazosin efflux by 5-ALA or Ko143 (positive control) in MDCK-BCRP cells are summarized in study report Table 10 below. 5-ALA did not significantly inhibit Ko143 transport by BCRP.

Compound ID	Mean IC_{50} (µM)
5-ALA	>300
Ko143	0.10

Table 10: IC_{50} values of prazosin transport in the presence of 5-ALA and Ko143 in MDCK-BCRP cells

Mean IC_{50} results for the inhibition of uptake transporters by 5-ALA in HEK293 cells cells are summarized in study report Table 11 below. 5-ALA was not a potent inhibitor of the human influx transporters evaluated.

Compound ID	Mean IC_{50} (µM)					
	OATP1B1	OATP1B3	OAT1	OAT3	OCT1	OCT2
5-ALA	>300	>300	>300	>300	>300	>300
Positive control	0.94	0.54	11	2.3	6.0	5.3

Table 11: IC_{50} (µM) for test and control compounds

6 General Toxicology

6.1 Single-Dose Toxicity

No adverse effects were observed in classic LD_{50} studies in mice (i.p.) and rats (p.o.) at doses up to 2500 mg/kg 5-ALA HCl.

6.2 Repeat-Dose Toxicity

Study title: 14-Day Subchronic Toxicity Study of MC-506/1 (5-ALA HCl) by Oral Administration to Sprague Dawley Rats

Study no.:	11651/98
Study report location:	Testing Facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 9, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 980923AA, 99.5% purity

Key Study Findings

This study was conducted in a room that did not emit UV light. In a 14 day toxicity study, rats were administered via gavage 0 (saline), 30, 100, or 300 mg (dissolved in water)/kg/day 5-ALA HCl once daily for 14 consecutive days. Minimal to moderate dose-related bile duct changes were observed in a few mid dose male rats and all high dose male rats. Changes included bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis/inflammation, intraductal bile plugs and peribiliar bile pigment accumulation. These findings were still present in recovery animals. The NOAEL for this study was 30 mg/kg/day.

Methods

Doses:	0 (saline), 30, 100, or 300 mg (dissolved in water)/kg/day
Frequency of dosing:	Once Daily for 14 consecutive days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/Water for injection
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	10/sex/group main study; 5/sex/group recovery
Age:	Approximately 30 days at initiation of dosing
Weight:	Weight at initiation of dosing not clearly stated.
Satellite groups:	6/sex/group (low and high dose only) for TK study
Unique study design:	The study was conducted in a dark room with a light source that did not emit UV light.
Deviation from study protocol:	None stated.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

There were no clinical signs noted.

Body Weights

There were no statistically significant effects on body weights.

Feed Consumption

There were no effects on feed consumption.

Ophthalmoscopy

Ophthalmic examinations were performed on Test Days 15 and 29. There were no ophthalmic findings observed in this study.

Hematology

Blood samples for hematology parameter analyses (including PT and TT) were collected via retro-orbital venous plexus on Test Day 13 (main study) and Test Day 29 (recovery). There were no toxicologically significant drug-related effects on hematology parameters observed in this study.

Clinical Chemistry

Blood samples for clinical chemistry parameter analyses were collected via retroorbital venous plexus on Test Day 13 (main study) and Test Day 29 (recovery). Mean total bilirubin was increased by 48% in high dose males compared to controls. This increase had reversed in recovery animals. There were no other toxicologically significant drug-related effects on clinical chemistry parameters observed in this study.

Urinalysis

Urine samples were collected overnight on fasting animals prior to blood collection. There were no drug-related adverse effects on urine parameters observed in this study. As shown in the study report Tables below, 5-ALA and urobilinogen excretion were increased with increasing dose.

Parameter	δ-aminolevulinic acid level in urine (mg/l urine) urobilinogen level in urine (mg/l urine)							
	control		30 mg/kg		100 mg/kg		300 mg/kg	
	♂	♀	♂	♀	♂	♀	♂	♀
δ-aminolevulinic acid	4.7	5.7	6.6	6.9	39.4	42.7	340.4	251.4
urobilinogen	8.6	19.0	10.2	20.7	18.1	24.8	37.8	41.8

Parameter	Increase in the δ-aminolevulinic and urobilinogen excretion with the urine (compared to the control)					
	30 mg/kg		100 mg/kg		300 mg/kg	
	♂	♀	♂	♀	♂	♀
δ-aminolevulinic acid	+40%	+21%	+738%*	+649%*	+7143%*	+4311%*
urobilinogen	+19%	+9%	+110%*	+31%	+ 340%*	+ 120%*

* = significantly different from the controls ($p \leq 0.01$)

Gross Pathology

Animals were euthanized and necropsied on Test Day 15 or 29. There were no drug-related macroscopic findings. The organs collected for microscopic evaluation are listed below.

adrenal (2)
*aorta abdominalis
blood smears (*in case of anaemia, enlarged thymus, lymphadenopathy*)
bone (*os femoris*)
bone marrow (*os femoris*)
brain
*caecum
epididymis (2)
eye with optic nerve and harderian gland (2)
gross lesions
heart
intestine, small (*duodenum, jejunum, ileum - swiss roll method*)
intestine, large (*colon, *rectum*)
kidney (2)
liver
lungs (*with mainstem bronchi*)
lymph node (*cervical*)
lymph node (*mesenteric*)
mammary gland
muscle (*skeletal, leg*)
*nerve (*sciatic*)
oesophagus
ovary (2)
pancreas
*pharynx
pituitary
prostate
salivary gland
*seminal vesicle
*skin (*left flank*)
spinal cord
spleen
stomach
*teeth (two incisors, two molars)
testicle (2)
thymus
thyroid (2) (*incl. parathyroids*)
tissue masses or tumours (*including regional lymph nodes*)
*tongue (*incl. base*)
trachea (*incl. larynx*)
urinary bladder
uterus (*incl. cervix*)
*vagina

Organ Weights

Weights were collected on the organs listed below. Relative mean liver weight was increased by 7% and 6% in mid dose males and females, respectively, and by 10% and 9% in high dose males and females, respectively.

adrenal (2)	kidney (2)	ovary (2)	testicle (2)
brain	liver	pituitary	thymus
heart	lungs	spleen	thyroid (incl. parathyroid) (1)

Adrenals, gonads and kidneys were weighed individually and identified as left or right.

Histopathology

Adequate Battery: Yes

Peer Review: Not stated

Histological Findings

Minimal to moderate dose-related bile duct changes were observed in a few mid dose male rats and all high dose male rats. Changes included bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis/inflammation, intraductal bile plugs and peribiliar bile pigment accumulation. These findings were still present in recovery animals.

Toxicokinetics

Blood samples for plasma drug level determination were collected predose and 20 min, 2, 6, and 24 hr after the first and last dose in TK animals. Mean TK results are summarized in the study report tables below. Drug systemic exposure (AUC) increased in a dose-related manner. There were no apparent gender differences in systemic exposure. Systemic exposure was similar on Day 14 compared to Day 1.

