

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-415

PHARMACOLOGY REVIEW(S)

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION
Memorandum**

NDA NUMBER: **21-415**
SERIAL NUMBER: **000 AZ**
DATE RECEIVED BY CENTER: **5/27/04**
DRUG NAME: **Methyl 5-aminolevulinate hydrochloride**
INDICATION: **Actinic keratoses**
SPONSOR: **PhotoCure ASA**
REVIEW DIVISION: **Division of Dermatologic and Dental
Drug Products (HFD-540)**
PHARM/TOX REVIEWER: **Jiaqin Yao, Ph.D.**
PHARM/TOX SUPERVISOR: **Paul C. Brown, Ph.D.**
DIVISION DIRECTOR: **Jonathan Wilkin, M.D.**
PROJECT MANAGER: **Melinda Harris**

Date of review submission to Division File System (DFS): June 24, 2004

The sponsor resubmitted this application in response to the Approvable Letter dated January 16, 2004. The NDA was approvable from a pharm/tox perspective and this current submission does not include any new nonclinical studies. Therefore, this NDA is approvable from a pharm/tox perspective and no additional nonclinical studies are recommended at this time. The labeling is identical to that previously mutually agreed to.

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this page is the manifestation of the electronic signature.**

/s/

Jiaqin Yao
6/24/04 11:06:52 AM
PHARMACOLOGIST

Paul Brown
6/24/04 05:35:58 PM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-415
SERIAL NUMBER: 000 (response to approvable letter)
DATE RECEIVED BY CENTER: 7/17/03
DRUG NAME: methyl 5-aminolevulinate hydrochloride
INDICATION: actinic keratoses
SPONSOR: PhotoCure ASA
DOCUMENTS REVIEWED: Vol. 1-2
REVIEW DIVISION: Division of Dermatologic and Dental
Drug Products (HFD-540)
PHARM/TOX REVIEWER: Paul C. Brown, Ph.D.
PHARM/TOX SUPERVISOR: Abby Jacobs, Ph.D.
DIVISION DIRECTOR: Jonathan Wilkin, M.D.
PROJECT MANAGER: Melinda Harris

Date of review submission to Division File System (DFS): October 1, 2003

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EXECUTIVE SUMMARY

1. Recommendations

a. Recommendation on approvability

The NDA is approvable from a pharm/tox perspective.

b. Recommendation for nonclinical studies

No additional nonclinical studies are recommended at this time.

c. Recommendations on labeling

i. The

may be removed if the medical officer finds that the human data sufficiently addresses the hepatotoxic potential of the drug product.

ii.

iii

iv. The two sentences describing the literature information on the genotoxic potential of ALA should be maintained in the label.

2. Summary of nonclinical findings

a. Brief overview of nonclinical findings

New nonclinical data in this submission includes the histopathology data from the 4 dose topical drug plus light minipig study. This data does not show any clear histopathological evidence of systemic toxicity.

b. Pharmacologic activity

No new information on the pharmacologic activity of methyl-ALA was included in the current submission.

c. Nonclinical safety issues relevant to clinical use

The animal toxicology data indicates that methyl ALA has the potential to induce hepatotoxicity at sufficient doses. The NDA did not originally contain sufficient data on the monitoring of liver enzymes in the human studies.

If the human data in the current submission is considered sufficient to address the potential for hepatotoxicity then the may be removed from the label.

PHARMACOLOGY/TOXICOLOGY REVIEW**3.1 INTRODUCTION AND DRUG HISTORY****NDA number:** 21-415**Review number:****Sequence number/date/type of submission:** 000/ 16 July 2003/ B2 complete response to approvable letter**Information to sponsor:** Yes () No (X)**Sponsor and/or agent:** Photocure ASA

Clementi & Associates, Ltd., Agent

Manufacturer for drug substance: —**Reviewer name:** Paul C. Brown**Division name:** Division of Dermatologic and Dental Drug Products**HFD #:** 540**Review completion date:** October 1, 2003**Drug:**

Generic name: methyl 5-aminolevulinate hydrochloride, 5-ALA methyl ester

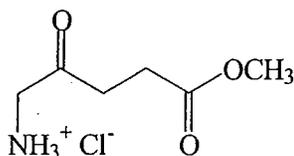
Code name: P-1202

Chemical name: 5-aminolevulinic acid methyl ester hydrochloride, methyl 5-aminolevulinate hydrochloride, 5-amino-4-oxo-pentanoic acid methyl ester hydrochloride, methyl 5-amino-4-oxo-pentanoate hydrochloride, methyl 5-amino-4-oxo-valeroate hydrochloride, 5-amino-4-oxo-valeric acid methyl ester hydrochloride