Dose level/ Sex	Test Day	Plasma elimination half-life	
		5-aminolevulinic acid (min)	Protoporphyrin-IX (min)
male rats:			
30 mg/kg	TD 1	70.8	345.3
	TD 14	88.1	460.0
300 mg/kg	TD 1	108.5	460.0
	TD 14	43.3	1205.2
female rats:			
30 mg/kg	TD 1	63.3	619.7
	TD 14	64.6	-
300 mg/kg	TD 1	143.4	435.8
	TD 14	43.1	-

AUC_{0-24h} / AUD_{0-24h} [mg * hour/L]	
5-Aminolevulinic acid	Protoporphyrin-IX
Group 2: 30 mg/kg	Group 2: 30 mg/kg
male animals	male animals
TD 1 24.1	TD 1 0.933
TD 14 34.2	TD 14 0.592 (AUD_{0-24h})
female animals	female animals
TD 1 26.7	TD 1 0.585 (AUD_{0-24h})
TD 14 23.2	TD 14 0.307 (AUD_{0-24h})
Group 4: 300 mg/kg	Group 4: 300 mg/kg
male animals	male animals
TD 1 260.2	TD 1 2.001 (AUD_{0-24h})
TD 14 233.2	TD 14 0.615 (AUD_{0-24h})
female animals	female animals
TD 1 246.8	TD 1 1.757
TD 14 224.4	TD 14 0.191

Dosing Solution Analysis

Dosing solutions were analyzed for homogeneity and concentration. Results were not clearly stated in the study report.

Study title: 14-Day Subchronic Toxicity Study of MC 506 (5-ALA HCl) by Intravenous Administration to Sprague Dawley Rats

Study no.:	10828/97
Study report location:	Testing facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, batch # 960315/3, 98.8% purity

Key Study Findings

This study was conducted with a light source that did not emit UV light. Rats were administered via intravenous injection 0, 125, 250, or 500 mg/kg/day 5-ALA HCl for 14

consecutive days. Minimal, mild, to moderated dose-related microscopic findings were observed in almost all rats at all dose levels that consisted of bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis and inflammatory infiltrates, intraductal bile plugs and peribiliar bile accumulation. These findings were still present in recovery animals. This study did not identify a NOAEL as the NOAEL is less that 125 mg/kg/day.

Methods

Doses:	0 (saline), 125, 250, or 500 mg/kg/day
Frequency of dosing:	Once daily for 14 consecutive days
Route of administration:	Intravenous
Dose volume:	10 mL/kg injected over a 60 sec period
Formulation/Vehicle:	Solution/5.7% sodium monohydrogenphosphate
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	10/sex/group main study and 5/sex/group recovery
Age:	42 (males) and 52 (females) days at the start of the 5 day adaption period
Weight:	175-194 (males) and 178-188 (females) grams at the start of the 5 day adaption period
Satellite groups:	6/sex/group (low and high dose only) for TK
Unique study design:	None
Deviation from study protocol:	None stated.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

Animals were observed at least once daily for clinical signs. Ataxia, dyspnea, and slow gait were observed in all high dose animals immediately after injection for approximately 1 minute from Day 5 through the treatment period. There were no clinical signs observed in recovery animals.

Body Weights

Body weights were recorded weekly. Mean body weights in high dose males were slightly but significantly decreased by 5% and 7% after Week 1 and Week 2, respectively. Body weights in high dose recovery males showed a tendency towards normalization. There were no other significant differences in mean body weights observed in this study.

Feed Consumption

Feed consumption was recorded weekly. There were no drug-related effects on feed consumption.

Ophthalmoscopy

Ophthalmic examinations were performed prior to dosing and just prior to sacrifice. There were no drug-related ophthalmic findings.

Hematology

Blood samples for hematology parameter analyses were collected prior to sacrifice. There were no toxicologically significant drug-related effect on hematology parameters observed in this study.

Clinical Chemistry

Blood samples for clinical chemistry parameter analyses were collected on fasted animals prior to sacrifice. Notable drug-related changes in mean clinical chemistry parameters are shown in the Study Report Table below. All clinical chemistry parameters were within normal ranges at the end of the recovery period.

Parameter	125 mg/kg		250 mg/kg		500 mg/kg	
	♂	♀	♂	♀	♂	♀
bilirubin (total)	+ 7%	+ 22%	+ 35%	+ 38%	+ 99%*	+ 44%*
cholesterol (total)	+ 14%	+ 18%	+ 36%	none	+ 43%*	+ 16%
creatinine	+ 10%	+ 12%	+ 21%*	+ 26%*	+ 15%	+ 21%*
protein (total)	+ 5%	+ 4%	+ 7%*	+ 5%	+ 7%*	+ 4%
urea (in blood)	none	none	+ 11%	+ 11%	+ 6%	+ 20%
calcium	none	none	none	none	+ 4%*	+ 4%*
ALAT activity	none	none	+ 119%	+ 70%	+ 446%*	+ 93%
ASAT activity	none	none	+ 75%	+ 30%	+ 319%*	+ 91%
LDH activity	+ 27%	none	+ 47%	+ 23%	+ 193%*	none

* = significantly different from the controls ($p \leq 0.01$)

Urinalysis

Urine samples were collected overnight on fasted animals. There were no drug-related effects on urinalysis parameters.

Gross Pathology

Main study animals were euthanized on Day 15 or 16 and recovery animals were euthanized on Day 29 and full necropsies were performed. There were no drug-related macroscopic findings.

Following organs or parts of organs of all animals were fixed in 7% buffered formalin:

adrenal (2)	*nerve (*sciatic*)
*aorta abdominalis	oesophagus
blood smears (*in case of anaemia, enlarged thymus, lymphadenopathy*)	ovary (2)
	pancreas
bone (*os femoris*)	*pharynx
bone marrow (*os femoris*)	pituitary
brain	prostate
*caecum	salivary gland
epididymis (2)	*seminal vesicle
eye with optic nerve and Harderian gland (2)	*skin (*left flank*)
	spinal cord
gross lesions	spleen
heart	stomach
injection sites (*earlier / last*)	*teeth (*two incisors, two molars*)
intestine, small (*duodenum, jejunum, ileum, Swiss roll method*)	testicle (2)
	thymus
intestine, large (*colon, *rectum*)	thyroid (2) (*incl. parathyroids*)
kidney (2)	tissue masses or tumours (*including regional lymph nodes*)
liver	
lungs (*with mainstem bronchi*)	*tongue (*incl. base*)
lymph node (*cervical*)	trachea
lymph node (*mesenteric*)	urinary bladder
mammary gland	uterus (*incl. cervix*)
*muscle (*skeletal, leg*)	*vagina

Organ Weights

Weights were collected on the organs shown below. There were no drug-related effects on organ weights.

adrenal (2)	kidney (2)	ovary (2)	testicle (2)
brain	liver	pituitary	thymus
heart	lungs	spleen	thyroid (incl. parathyroids) (1)

Adrenals, gonads and kidneys were weighed individually and identified as left or right.

Histopathology

Adequate Battery: Yes

Peer Review: Not stated.

Histological Findings: Minimal, mild, to moderated dose-related microscopic findings were observed in almost all rats at all dose levels that consisted of bile duct proliferation, enlargement of the biliary epithelium, peribiliar fibrosis and inflammatory infiltrates, intraductal bile plugs and peribiliar bile accumulation. These findings were still present in recovery animals.

Toxicokinetics

Blood samples for plasma 5-ALA determination were collected predose, 1 min, 2, 4, 8, and 24 hr after dosing. Mean systemic exposure (AUC) is summarized in the Study Report table below. 5-ALA systemic exposure increased in a dose-related fashion. There were no gender-related effects on systemic exposure. Mean systemic exposure was slight decreased on Day 14 relative to Day 1 in high dose groups.

AUC_{0-24h} / AUD_{0-24h} [mg * hour/L]			
5-Aminolevulinic acid		Protoporphyrin-IX	
Group 2: 125 mg/kg		Group 2: 125 mg/kg	
male animals		male animals	
TD 1	517.6	TD 1	0.240
TD 14	661.3	TD 14	0.587 (AUD_{0-24h})
female animals		female animals	
TD 1	832.9	TD 1	0.233
TD 14	686.1	TD 14	0.443
Group 4: 500 mg/kg		Group 4: 500 mg/kg	
male animals		male animals	
TD 1	2716.5	TD 1	0.548 (AUD_{0-24h})
TD 14	1359.6	TD 14	1.986 (AUD_{0-24h})
female animals		female animals	
TD 1	3100.9	TD 1	0.533 (AUD_{0-24h})
TD 14	2051.3	TD 14	0.305

Dosing Solution Analysis

Not performed.

Study title: 4-Week Repeated Dose Oral Toxicity Study and 4-Week Recovery Study of 5-ALA HCl in Dogs

Study no.:	DM10262
Study report location:	Not stated
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 21, 2010
GLP compliance:	Yes (Japan Guidelines)
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 10809, 99.3% purity

Key Study Findings

This study was conducted in a room that did not emit UV light. In a 4-week toxicity study, dogs were administered orally 0, 1, 3, or 10 mg/kg/day 5-ALA. The NOAEL was 3 mg/kg/day due to a combination of increased vomiting, ALT and the presence of brown pigment in the liver in high dose animals.

Methods

Doses:	0 (vehicle), 1, 3, and 10 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral using a gastric catheter
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/water for injection
Species/Strain:	Dog/Beagle
Number/Sex/Group:	N = 3/sex/main study group; 1/animal/sex/recovery group (control and high dose only)
Age:	8-9 months at initiation of dosing
Weight:	8.1-10.5 kg for males and 7.1- 8.6 kg for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None stated.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

Clinical signs were monitored twice daily during the dosing period. A dose-related increase in vomiting and vomitus was observed from occasionally in low dose animals to all high dose animals for 1 to 7 days. There were no other drug-related clinical signs noted.

Body Weights

Body weights were recorded predose and on Days 1, 4, 8, 11, 15, 18, 22, 25 and 28 for both the main study and recovery periods. There were no significant changes in body weights observed in this study.

Feed Consumption

Feed intake was recorded daily. There were no significant changes in feed consumption observed in this study.

Ophthalmoscopy

Ophthalmic examinations were performed predose and during week 4. There were no drug-related ophthalmic findings.

ECG

ECGs were performed predose and during weeks 2 and 4. There were no drug-related EKG changes.

Hematology

Blood samples for hematology parameter analyses (including PT and aPTT) were collected predose, Week 2, and prior to sacrifice. There were no toxicologically significant drug-related effects on hematology parameters.

Clinical Chemistry

Blood samples for clinical chemistry parameter analyses were collected predose, Week 2, and prior to sacrifice. Mean AST was significantly increased in high dose males by approximately 44% in Week 2 and 67% in Week 4 (relative to control animals). Mean ALT was significantly increased in high dose males by 7-fold in Week 2 and 9-fold in Week 4. Mean ALT was significantly increased in high dose females by 4-fold in Week 2 and 6-fold in Week 4. There were no toxicologically significant drug-related effects on clinical chemistry parameters. AST and ALT had returned to near predose levels in recovery animals.

Urinalysis

Urine samples were collected predose and during weeks 2 and 4. There were no apparent drug-related changes in urinalysis parameters.

Gross Pathology

All animals were euthanized at the end of the main study or recovery periods (28 days for both). Dark brown pigment was observed in all high dose males and 2/3 high dose females. There were no other drug-related macroscopic findings and no macroscopic findings were observed in recovery animals. The organs/tissues collected are listed below.

Organs/Tissue	Fix	Weight	Specimen preparation	
			HE specimen	Remark
Heart	O	O	O	Including the left ventricular papillary muscle, right ventricular wall, coronary artery, and aortic valve
Aorta (thoracic)	O	–	O	
Sternum	O	–	O	
Sternum bone marrow		–		
Femur	O L & R	–	O L	
Femoral bone marrow	O R	–	O R	
Thymus	O	O	O	
Spleen	O	O	O	
Submandibular lymph node	O L & R	–	OL	
Mesenteric lymph node	O	–	O	
Trachea	O	–	O	
Bronchus	O L & R	O Individually (L, R)	O L & R	Left anterior lobe, right posterior

Organs/Tissue	Fix	Weight	Specimen preparation	
			HE specimen	Remark
Lung				lobe
Tongue	O	–	O	
Submandibular gland	O L & R	O Total (L+R)	O L	
Parotid gland	O L & R	–	O L	
Esophagus	O	–	O	
Stomach	O	–	O	Cardia, corpus, pylorus
Duodenum	O	–	O	
Jejunum	O	–	O	
Ileum Peyer's patch	O	–	O	
Cecum	O	–	O	
Colon	O	–	O	
Rectum	O	–	O	
Liver	O	O Including gallbladder void of bile	O	Lateral left lobe, medial right lobe including gallbladder
Gallbladder			O	
Pancreas	O	O	O	
Kidney	O L & R	O Individually (L, R)	O L & R	PAS stained specimen (left) is also prepared
Bladder		–	O	
Pituitary gland		O	O	
Thyroid gland	O L & R	O Individually (L, R)	O L & R	
Parathyroid gland			O L	
Adrenal gland	O L & R	O Individually (L, R)	O L & R	
Testis	O L & R	O Individually (L, R)	O L & R	
Epididymis	O L & R	O Individually (L, R)	O L & R	
Prostate	O	O	O	
Ovary	O L & R	O Individually (L, R)	O L & R	
Uterus	O	O	O	
Vagina	O	–	O	
Brain	O	O	O	Cerebrum [frontal lobe, parietal lobe (including basal nucleus and hippocampus), occipital lobe], cerebellum., pons, medulla oblongata
Spinal cord (thoracic)	O	–	O	
Sciatic nerve	O L	–	O L	
Eye	O L & R	–	O L & R	
Optic nerve	O L & R	–	O L & R	
Lacrimal gland	O L & R	–	O L	
Skeletal muscle (femoral muscle)	O L	–	O L	
Skin (abdominal)	O	–	O	
Mammary gland (female only)	O	–	O	
Organs and tissues that were abnormal at autopsy	O	–	O	

O: Examined –: Not examined

Organ Weights

Organs weighed are shown above. Mean absolute and relative submandibular gland weight was significantly increased by approximately 30% in high dose females relative to control. This finding was not considered adverse since it was not correlated with any microscopic changes. Unable to determine if there was recovery due to n =1/sex for recovery groups. There were no other significant effects on organ weights observed in this study.

Histopathology

Adequate Battery: Yes

Peer Review: Not stated

Histological Findings

Mild to moderate yellowish brown pigmentation of the bile canaliculi, Kupffer cells and hepatocytes was observe in all high dose animals. This finding was still present in recovery animals. The pigment was thought to be due to protoporphyrin deposition in the liver as a result of excessive 5-ALA metabolism. There were no other drug-related microscopic findings observed in this study.

Toxicokinetics

Blood samples for plasma drug level determination were collected predose, and 0.5, 1, 2, 4, 8, and 24 hours after dosing. Systemic exposure (AUC) increased in a dose related manner. There were no apparent gender related differences in systemic exposure and systemic exposure was similar on Day 28 compared to Day 1.

Dosing Solution Analysis

Dosing solution analysis was performed using HPLC. All dosing solutions were within 2% of the nominal concentration.

Study title: 14-Day Subchronic Toxicity Study of MC 506 (5-ALA HCl) by Intravenous Administration to Beagle Dogs

Study no.:	11664/98
Study report location:	Testing Facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 22, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCL, Batch # 980923AA, 99.5% purity

Key Study Findings

This study was conducted in a room that did not emit UV light.