CAS registry number: 79416-27-6

Molecular formula/molecular weight: C₆H₁₂NO₃Cl; MW=181.62

Structure:

**Relevant INDs/NDAs/DMFs:**

IND 59,756

Metvix for PDT of actinic keratoses

Drug class: photodynamic therapy agent**Indication:** for the photodynamic therapy of non-hyperkeratotic actinic keratoses

Clinical formulation:

<u>Ingredient</u>	<u>percent (w/w)</u>	<u>mg/g</u>
methyl 5-aminolevulinate hydrochloride equivalent to methyl 5-aminolevulinate	16.8	168
glyceryl monostearate, BP		
cetostearyl alcohol, NF		
polyoxyl stearate, NF		
methylparaben, NF		
propylparaben, NF		
edetate disodium, USP		
glycerin, USP		
white petrolatum, USP		
cholesterol, NF		
isopropyl myristate, NF		
peanut oil, NF		
refined almond oil, Ph. Eur.		
oleyl alcohol, NF		
Total	100	1000

The sponsor has previously indicated that the peanut and almond oils are processed to adequately denature proteins to which patients might be allergic.

Route of administration: topical to lesion surface

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Introduction:

The original pharm/tox review of this NDA found it to be approvable. The only pharm/tox comment in the approvable letter (9/19/02) was: "Tissues collected in the repeated dose dermal toxicology study in minipigs should be evaluated histologically." The histopathology data from the repeat dose minipig study are included in the current submission.

The label for the drug in the approvable letter included

studies was not submitted. The sponsor was told that the _____ may be removed if adequate human data was submitted.

The label for the drug in the approvable letter also had two sentences describing some published literature information on the genotoxicity of ALA. The sponsor requests that this be removed. This issue is discussed below.

Studies reviewed within this submission:

Repeated dose dermal toxicity study with integral photoactivation in minipigs (Study no.: 35635) *Note: This study was previously reviewed in the original review of this NDA, but complete histopathology was not submitted at that time. The histopathology of the collected tissues has now been submitted and so these have been reviewed below.*

Studies not reviewed within this submission:

None

3.2 PHARMACOLOGY**3.2.1 Brief summary**

Methyl-ALA (P-1202) is taken up into cells and after de-esterification to ALA is metabolized to form PpIX, a photoreactive compound that is an intermediate in the synthesis of heme. Illumination of PpIX results in fluorescence and the production of reactive oxygen species with resulting cellular damage and death. Studies *in vivo* and *in vitro* have demonstrated this effect and a dose-related production of PpIX after topical treatment with 2% and 20% concentrations of the active ingredient in various vehicle creams. However, no difference was seen in the time courses and quantity of fluorescence following treatment with 10% and 20% concentrations. Maximum fluorescence was reported to be at 10-12 hours. *In vitro*, there was no difference in cellular uptake between ALA and P-1202. *In vivo*, PpIX localized in epidermis after topical application of P-1202.

3.2.2 Primary pharmacodynamics

No new information on the primary pharmacodynamics of methyl-ALA has been submitted at this time.

3.2.3 Secondary pharmacodynamics

No new information on the secondary pharmacodynamics of methyl-ALA has been submitted at this time.

3.2.4 Safety pharmacology

No safety pharmacology studies were performed.

3.2.5 Pharmacodynamic drug interactions

No studies on the pharmacodynamic interaction of methyl-ALA with other drugs have been performed.

3.3 PHARMACOKINETICS/TOXICOKINETICS**3.3.1 Brief summary**

After topical application of ALA or methyl-ALA (20% in Unguentum Merck), both ALA and methyl-ALA produced porphyrins in normal mouse skin, mostly localized in the epidermis, epithelial hair follicles and sebaceous glands. There did not appear to

be any difference in skin fluorescence between ALA and methyl-ALA; both were converted to photoactive porphyrins. No fluorescence was detected with either treatment at any time point in the dermis. The fluorescence intensity increased with time up to 6 hours (last time evaluated).

The rates of penetration through human and rat skin of (¹⁴C)-P-1202 cream were determined *in vitro*. (¹⁴C)-P-1202 20% cream was applied 1 mm thick without occlusion for 24 hours. The receptor fluid in the Franz cells was sampled pre-dose and up to 24 hours after dose application. By 24 hours, the mean cumulative absorption through rat skin was 2.1% and the mean cumulative absorption through human skin was 0.26% of the administered dose. The mean rate of penetration was 17.4 µg-equivalents of ALA/cm²/hour for rat skin and 1.50 µg-equivalents of ALA/cm²/hour for human skin. After a 2-hour lag phase, the relationship between absorption of radioactivity and time was linear for skin from both species. The permeability coefficient for P-1202 through rat skin was 1.083 x 10⁻⁴ cm/h, approximately 10 times the value for human skin (0.936 x 10⁻⁵ cm/h). A portion of the radioactivity remained as a depot in the skin (9.45% for rat and 4.91% for human) and could not be removed by washing.

A study of quantitative whole body autoradiography (QWBA) and excretion of radioactivity following topical application of (¹⁴C)-P-1202 cream to abraded and non-abraded skin of male Sprague-Dawley rats was performed. Excreta (urine, feces, expired air traps and cage washings) were collected for 48 hours. In animals with abraded skin sites, the total absorption was 13.1%. In animals with nonabraded sites, total absorption was 6.4% of dose. For both groups, approximately 50% of the absorbed dose was found in the urine and 30% remained in the carcass. Distribution was widespread, but concentrations in tissues were low. The highest radioactivity was found in the kidney cortex. Peak radioactivity concentrations were seen at 24 hours. Drug remaining as a depot in the skin may allow for continued systemic exposure over time. In animals with abraded treatment sites, radioactivity was quantifiable at 24 hours in the kidney cortex (19.79 µg-equiv/g) and Harderian gland (6.62 µg-equiv/g), brown fat, exorbital lachrymal gland, kidney medulla, liver, lung, salivary glands and thyroid. In animals with nonabraded treatment sites, the only quantifiable radioactivity at 3 and at 8 hours was in the kidney cortex. At 24 hours, tissue radioactivity had increased, but the only quantifiable radioactivity was in the kidney medulla (5.59 µg-equiv/g). Note that that, the clinical use calls for preparation of the skin surface, which may be similar to the abraded skin situation in the rat study. This appears to allow for deeper penetration of the drug substance, as well as enhanced systemic exposure.

3.3.3 Absorption

See summary above.

3.3.4 Distribution

See summary above.

3.3.5 Metabolism

Not assessed.

3.3.6 Excretion

See summary above.

3.3.7 Pharmacokinetic drug interactions

Not assessed.

3.3.10 Tables and figures to include comparative TK summary

Not applicable.

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology:

Single dose oral studies of P-1202 were conducted in mice and rats with a 14-day observation period. At doses of 2000 mg/kg, piloerection was observed in mice. No clinical signs were observed in rats, and no deaths occurred in either species.

Single dose studies were conducted in mice and rats with intravenous administration of P-1202. In mice at doses of 700 mg/kg and above, signs included lethargy, piloerection, and gasping. Deaths were seen at doses of 840 mg/kg and above. In rats, observations after doses of 1000 mg/kg and above included lethargy, salivation, chromodacryorrhea, snout staining, breathing pattern changes, piloerection, and anogenital soiling. Two of two animals administered 2000 mg/kg died. At 1500 mg/kg, one of five males died during drug administration. The remaining nine animals at that dose recovered, showing no clinical signs at two hours after dosing. The acute minimal lethal doses were 840 mg/kg iv in mice and 1500 mg/kg iv in rats.

A single dose dermal toxicity study with photoactivation was performed in rats. Shaved skin sites were treated with vehicle (Unguentum Merck®), 2, or 20% P-1202 for 12 or 36 hours, then illuminated with 100 or 200 J/cm². Signs and significant necropsy findings were limited to dermal reactions at the treatment site and some initial weight losses. Dermal observations in treated animals consisted of erythema and edema within the first few hours after photoactivation, followed by hyperkeratinization, hardening of the test site, desquamation and scab development, occasionally followed by eschar, necrosis or fissuring. Evidence of repair consisting of exfoliation to reveal new skin followed. Effects were dose-dependent in severity with respect to P-1202 concentration, duration of skin exposure, and light dose. Histological examination revealed acute and progressive inflammatory lesions in the treated skin with healing underway by day 15.