Methods

Doses:	0 (saline), 3, 9, and 27 mg/kg/day
Frequency of dosing:	Once daily for 14 consecutive days
Route of administration:	Intravenous
Dose volume:	5 mL/kg
Formulation/Vehicle:	Solution/5.7% sodium monohydrogenphosphate
Species/Strain:	Dog/beagle
Number/Sex/Group:	3/sex/group main study and 2/sex/group recovery
Age:	7 months at initiation of dosing
Weight:	6.7-8.3 (males) and 5.0-6.3 (females) kg at initiation of dosing
Satellite groups:	None
Unique study design:	Blood pressure was recorded on Days 1, 14, and 28 before and 10 min after dosing.
Deviation from study protocol:	None stated.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

Animals were observed daily after dosing for clinical signs. Emesis was observed in most high dose males at least once during the dosing period. There were no other drug-related clinical signs noted.

Body Weights

Body weights were recorded weekly. Mean body weights were decreased relative to control in mid and high dose animals. Results are summarized in the study report table below. Body weights were still decreased compared to controls at the end of recovery period (decreased by 13-15% in mid dose animals and 28-35% in high dose animals).

Group / Dose level	Test Week 1		Test Week 2	
	♂	♀	♂	♀
3 9 mg/kg b.w./day	minus 7%	minus 3%	minus 10%	minus 4%
4 27 mg/kg b.w./day	minus 5%	minus 11%	minus 19%*	minus 23%

* = significantly different from the controls ($p \leq 0.01$)

Feed Consumption

Feed consumption was recorded daily. Feed consumption was significantly decreased in high dose animals. Mean results are summarized in the study report table below. Feed intake was still decreased in high dose animals by 24-26% relative to controls at the end of the recovery period.

Dose level	Test Week 1		Test Week 2	
	♂	♀	♂	♀
27 mg/kg b.w./day	minus 14%	minus 33%*	minus 53%*	minus 75%*

* = significantly different from the controls ($p \leq 0.01$)

Ophthalmoscopy

Ophthalmic examinations were performed on Days 15 and 29. There were no drug-related ophthalmic findings.

ECG

ECGs were recorded pm Days 1, 14, and 28 before and 5 minutes after dosing. There were no drug-related effects on ECG parameters observed in this study.

Hematology

Blood samples for hematology parameter analyses were collected prior to first dose and on Days 14 and 28. There were no toxicologically significant drug-related effects on hematology parameters observed in this study.

Clinical Chemistry

Blood samples for clinical chemistry parameter analyses were collected prior to first dose and on Days 14 and 28. Drug-related changes in clinical chemistry parameters are summarized in the study report table below. ALAT activity was still elevated but showed a tendency towards normalization at the end of the recovery period. All other

clinical chemistry parameters were within normal limits at the end of the recovery period.

Parameter	Sex	Difference from the controls [%] Group / Dose	
		Group 3 9 mg/kg	Group 4 27 mg/kg
bilirubin (total)	♂	+ 17	+ 54
	♀	-	+ 60*
ALAT activity	♂	+ 442	+ 1621*
	♀	+ 689	+ 1187*
ASAT activity	♂	+ 15	+ 198*
	♀	+ 57	+ 58
LDH activity	♂	-	+ 96
	♀	-	+ 42

* = significant at $p \leq 0.01$

Urinalysis

Urine samples for urinalysis were collected prior to first dose and on Days 14 and 28. There were no drug-related changes in urinalysis parameters.

Gross Pathology

Animals were sacrificed on Day 15 (main study) or Day 29 (recovery) and full necropsies were performed. Multiple macroscopic findings were observed in high dose animals. These findings are summarized in the study report table below.

Group/ Dose	Animal No./ Sex	Affected Organ/Tissue / Findings
4 27 mg MC 506/1/ kg b.w./day	30 ♀ (R)	**lungs:** multiple white foci
	31 ♂	salivary gland (submandibular, left): dark-red to black discoloured, solid
	34 ♂ (R)	**lungs:** margin of left lung dark-red discoloured
	36 ♀	**liver:** black discoloured
	38 ♀	**uterus, gastro-intestinal tract, gastric mucosa, mucosa of urinary bladder:** reddened **liver:** black discoloured **lungs:** not collapsed, multiple haemorrhagic foci (Ø: approx. 3 mm) **intestine, small and large:** bloody contents
	39 ♀ (R)	**lungs:** multiple white foci

(R) = recovery animal

Organ Weights

Weights were collected on the organs shown below. Mean liver weights were decreased in high males (19%) relative to controls, but the decrease was not statistically significant. There were no other drug-related changes in organs weights observed in this study.

adrenal (2)	ovary (2)	heart	testicle (2)
liver	pituitary	kidney (2)	thymus
lungs	brain	spleen	thyroid

Adrenals, gonads, kidneys and thyroids were weighed individually and identified as left or right.

The organs/tissues collected are shown below. Those marked with * were not examined histologically.

adrenal (2)
*aorta abdominalis
blood smears (*in case of anaemia, enlarged thymus, lymphadenopathy*)
bone (*os femoris*)
bone marrow (*os femoris*)
brain (*coronal sections at 3 levels*)
*caecum
epididymis (2)
eye with optic nerve (2)
gall bladder
gross lesions
heart
injection site (*earlier / last*)
intestine, small (*duodenum, jejunum, ileum*)
intestine, large (*colon, *rectum*)
kidney (2)
*lacrimal gland
liver
lungs (*with mainstem bronchi*)
lymph node (*mandibular*)
lymph node (*mesenteric*)
mammary gland
*muscle (*skeletal, leg*)
*nerve (*sciatic*)
oesophagus
ovary (2)
pancreas
*pharynx
pituitary
prostate
salivary gland
*seminal duct
*skin (*left flank*)
spinal cord
spleen
stomach
*teeth (two incisors, two molars)
testicle (2)
thymus
thyroid (2) (*incl. parathyroid*)
tissue masses or tumours (*incl. regional lymph nodes*)
tongue (*incl. base*)
trachea
urinary bladder
uterus (*incl. cervix*)
vagina

Histopathology

Adequate Battery: Yes

Peer Review: Not stated

Histological Findings: The study report summary of drug-related microscopic findings is shown below.

Microscopically, intrahepatic cholestasis was noted, dose dependent in severity, in animals treated with **3, 9** or **27 mg/kg** b.w./day. This finding, characterised by bile plugs in the extended bile canaliculi, is considered to be related to the treatment with MC 506/1. At the **low dose**, the effect was only marginal (grade 1).
The finding is not completely reversible during the 14-day treatment-free recovery period at **9** and **27 mg/kg** b.w./day, however, in the **low dose group** the effect had completely subsided during the recovery period.

Special Evaluation

There were no drug-related effects on peripheral arterial blood systolic blood pressure observed in this study.

Toxicokinetics

Blood samples for plasma drug level determination were collected predose, 1 min, 2, 4, 8, and 24 hours after dosing on Days 1 and 14. Mean systemic exposure (AUC) results are shown in the study report table below. Systemic exposure increased in a dose-related fashion. There were no apparent gender differences and mean values on Day 14 were similar to Day 1 values.

$AUC_{0\text{-}24h}$ [mg * hour/L]			
5-Aminolevulinic acid		Protoporphyrin-IX	
Group 2: 3 mg/kg		Group 2: 3 mg/kg	
male animals		male animals	
TD 1	11.6	TD 1	-
TD 14	9.0	TD 14	-
female animals		female animals	
TD 1	8.2	TD 1	-
TD 14	10.2	TD 14	-
Group 4: 27 mg/kg		Group 4: 27 mg/kg	
male animals		male animals	
TD 1	71.0	TD 1	0.165
TD 14	85.6	TD 14	0.129
female animals		female animals	
TD 1	80.2	TD 1	-
TD 14	87.7	TD 14	-

Dosing Solution Analysis

Not performed.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity Study of MC-506 (5-ALA HCl) in the *S. typhimurium* Reverse Mutation Assay (*In Vitro*)

Study no.:	10112/96
Study report location:	Testing Facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 7, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 15503, 99.84% purity

Key Study Findings

5-ALA HCl was not mutagenic in the Ames Assay.