A repeated dose dermal toxicity study in rats with photoactivation was also performed. Vehicle, 2, 10, or 20% P-1202 in what may be the clinical formulation was applied to clipped sites for 24-hours and illuminated with 100 J/cm². Dermal reactions increased in duration, persistence, or incidence with repeated dosing. During the first few hours after photoactivation, erythema and edema were seen that resolved in a few days. Blanching and atonia were seen in the mid-dose group. Initial effects were followed by induration, hyperkeratinization, hardening, eschar formation, hemorrhage, and bruising at the treatment site (also blistering with discharge, some fissuring and petechiation in mid- and high dose animals and necrosis in mid-dose animals). These signs increased in incidence in the first 2-3 days after photoactivation, then progressed to the formation of scabs that peeled off, and exfoliation to reveal new intact skin. Subsequent doses were administered only after the test sites were near full resolution. After recovery period after the fourth dose, residual effects were present in both the mid- and high dose groups. Those effects were considered to be marked in females at the 10% concentration. Systemic findings that may have been treatment-related included increased PT or APTT

in mid- and high dose males, dose-related increases in alkaline phosphatase in females and glucose in males, and decreased total protein and/or albumin in mid- and high dose animals. Spleen weights were slightly increased and livers were increased in weight in females at the mid- and high doses. Splenic hematopoiesis was seen in high dose females. Serum concentrations of ALA were increased relative to control after the fourth dose.

A seven-day repeated dose intravenous study was performed in rats at doses of 0, 250 and 750 mg/kg/day. Signs observed included transient dose-related red-brown staining of the nose and mouth and one incidence of salivation at the high dose. Dose-related decreases were seen in red blood cell count, hemoglobin and hematocrit. Bilirubin was increased in high dose animals. There were apparent increases in AST and ALT but they were not reported to be statistically significant. There were nonsignificant increases in liver and kidney weights in treated animals, a significant decrease in relative testis and epididymis weights and an apparent decrease in prostate weights.

14-day repeated dose intravenous study was performed in rats at doses of 50, 200, and 800 mg/kg/day. The high dose was decreased to 600 mg/kg/day after day 2. At the high dose, observations included labored noisy respiration, salivation, ataxia, protruding eyes, red/brown staining of the nose and mouth, and piloerection. Red blood cell count, hemoglobin, and hematocrit were decreased. Bilirubin and ALT were increased. Livers were enlarged and liver weight was increased. Histological examination revealed cholangitis and peri-cholangitis in high dose animals. The only finding at 200 mg/kg/day was a small increase in liver weight. The NOEL was considered to be 50 mg/kg/day (HED=8.3 mg/kg/day).

A dermal study in minipigs was performed, consisting of four sequential treatments with P-1202 cream followed by photoactivation. This was a small study with only 2 animals/sex/group. Results were limited to marked chronic dermatitis that persisted through the 15-day recovery period. Photoactivation of the areas treated with the drug product resulted in acute wounds. Areas treated with the drug product but not illuminated exhibited slight chronic dermatitis that was mostly reversed in the recovery period. Clinical pathology findings consistent with systemic ALA exposure in the sponsor's studies included increased clotting times, and increased serum ALT, although the values were within the range of historical controls for the laboratory. Histopathology results revealed no indication of systemic toxicity.

Genetic toxicology:

P-1202 was studied in the Ames assay with and without photoactivation, in CHO cells in the presence and absence of visible light, and in an in vivo micronucleus assay in the rat. All studies were negative for genotoxic effects.

There are studies in the literature of 5-aminolevulinic acid (5-ALA), an intermediate metabolite of the drug substance, that indicate that 5-ALA may be positive in photo-genotoxicity tests (Fiedler et al., *J. Photochem. Photobiol. B: Biol.* 33:39-44, 1996; Onuki et al., *Biochimica et Biophysica Acta* 1225:259-263, 1994; Fraga et al., *Carcinogenesis* 15:2241-2244, 1994). Two sentences referring to these findings were included in the label in the approvable letter for this NDA. This was consistent with the label for Levulan® (5-ALA HCl).

Carcinogenicity:

No carcinogenicity studies were performed.

Reproductive toxicology:

No reproductive toxicology studies were performed.

Special toxicology:

The drug substance, methyl-ALA (P-1202) is a contact sensitizer to guinea pig skin. While P-1202 in a cream vehicle did cause an initial sting response and conjunctival injection in rabbit eyes, these effects were transient, and the drug product was not considered to be a primary ocular irritant.

3.4.2 Single-dose toxicity

No new single-dose toxicity studies have been submitted.

3.4.3 Repeat-dose toxicity

Note: The following study was previously reviewed in the original NDA submission but only the histopathology of the skin was submitted at that time. The approvable letter sent to the sponsor requested that the histopathology for all collected tissues in this study be submitted. Therefore, the study has been reviewed again below with the histopathology data. Much of the previous review is incorporated into this review.

Study title: Repeated dose dermal toxicity study with integral photoactivation in minipigs

Study no.: 35635

Volume #, and page #: volume 1.12, page 1-329 in original submission, the histopathology data is in volume 2 page 16-50 of the response to the approvable letter

Conducting laboratory and location:

Date of study initiation: 5 July 2000

GLP compliance: Yes (OECD)

QA report: Yes

Drug, lot #, and % purity: P-1202 (Metvix®) cream, batch no. 0080S

Methods

Doses: Group 1 – placebo control

Group 2 – P-1202 cream (containing 168 mg methyl aminolevulinate/g)

Species/strain: SPF minipigs

Number/sex/group or time point (main study): 2/sex/group

Route, formulation and volume: topical, clinical Metvix® formulation, 2g per site

Satellite groups used for toxicokinetics or recovery: an additional 2/sex/group for recovery

Age: 3-4 months

Weight: 5.5-7.5 kg

Unique study design or methodology (if any):

Drug was applied to shaved 50 mm diameter sites on the back. Each animal had two treatment sites, one on each side of the back. The right side was not illuminated, and the left side was illuminated. Two g of placebo or test article was applied to each of the two sites, covered with Tegaderm® dressing and bandaging, for three hours. The material was then wiped off of both sites, and the site on the right side of the back was again covered with the lightproof dressing. The site on the left was exposed to 75 J/cm² (570-670 nm) light, then recovered. Both dressings were removed after 24 hours.

It was intended that the total of four treatments were to be given at eight day intervals, but those intervals were extended to 12, 23, and 26 days, respectively, due to the severity of the reactions at the treatment sites.

The animals were anesthetized with an intramuscular cocktail of tiletamine, zolazepam, xylazine, ketamine, and methadon for these procedures. Buprenorphine was administered for pain management.

Observation times and results

Mortality: All animals survived to scheduled sacrifice.

Clinical signs: Clinical signs were recorded daily. Skin reactions were observed and scored at 3 and 6 hours after treatment and daily thereafter. Erythema and edema were scored on a scale of 0-4 (OECD guidelines).

Treatment site observations in the placebo group noted no effect on the non-illuminated site. At the illuminated site, erythema and, in a few animals, edema and/or wound formation were observed.

In the group treated with P-1202 cream, erythema was seen after treatment that became more severe in grade with repeated treatments, with edema in some animals at the non-illuminated site. At the illuminated site, erythema and edema were more severe in grade (statistically significant), and wound formation with necrosis or crusting was observed in most animals.

Body weights: Body weights were measured one week before the start of dosing (day – 7), on the first day of dosing (day 1), weekly thereafter, and at necropsy.

All animals gained weight during the study. There were no treatment-related effects on body weight.

Food consumption: Not assessed.

Ophthalmoscopy: Not assessed.

EKG: Not assessed.

Hematology: Blood samples were taken prior to treatment and before the termination of treatment from overnight fasted animals for hematology and clotting function.

After the last treatment, thrombin time was higher in treated males than in control males. The value was within the range of historical controls for the laboratory and was considered an incidental finding.

Clinical chemistry: Blood samples were taken prior to treatment and before the termination of treatment from overnight fasted animals for clinical chemistry determinations.

After the last treatment, serum ALT was higher and blood glucose was lower in treated males than in control males. However, these values were within the range of historical controls for the laboratory and were considered incidental findings. In 3 of 4 females the ALT values were within historical control but the value for one female was more than twice the upper limit.

Urinalysis: Urine samples were collected prior to the start and prior to the termination of treatment overnight in clean stainless steel trays under cages.

There were no treatment-related effects.

Gross pathology: Animals were killed by exsanguination and were subjected to a full macroscopic examination. Main study animals were sacrificed three days after the last treatment, and recovery animals were sacrificed 15 days after the last treatment.

There were no treatment-related macroscopic findings other than effects at the treatment site. In the placebo group, there were no findings at either treatment site. In the P-1202-treated group, two animals had reddening at the non-illuminated treatment site, and all four had wounds at the illuminated treatment site.

In the recovery animals, one placebo animal had a wound at the illuminated site. One P-1202-treated animal had red discoloration at the non-illuminated site. Three animals had wounds at the illuminated treatment site; one of these also had red discoloration at the illuminated site.

Organ weights: See histopath table for organs weighed.

No treatment-related effects were reported. There was no statistical treatment of the data. Values were somewhat variable, especially at the sacrifice at the end of treatment; this was likely due to the small sample size and the young age of the animals. Uterine weights were increased in one of two females in the treated group at the end of treatment and in one of two control and one of two treated females at the end of the recovery period. Ovarian weight was also increased in that treated recovery female. These findings are likely to be due to the estrous status of the animals, but there are no remarks in the clinical observations or necropsy findings to that effect. The thymus weight was somewhat greater in one of the recovery treated females than in the other recovery females; this may be related to the age of the animal.