Methods

Strains: *S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537

Concentrations in definitive study: 100, 316, 1000, 3160 and 10,000 µg/plate

Basis of concentration selection: Preliminary study were no cytotoxicity was observed.

Negative control: Vehicle

Positive control: The positive controls used in this study are shown below.

a) without metabolic activation	
1. sodium azide[4] in H_2O (10 µg/plate)	TA 1535, TA 100
2. 2-nitro-9H-fluorene[4] in DMSO (10 µg/plate)	TA 98
3. 9-amino-acridine[4] in ethanol (100 µg/plate)	TA 1537
4. methyl methane sulfonate[4], MMS in DMSO (1300 µg/plate)	TA 102
b) with metabolic activation	
2-aminoanthracene[4] in DMSO (2 µg/plate)	TA 98, TA 100, TA 102, TA 1535, TA 1537

Formulation/Vehicle: Solution/water for injection

Incubation & sampling time: Aliquots of top agar (2.0 mL + 0.5 mL S9 mix or 2.5 mL without S9 mix) were mixed with 100 µL of tester strain and 100 µL of test article or vehicle. The mixture was poured over minimal bottom agar and inverted plates were incubated in the dark for 48 hours at 37ºC.

Study Validity

Two identical assays were performed. Three replicate plates were used at each concentration with and without metabolic activation for all definitive/confirmatory assays. Revertant colonies were counted by automated colony counter and/or by hand. A response was considered positive if: 1) the number of revertants is significantly increased to at least 2-fold compare to solvent control, 2) a dose-related increase is observed and 3) positive results are reproducible and the histidine independence is confirmed.

Results

There was no evidence of cytotoxicity or precipitation. Results are summarized in the study report Tables below. Positive controls produced a marked increase in the number of revertants/plate. 5-ALA was not mutagenic in this study.

Summarized data
without metabolic activation

Substance (µg/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		1st experiment				
MC 506						
10000	M	15.0	72.0	134.0	4.0	3.3
	±SD	6.2	17.3	13.0	2.6	3.6
3160	M	16.0	71.7	143.0	5.0	1.7
	±SD	1.7	1.5	11.3	2.6	1.2
1000	M	14.0	67.3	101.7	4.0	2.3
	±SD	3.6	3.8	18.2	2.0	1.2
316	M	18.3	76.0	144.3	5.0	3.7
	±SD	4.7	1.7	18.6	2.0	2.1
100	M	16.0	78.0	120.0	2.3	1.3
	±SD	3.5	13.1	35.6	1.2	0.6
Negative control: 100 µl/plate						
	M	18.0	70.7	170.0	6.7	1.7
	±SD	4.4	11.4	13.7	1.5	0.6
Positive control substance:		2-Nitro-fluorene	Sodium azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acricine
Concentration: (µg/plate)		10	10	1300	10	100
	M	713.0	1291.0	2284.0	1053.3	1563.3
	±SD	122.5	85.9	314.0	115.0	59.9

M = mean number of revertants
SD = standard deviation

Summarized data
without metabolic activation

Substance (µg/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		2nd experiment				
MC 506						
10000	M	23.3	90.7	260.3	10.0	2.7
	±SD	3.1	2.3	8.1	5.3	1.2
3160	M	18.7	88.3	257.0	10.3	3.0
	±SD	0.6	2.3	44.2	2.1	2.0
1000	M	19.0	87.0	266.0	7.0	2.0
	±SD	1.0	6.6	11.3	1.7	1.7
316	M	18.7	100.0	247.3	9.3	3.7
	±SD	1.5	1.0	22.3	4.2	2.3
100	M	21.3	89.3	234.3	6.3	2.7
	±SD	1.5	2.3	31.1	2.5	2.1
Negative control: 100 µl/plate						
	M	23.7	93.0	272.7	10.0	8.3
	±SD	1.5	4.4	24.7	3.6	0.6
Positive control substance:		2-Nitro-fluorene	Sodium-azide	Methyl methane sulfonate	Sodium-azide	9-Amino-acridine
Concentration: (µg/plate)		10	10	1300	10	100
	M	1191.7	1723.7	2156.3	1429.3	2880.0
	±SD	122.8	321.1	172.3	136.5	714.0

M = mean number of revertants
SD = standard deviation

Summarized data
with metabolic activation

Substance (µg/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		1st experiment				
MC 506						
10000	M	20.3	86.3	165.0	2.7	0.7
	±SD	4.0	12.4	15.1	1.2	1.2
3160	M	21.7	107.3	206.7	4.7	0.7
	±SD	3.2	4.7	43.7	2.1	1.2
1000	M	18.0	105.0	261.7	6.3	1.3
	±SD	3.6	6.2	27.0	3.5	0.6
316	M	22.3	115.7	220.7	5.3	2.7
	±SD	4.6	5.0	31.7	2.5	0.6
100	M	25.0	113.7	269.7	3.3	2.7
	±SD	6.1	10.0	6.4	1.5	1.2
Negative control: 100 µl/plate						
	M	21.7	117.0	287.0	4.0	3.7
	±SD	3.5	7.6	25.5	1.7	1.5
Positive control substance:			2-Aminoanthracene			
Concentration: (µg/plate)		2	2	2	2	2
	M	588.3	677.3	188.0	88.0	24.0
	±SD	60.5	111.5	22.3	29.9	6.2

M = mean number of revertants
SD = standard deviation

Summarized data
with metabolic activation

Substance (µg/plate)		revertants per plate TA 98	TA 100	TA 102	TA 1535	TA 1537
		2nd experiment				
MC 506						
10000	M	26.3	93.3	299.3	6.7	3.0
	±SD	5.7	3.5	23.9	2.5	1.7
3160	M	23.3	92.7	324.0	8.3	4.7
	±SD	3.5	3.8	2.6	0.6	1.5
1000	M	24.7	110.7	349.7	9.0	4.3
	±SD	3.1	4.6	3.8	2.0	3.2
316	M	27.3	97.3	340.7	7.0	3.7
	±SD	2.1	5.5	2.1	3.6	1.5
100	M	27.7	104.7	311.3	9.0	4.3
	±SD	5.1	3.8	31.7	1.0	2.1
Negative control: 100 µl/plate						
	M	35.7	99.7	332.0	12.3	6.3
	±SD	2.1	5.5	12.3	3.5	2.1
Positive control substance:		2-Aminoanthracene				
Concentration: (µg/plate)		2	2	2	2	2
	M	936.3	1071.0	808.3	169.0	100.0
	±SD	21.5	124.8	92.7	17.1	1.0

M = mean number of revertants
SD = standard deviation

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Mutagenicity Study of MC-506 in Mammalian Cells (V79) in the *In Vitro* Gene Mutation Assay

Study no.:	10113/96
Study report location:	Testing Facility
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 31, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 15503, 99.84% purity

Key Study Findings

5-ALA HCl was not mutagenic in the hamster V79 cell gene mutation assay.

Methods

Cell line:	V79
Concentrations in definitive study:	312.5, 625, 1250, 2500 and 5000 µg/mL
Basis of concentration selection:	Lack of cytotoxicity in a preliminary study at doses up to 1000 µg/mL 5-ALA HCl.
Negative control:	Vehicle
Positive control:	Ethyl Methanesulfonate (EMS)
Formulation/Vehicle:	Solution/water for injection
Incubation & sampling time:	Cells were incubated 24 h without and 4 h with S9 mix.