Histopathology: Only a limited set of tissues was collected in this study. In the original submission only the skin samples were examined microscopically. The sponsor has now submitted the histopathology analysis of the following tissues: adrenal glands, brain, heart, kidneys, liver, lung, ovaries, pituitary, prostate, skin (untreated), skin (treatment

site 1, not illuminated), skin (treatment site 2, illuminated) spleen, testes, thymus, thyroid, uterus

Peer review: yes (), no (X)

Treatment sites:

In the placebo main study group, minimal epidermal crusts and minimal to moderate dermal/epidermal inflammation were noted in both illuminated and non-illuminated sites. One illuminated site had minimal dermal hemorrhage.

In the P-1202 cream-treated main study group, minimal to slight epidermal crusts, epidermal hyperplasia and dermal/epidermal inflammation were recorded at the non-illuminated site. The incidence and grades of these findings were significantly different from control. (*Reviewer's comment: This may represent some degree of phototoxicity from ambient lighting after removal of the dressings from the non-illuminated site.*)

Minimal to slight dermal hemorrhage was recorded in three of the main study animals.

In the P-1202 cream-treated main study group, at the illuminated treatment site, the incidence and severity of epidermal crusts, epidermal hyperplasia, dermal/epidermal inflammation, and dermal hemorrhage were increased, relative to non-illuminated sites in both groups. Three animals had acute wounds (epidermal/dermal coagulation necrosis); two of these also had subacute or healed wounds. The fourth animal had a chronic partially healed wound.

In recovery animals, one placebo-treated female had slight to marked epidermal crusts, epidermal hyperplasia and dermal/epidermal inflammation in both the illuminated and non-illuminated sites. At the non-illuminated site in P-1202 treated animals, there were epidermal crusts, dermal inflammation and hemorrhage. The incidence and severity of these were low and not significantly different from placebo. At the illuminated site in P-1202 treated animals, the incidence and severity of epidermal crusts, epidermal hyperplasia, dermal/epidermal inflammation, dermal hemorrhage, chronic partly healed wounds and healed wounds were significantly greater than at the illuminated site in placebo-treated animals.

Other tissues:

In the main study group treated with vehicle, one female had alveolar macrophages in the lung, mononuclear cell infiltration in the liver and tubular basophilia in the kidney. One male in this group had tubular hypoplasia in the testes and mononuclear cell infiltration in the adrenal cortex.

In the main study group treated with P-1202, one male had mononuclear cell infiltration in the kidneys and tubular hypoplasia in the testes. The other male in this group had mononuclear cell infiltration of the adrenal cortex and medulla.

In the recovery groups treated with vehicle, both males had mononuclear cell infiltration in the kidney and one male also had tubular basophilia of the kidney and mononuclear cell infiltration of the liver and adrenal cortex. One female had mononuclear cell infiltration of the adrenal cortex in this group.

In the recovery group treated with P-1202, one male had mononuclear cell infiltration in the liver and slight tubular hypoplasia in the testes. One female in this group had mononuclear cell infiltration in the kidney and adrenal medulla.

Toxicokinetics: Not evaluated.

Summary of individual study findings:

Four sequential treatments with P-1202 cream followed by photoactivation resulted in marked chronic dermatitis that persisted through the 15-day recovery period. (Reviewer's comment: Since the interval between treatments had to be extended to up to 26 days to allow sufficient recovery for re-treatment, perhaps a longer recovery period would have been more appropriate.) Photoactivation of the areas treated with the drug product resulted in acute wounds. Areas treated with the drug product but not illuminated exhibited slight chronic dermatitis that was mostly reversed in the recovery period. After the last treatment, thrombin time was higher in treated males than in control males, and serum ALT was higher and blood glucose was lower in treated males than in control males. These values were within the range of historical controls for the laboratory and were considered incidental findings by the sponsor. However, they are consistent with signs of systemic exposure observed in the sponsor's studies in other species.

Histopathology of tissues other than the skin was not originally submitted with this study. The histopathology has now been submitted and there do not appear to be any obvious histopathological findings that are due to the drug plus light treatment in any tissues or organs other than skin. The conclusions for this study are somewhat weakened by the fact that there were only 2 animals per sex per dose per time point. This makes statistical evaluations impossible.

Histopathology inventory

Study	14 day iv 1555/8	4 dose topical 35635
Species	Rat	Minipig
Adrenals	X*	X*
Aorta		
Bone Marrow smear		
Bone (femur)		
Brain	X*	X*
Cecum		
Cervix		
Colon		
Duodenum		
Epididymis	X*	
Esophagus		
Eye		
Fallopian tube		
Gall bladder		
Gross lesions		
Harderian gland		
Heart	X*	X*
Ileum		
Injection site		
Jejunum		
Kidneys	X*	X*

Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs		X*
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric		
Mammary Gland		
Nasal cavity		
Optic nerves		
Ovaries	X*	X*
Pancreas		
Parathyroid	X*	
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum		
Salivary gland		
Sciatic nerve		
Seminal vesicles		
Skeletal muscle		
Skin		
Spinal cord		
Spleen	X*	X*
Sternum		
Stomach		
Testes	X*	X*
Thymus		X*
Thyroid	X*	X*
Tongue		
Trachea		
Urinary bladder		
Uterus		X*
Vagina		
Zymbal gland		

X, histopathology performed
 *, organ weight obtained

3.4.4. Genetic toxicology

No new genetic toxicology studies have been submitted.

3.4.5. Carcinogenicity

No carcinogenicity studies have been performed with 5-methyl ALA. The drug product is proposed for short-term use, and does not warrant carcinogenicity testing.

3.4.6. Reproductive and developmental toxicology

No reproductive or developmental toxicity studies have been performed with 5-methyl ALA. Because of the likely low systemic exposure and the lack of historical data regarding adverse reproductive or developmental effects in patients with porphyrias (who

would have elevated systemic levels of ALA), reproductive and developmental toxicity studies were not considered necessary.

3.4.7 Local tolerance

No new studies on local tolerance have been submitted.

3.4.8 Special toxicology studies

No new special toxicology studies have been submitted.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The histopathology information for the minipig study does not show any indication of systemic toxicity. Changes to the label proposed by the Applicant are discussed below.

Unresolved labeling issues:

Genetic toxicology:

The Applicant requested that the genotoxicity information from published literature on ALA be removed from the label. This Applicant's comments on this issue and my response are listed below.

Applicant's position:

- The Applicant wishes to delete the following text from the mutagenesis section of the label:

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.
- The Applicant states that they assume that the statement is derived from two published reports:
 - Fiedler DM et al., J Photochem Photobiol B: Biology, 33:39-44, 1996.
 - Kersten B et al., Environ Mutagen Res, 23: 89-94, 2001.
- The Applicant states that several published reports have concluded that there is no evidence of ALA being genotoxic and photogenotoxic. As an example the Applicant refers to a review article by Fuchs et al. (2000).
- The sponsor acknowledges that it is plausible that ALA could lead to genetic damage since the mechanism of action is the production of reactive oxygen species by PpIX. However, they suggest that DNA damage is unlikely since the PpIX is primarily localized in the mitochondria and reactive oxygen species like singlet oxygen are so reactive that they will not be able to diffuse to the nucleus to cause DNA damage.

- The sponsor notes that the standard battery of genotoxic tests have been conducted and these were negative.
- The sponsor notes that the published studies were not conducted with methyl-ALA but with ALA.
- The sponsor notes that the published studies are not normally accepted by regulatory authorities and the studies have major shortcomings.

Response:

- There are several additional published reports, not cited by the sponsor, that were consulted in the original review of this NDA to support this statement (Onuki et al., *Biochimica et Biophysica Acta* 1225:259-263, 1994; Fraga et al., *Carcinogenesis* 15:2241-2244, 1994). There are also other studies in the literature that show the genotoxic potential of ALA but were not cited in the original review of this NDA (e.g. Onuki et al., *Environ. Mol. Mutagen.* 40:63-70, 2002).
- The paper by Fuchs et al. (2000) does not support the Applicant's statement that "there is no evidence of ALA being genotoxic or photogenotoxic." In fact, Fuchs et al. state "ALA causes genotoxic effects in the absence as well as in the presence of activating light and ALA induced PP is localized in the perinuclear region."
- The possibility of DNA damage from reactive oxygen species produced from photodynamic therapy and other mechanisms is not controversial. Photodynamic therapy can produce singlet oxygen and superoxide anion. These species can, through a variety of reactions, lead to the production of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical and lipid peroxides. Some of these species have relatively long half-lives and may be able to diffuse from the site of formation to the nucleus where they may cause DNA damage. (For example, see paper by Ouédraogo and Redmond, Secondary reactive oxygen species extend the range of photosensitization effects in cells: DNA damage produced *via* initial membrane photosensitization. *Photochem. Photobiol.* 2003, 77(2):192-203.)
- The results of the standard battery of genotoxicity tests are described in the label. The extra statement is meant to convey the understanding that reactive oxygen species production from photodynamic activation of PpIX may cause genetic damage.
- The fact that the published studies were conducted with ALA seems irrelevant since the methyl-ALA ester is converted to ALA in the body and the photodynamic activity is mediated by the resulting PpIX in either case.
- The Agency accepts any study type and judges it based on its scientific merits. That there may be shortcomings in published studies is understood; however, these shortcomings are generally more of a problem in establishing conclusions from negative results than from positive results.