Study Validity

Two assays were performed. Triplicate cultures were used at each concentration with and without metabolic activation for both assays. Approximately 1.5 million cells were exposed to each concentration in a culture dish. The media was replaced after 4 h exposure with S9 mix and all cultures were incubated in the dark for a total of 24 h. Cells were then trypsinized and relative plating efficiency (PE1) was determined for each dose to measure toxicity. Cells were replated and further incubated until Day 8. Afterwards cells were harvested, replated to 1 million cells/dish in 6-thioguanine containing media for selection of mutants (5 replicate plates) or without 6-thioguanine for determining relative plating efficiency (PE2). Plates were fixed and stained after 8 days (without 6-thioguanine) or 12 days (with 6-thioguanine). The assay is considered positive if negative and positive controls are within historical ranges and a dose-dependent increase in mutation frequency is observed in both independent experiments at similar concentrations to at least 2-fold solvent control.

Results

As shown in the study report Tables below, cytotoxicity was observed at the 2500 and 5000 µg/mL concentrations as demonstrated by lower PE1 values. A marked increase in mutation frequency was observed with cells incubated in the presence of EMS with or without S9 mix. Mutation frequency was not increased by 2-fold at any 5-ALA HCl concentration.

MC 506
Results of the *in vitro* HPRT point mutation assay
(1st experiment) without S9 mix

TABLE 2

Compound (µg/ml medium)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^6 (e)
Control: Aqua ad iniectabilia											
0	1.0	1 : 20	0.88	0.91	5	8	6	6	8	4.55	7.3
MC 506											
312.5	1.0	1 : 20	0.91	1.04	6	11	10	4	6	5.20	7.1
625	1.0	1 : 20	0.82	0.89	5	5	5	7	4	4.45	5.8
1250	1.0	1 : 20	0.86	0.97	6	13	7	4	8	4.85	7.8
2500	1.0	1 : 20	0.49	0.96	2	4	7	2	4	4.80	4.0
5000	1.0	change of medium	0.23	1.03	10	7	10	7	11	5.15	8.7
EMS											
600	1.0	1 : 20	0.03	0.36	332	284	314	271	289	1.80	827.8
700	1.0	1 : 20	0.01	0.32	380	368	303	333	348	1.60	1082.5

for a, b, c, d, and e please see page 18

MC 506
Results of the *in vitro* HPRT point mutation assay
(2nd experiment) without S9 mix

TABLE 3

Compound (µg/ml medium)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^6 (e)
Control: Aqua ad iniectabilia											
0	1.0	1 : 20	0.99	0.93	8	13	11	10	5	4.85	10.1
MC 506											
312.5	1.0	1 : 20	1.05	1.02	12	11	15	12	11	5.10	12.0
625	1.0	1 : 20	0.82	0.80	8	8	7	6	7	4.00	9.0
1250	1.0	1 : 20	0.82	0.88	7	3	9	11	7	4.40	8.4
2500	1.0	1 : 20	0.92	0.94	6	5	10	5	6	4.70	6.8
5000	1.0	change of medium	0.25	0.83	0	0	0	0	1	4.15	0.2
EMS											
600	1.0	1 : 20	0.05	0.36	314	366	292	270	326	1.80	871.1
700	1.0	1 : 20	0.01	0.31	232	326	318	297	349	1.55	981.9

for a, b, c, d, and e please see page 18

MC 506
Results of the *in vitro* HPRT point mutation assay
(1st experiment) with S9 mix

TABLE 4

Compound (µg/ml medium)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^6 (e)
Control: Aqua ad iniectabilia											
0	1.0	1 : 20	0.96	0.85	1	5	2	5	9	4.25	5.2
MC 506											
312.5	1.0	1 : 20	0.94	0.91	12	14	13	4	3	4.55	10.1
625	1.0	1 : 20	0.97	0.83	9	8	10	9	8	4.15	10.6
1250	1.0	1 : 20	0.69	0.92	5	4	12	8	2	4.60	6.7
2500	1.0	1 : 20	0.003	1.03	2	2	4	5	5	5.15	3.5
5000	1.0	change of medium	0.00	0.52	2	*	*	*	*	2.60	3.8
DMBA											
20	1.0	1 : 20	0.87	0.93	322	229	410	382	205	4.65	332.9
30	1.0	1 : 20	0.83	0.97	395	411	419	365	390	4.85	408.2

* not sufficient material available for evaluation

for a, b, c, d, and e please see page 18

MC 506
Results of the *in vitro* HPRT point mutation assay
(2nd experiment) with S9 mix

TABLE 5

Compound (µg/ml medium)	Solvent (% in the medium, v/v)	Dilution at subculture during expression time	Plating Efficiencies PE_1 (a)	Plating Efficiencies PE_2 (b)	Thioguanine-resistant colonies (c)					Total number of viable cells exposed to thioguanine x 10^6 (d)	Mutation frequency x 10^6 (e)
Control: Aqua ad iniectabilia											
0	1.0	1 : 20	0.84	0.81	9	11	8	12	11	4.05	12.6
MC 506											
312.5	1.0	1 : 20	0.67	1.06	15	15	12	9	12	5.30	11.9
625	1.0	1 : 20	0.85	0.84	11	16	11	14	13	4.20	15.5
1250	1.0	1 : 20	0.91	1.04	5	2	6	3	8	5.20	4.6
2500	1.0	change of medium	0.00	0.50	0	*	*	*	*	0.50	0.0
5000	1.0	change of medium	0.00	0.17	*	*	*	*	*	-	-
DMBA											
20	1.0	1 : 20	0.05	0.31	365	313	338	303	354	1.55	1079.4
30	1.0	1 : 20	0.17	0.70	404	363	359	356	410	3.50	540.6

* not sufficient material available for evaluation

for a, b, c, d, and e please see page 18

Study title: *In Vitro* Assessment of the Clastogenic Activity of MC506 (5-ALA) in Cultured Human Peripheral Lymphocytes

Study no.:	10114/96
Study report location:	Testing facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 31, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 15503, 99.84% purity

Key Study Findings

5-ALA HCl was not mutagenic in the human peripheral lymphocyte chromosomal aberration assay.

Methods

Cell line:	Peripheral human lymphocytes
Concentrations in definitive study:	250, 500, 1000, 2000, and 4000 µg/mL for 4 h with and without S9 mix incubations. 125 to 2000 µg/mL for 24 h without S9 mix incubations
Basis of concentration selection:	Preliminary cytotoxicity study.
Negative control:	Vehicle
Positive control:	Mitomycin C without and cyclophosphamide with S9 mix
Formulation/Vehicle:	Solution/water for injection
Incubation & sampling time:	PHA treated lymphocytes were incubated for 4 hours at 37ºC in both the presence and absence of metabolic activation, and for 24 hours at 37ºC in the absence of activation. The 4-hr incubation cultures were washed, fed untreated culture media, and allowed to incubate 20 additional hours at 37ºC. Colcemid (0.1 µg/mL) was added the last 2 hours of incubation. All the above incubations were in the dark. Cells were then harvested, fixed, dropped onto glass slides, and stained with 10% Giemsa.

Study Validity

Two assays were performed. Duplicate cultures were used at each concentration with and without metabolic activation for both assays. Mitotic index (MI) was determined by examination of at least 1000 cells/culture. A total of 100 metaphases/dose level at the highest analyzable concentrations were then evaluated manually using a light

microscope for the presence of chromosomal aberrations. The positive response criteria was not clearly stated.

Results

Results are summarized in the study report tables below. The number of cells with chromosomal aberrations excluding gaps were within historical ranges for negative control cultures and were significantly increased in positive control cultures. The number of cells with chromosomal aberrations excluding gaps were significantly increased at the 4000 µg/mL dose level with S9 mix. Severe cytotoxicity was also observed at the 4000 µg/mL dose level with S9 mix, thus the increase was not considered a positive response. There were no other drug-related significant increases observed in this study.

Table 2

MC 506
Chromosome analysis in human peripheral lymphocytes in vitro
S U M M A R Y

Treatment (µg/ml medium)	4-h exposure			24-h exposure		
	Mitotic index*	Number of metaphases scored	% of cells with aberrations excluding gaps	Mitotic index*	Number of metaphases scored	% of cells with aberrations excluding gaps
without metabolic activation						
aqua ad iniectabilia						
0	1.00	200	0.5	1.00	200	2.0
MC 506						
125	-	-	-	0.91	200	1.5
250	-	-	-	0.98	200	4.0
500	0.93	200	3.0	0.69	200	2.0
1000	0.95	200	3.0	0.28	152	5.9
2000	0.43	200	4.0	-	#	-
4000	-	#	-	-	-	-
Mitomycin C						
0.1	0.93	200	9.5##	0.64	200	19.0##

* = mitotic index: number of metaphases/1000 cells; negative control = 1.00
\# = no metaphases of sufficient quality for evaluation due to cytotoxicity of MC 506
= significantly different from negative control (p ≤ 0.05)

Table 2 MC 506
Chromosome analysis in human peripheral lymphocytes in vitro
S U M M A R Y

Treatment (µg/ml medium)	4-h exposure		
	Mitotic index*	Number of metaphases scored	% of cells with aberrations excluding gaps
with metabolic activation			
aqua ad iniectabilia			
0	1.00	200	1.5
MC 506			
250	-	-	-
500	0.92	200	1.5
1000	0.84	200	1.5
2000	0.59	200	1.0
4000	0.03	101	17.8#
Cyclophosphamide			
10.0	0.64	200	13.0#

* = mitotic index: number of metaphases/1000 cells; negative control = 1.00
\# = significantly different from negative control (p = 0.05)

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus Test of 5-ALA in Bone Marrow Cells of the NMRI Mouse following Oral Administration

Study no:	17551/03
Study report location:	Testing Facility
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 26, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # M30906 AC, 101% purity

Key Study Findings

5-ALA HCl was not mutagenic in the mouse micronucleus assay. The study was performed using subdued light to prevent phototoxicity from occurring.

Methods

Doses in definitive study:	400, 800 and 1600 mg/kg
Frequency of dosing:	once
Route of administration:	gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/0.8% hydroxypropylmethylcellulose gel
Species/Strain:	Mice/NMRI
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	Lethality observed at the 2000 mg/kg dose level in a dose range-finding study.
Negative control:	Vehicle
Positive control:	27 mg/kg cyclophosphamide administered once i.p.

Study Validity

Bone marrow smears were prepared from each femur. The slides were fixed and H&E stained. Two thousand polychromatic erythrocytes (PCE) were scored for incidence of micronuclei. One thousand erythrocytes were scored for PCE/normochromatic erythrocyte ratio.

Results

Results are summarized in the study report table below. Vehicle control values were within historical ranges. The frequency of micronucleated PCEs was significantly increased in positive control treated animals. Treatment with 5-ALA did not increase the frequency of micronucleated PCEs in this study.

Table 1 SUMMARISED DATA OF THE MUTAGENICITY STUDY

Test item mg/kg b.w., p.o.	Sampling time (h)	Number of polychromatic erythrocytes scored per group*	Ratio PCE/NCE#			Micronucleated polychromatic erythrocytes mean frequency per 1000 PCE			Significances
			males	females	mean*	males	females	mean*	
5-ALA-HCl									
0	24	20000	1.29	1.35	1.32	1.1	1.0	1.1	-
400	24	20000	1.25	1.29	1.27	0.7	1.2	1.0	n.s.
800	24	20000	1.11	1.30	1.20	1.2	0.9	1.1	n.s.
1600	24	20000	1.09	0.89	0.99	0.9	0.7	0.8	n.s.
0	48	20000	1.07	0.88	0.97	0.5	1.0	0.8	-
1600	48	20000	0.86	0.90	0.88	0.2	0.8	0.5	n.s.
Cyclophosphamide									
27 mg/kg b.w., i.p.	24	20000	1.10	1.02	1.06	11.4	8.9	10.2	s.

s. significant at $p \leq 0.05$ PCE polychromatic erythrocytes
n.s. not significant at $p \leq 0.05$ NCE normochromatic erythrocytes
* males and females combined # per 1000 counted cells
m male f female

7.4 Other Genetic Toxicity Studies

5-ALA was not mutagenic in the standard genotoxicity battery when performed in the dark or under subdued light. However, there have been positive findings in non-GLP nonstandard genetic toxicology studies reported in the literature and when genotoxicity studies with 5-ALA have been performed in the light. Overall, the studies suggest that PpIX may have mutagenic potential.

8 Carcinogenicity

Carcinogenicity study reports were not submitted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and early embryonic development study reports were not submitted.

9.2 Embryonic Fetal Development

Study title: Embryo-Fetal Development Study of 5-Aminolevulinic Acid Hydrochloride in Rabbits

Study no.:	DM10264
Study report location:	Conducting Laboratory
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 2, 2010
GLP compliance:	Yes (Japanese Guidelines)
QA statement:	Yes
Drug, lot #, and % purity:	5-ALA HCl, Batch # 10809, 99.3% purity

Key Study Findings

Pregnant rabbits were administered orally 0 (vehicle), 15, 50, or 150 mg/kg/day 5-ALA daily from Gestation Day 6-18. Decreased maternal feed intake and weight loss were observed at the 150 mg/kg/day dose. There were no adverse effects in maternal reproduction function and embryo-fetal development observed in this study. The NOAEL for maternal toxicity was 50 mg/kg/day and the NOAEL for maternal reproduction function and embryo-fetal development was 150 mg/kg/day.

Methods

Doses:	0 (vehicle), 15, 50, or 150 mg/kg/day
Frequency of dosing:	Single Daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	Solution/Sterile water
Species/Strain:	Rabbit/Kbl:NZW
Number/Sex/Group:	20/F/group
Satellite groups:	None
Study design:	Successfully mated on Gestation Day 0. Administered drug or vehicle from Gestation Day 6-18. C-sections were performed on Gestation 29.
Deviation from study protocol:	There were no major protocol deviations.

Observations and Results

Mortality

There were no deaths among maternal animals. One animal each in the 50 and 150 mg/kg groups aborted on Gestation Day 21. The abortion in the high dose animal was associated with significant and sustained decreased feed intake suggesting the abortion was due to malnutrition. The cause of abortion in the mid dose animal was unknown. The abortion rate for historical controls was 2 per 18 control animals, thus the abortions were not considered drug-related.

Clinical Signs

Clinical signs are summarized in study report Table 1 below. There was a dose-related increase in scant or no feces that was associated with reduction in feed intake. Reddish brown urine was attributed to drug excretion.

Table 1 Study for the effects of 5-aminolevulinic acid hydrochloride on embryo-fetal development in rabbits

Clinical Observations

Generation: F0 Sex: Female Gestation period

Group	Control 0 mg/kg/day	Low 15 mg/kg/day	Mid 50 mg/kg/day	High 150 mg/kg/day
Number of animals	20	20	21	20
Number of dams	20	20	15	19
Number of pregnant animals at Cesarean section	20	20	14	18
Number of dams with abnormal signs	3	3	6	12
Number of dams with the following signs				
Scant or no feces	3	3	6	11
Scant or no urine	0	1	1	1
Reddish brown urine	0	0	0	8
Number of dams which aborted	0	0	1	1
Number of dams with abnormal signs	-	-	1	1
Number of dams with the following signs				
Scant or no feces	-	-	0	1
Scant or no urine	-	-	0	1
Reddish brown urine	-	-	1	1
Number of animals which did not become pregnant	0	0	6	1
Number of animals with abnormal signs	-	-	2	0
Number of animals with the following signs				
Scant feces	-	-	2	0
No urine	-	-	1	0

Body Weight

Mean body weight gain was significantly decreased in the 150 mg/kg/day group from Gestation Day 10 to the end of study. Mean body weight gain for the other treatment groups was similar to control.

Feed Consumption

Mean feed consumption was significantly decreased in the 150 mg/kg/day group from Gestation Day 8-19. Mean feed consumption for the other treatment groups was similar to control.

Toxicokinetics

Blood samples (n = 5/group) for plasma drug level determination were collected at 0.5, 1, 2, 4, 8, and 24 h after dosing on the first and last dosing days. Mean TK results are summarized in study report Table 5 below. Systemic exposure (AUC) increased in a greater than dose proportional fashion. There were no gender related differences in TK parameters. Mean TK results were similar on Day 18 compared to Day 6.

Table 5 Study for the effects of 5-aminolevulinic acid hydrochloride on embryo-fetal development in rabbits

Plasma Concentrations and Toxicokinetic Parameters of 5-aminolevulinic acid

Generation: F0 Sex: Female Day 6 of gestation

	Plasma concentration (μg/mL) Time post-dosing						C_{max}	T_{max}	$AUC_{0\text{-}24h}$
Group	0.5 h	1 h	2 h	4 h	8 h	24 h	(μg/mL)	(h)	(μg·h/mL)
Low									
15 mg/kg/day	1.73 ± 0.18	1.52 ± 0.45	0.600 ± 0.142	0.0711 ± 0.0876	BLQ ± BLQ	BLQ ± BLQ	1.75 ± 0.22	0.7 ± 0.3	3.13 ± 0.47
Mid									
50 mg/kg/day	6.23 ± 2.66	9.02 ± 0.41	4.62 ± 1.62	0.454 ± 0.176	0.0210 ± 0.0060	BLQ ± BLQ	9.05 ± 0.36	0.9 ± 0.3	18.4 ± 1.7
High									
150 mg/kg/day	28.5 ± 13.0	45.2 ± 16.2	24.6 ± 5.8	4.25 ± 1.72	0.123 ± 0.063	0.0558 ± 0.0311	45.2 ± 16.2	1.0 ± 0.0	99.6 ± 25.6

Day 18 of gestation

Group	Plasma concentration (µg/mL) Pre-dosing	Time post-dosing 0.5 h	1 h	2 h	4 h	8 h	24 h	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-24h} (µg·h/mL)
Low										
15 mg/kg/day	0.00990 ± 0.00557	2.00 ± 0.40	1.59 ± 0.29	0.513 ± 0.128	0.0435 ± 0.0176	BLQ ± BLQ	0.00724 ± 0.00674	2.00 ± 0.40	0.5 ± 0.0	3.20 ± 0.50
Mid										
50 mg/kg/day	0.0157 ± 0.0090	9.00 ± 1.49	10.6 ± 1.2	4.54 ± 0.74	0.664 ± 0.294	0.0245 ± 0.0103	0.00928 ± 0.00632	10.6 ± 1.2	1.0 ± 0.0	21.6 ± 1.1
High										
150 mg/kg/day	0.0345 ± 0.0507	24.6 ± 11.6	34.7 ± 11.4	23.7 ± 5.8	5.79 ± 1.64	0.755 ± 0.809	0.0257 ± 0.0285	35.6 ± 10.2	1.2 ± 0.4	99.1 ± 14.1

Mean ± S.D. (15 and 150 mg/kg/day: n = 5, 50 mg/kg/day: n = 4)

Dosing Solution Analysis

Dosing solutions were shown to be within 10% of the nominal value and stable after 8 days when store in an air-tight, light shielded container.

Necropsy

There were no drug-related macroscopic findings observed in dams.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no drug-related effects on the number of corpora lutea, number of implantations, number of surviving fetuses, sex ratios of surviving fetuses, fetal weight, and placental weight.

Offspring (Malformations, Variations, etc.)

There were no drug-related external, visceral or skeletal abnormalities/variations observed in this study.

ADDITIONAL REPRODUCTIVE TOXICOLOGY DATA

A number of reproductive toxicology studies were reported in the literature. However, these studies were generally non-GLP, not adequately designed and/or only summaries were provided. These studies were not reviewed. A number of studies suggested that 5-ALA administration plus direct light exposure to reproductive organs exerts embryotoxic activity. This is likely due to the phototoxicity of PpIX. Reproductive organs will not be exposed to direct light during or after glioma surgery.

9.3 Prenatal and Postnatal Development

Prenatal and postnatal development study reports were not submitted.

10 Special Toxicology Studies

Phototoxicity Study of MC-506 (5-ALA HCl) by Intravenous Administration to Mice (Study # 10121/96)

In this GLP study, the shoulder region of female NMRI mice (n = 5/group) was clipped and depilated the day before drug administration. On the day of drug administration, animals were administered via intravenous injection 0 (vehicle) 250, or 750 mg/kg 5-ALA HCl. Positive control animals were administered orally 40 mg/kg 8-methoxypsoralen. Animals were then exposed to 1 h UV-irradiation (30 J UV-A/cm^2

and 0.3 J UV-B/cm^2) using a mercury lamp at a distance of 80 cm at 4 h and 24 h after dosing. Skin was evaluated for the presence of edema at 24, 48 and 72 h post UV-irradiation. Animals were euthanized after 72 h. The eye and clipped skin was collected and examined microscopically. These tissues were collected as soon as possible after an animal died and examined microscopically.

There were no phototoxicity reactions in vehicle control animals. None of the positive control animals died, but all 5 exhibited grade 1-3 edema. In 4 h post UV-irradiation groups, 2 low-dose animals died between 48-72 h post-irradiation and all 5 high-dose animals died within 24 h post irradiation. The 3 surviving low dose mice exhibited grade 2 edema. In 24 h post UV-irradiation groups, no phototoxic effects were observed in low dose animals. All 5 high dose animals exhibited grade 1-2 edema at 24 h post irradiation, 3 animals exhibited grade 1-2 edema at 48 h post irradiation, and 2 animals exhibited grade 2-3 edema at 72 h post irradiation. Inflammatory reactions were observed in the eyelids and the shoulder skin of all positive control animals, at both dose levels in animals from 4 h post UV-irradiation groups, and at the high dose level in animals from the 24 h post UV-irradiation group.

11 Integrated Summary and Safety Evaluation

The Sponsor submitted this application as a 505(b)2 submission. From a nonclinical perspective, the submitted nonclinical study reports would have supported a standard NDA application. The nonclinical safety studies identified the target organs of toxicity as being the liver and skin (if exposed to direct light following 5-ALA administration due to PpIX-induced phototoxicity).

The Sponsor submitted only one reproductive and developmental toxicity study report. In a rabbit embryo-fetal developmental toxicity study, the NOAEL for maternal toxicity was 50 mg/kg/day and the NOAEL for maternal reproduction function and embryo-fetal development was 150 mg/kg/day for orally administered 5-ALA HCl. The lack of Segment I and III reproductive toxicology studies is not an issue for this application considering the intended patient population and that 5-ALA is an endogenous compound.

From a nonclinical perspective, the available nonclinical data and the available clinical data make this application approvable.

12 Appendix/Attachments

None.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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04/28/2017

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