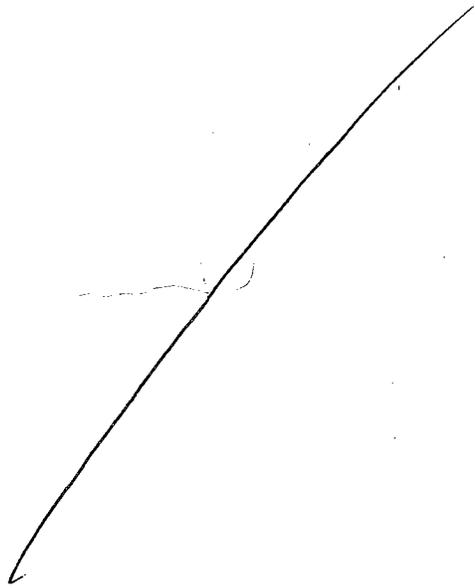
- The statement in the label is identical to the one in the label of topical ALA (Levulan). It is recommended that this statement be kept in the methyl-ALA label.

Hepatotoxic potential:

As noted in the introduction to this review, the label for the drug in the approvable letter included _____

_____ because hepatotoxicity monitoring data from the human clinical studies was not submitted to the NDA. The sponsor was previously told that the _____ may be removed if adequate human data was submitted. The current submission also appears to contain some data on monitoring of hepatic serum enzymes in clinical studies. If this information is determined to be sufficient by the medical officer, then the wording describing the _____ may be removed from the label.

In the current submission, the Applicant reviews the _____ that are the basis of the _____ label. The Applicant concludes that the wording does not reflect the data _____ and they request that _____ be removed from the label. The sponsor's position on each study is discussed below. If the human data is found to be sufficient and the _____ is removed from the label on that basis then the discussion below becomes moot.

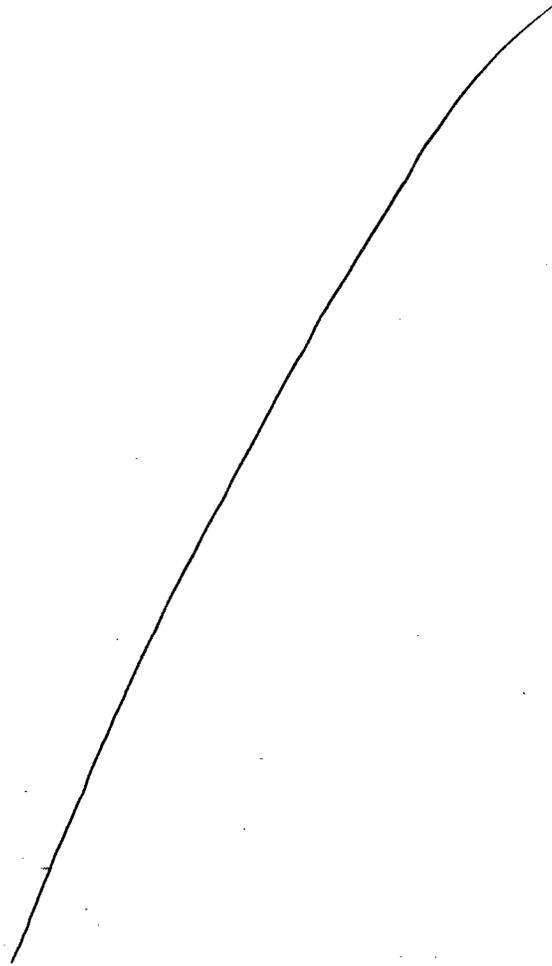


3 Page(s) Withheld

§ 552(b)(4) Trade Secret / Confidential

§ 552(b)(5) Deliberative Process

§ 552(b)(5) Draft Labeling



Recommendations:

1.
 - a. The _____ may be removed if the medical officer finds that the human data sufficiently addresses the hepatotoxic potential of the drug product.
 - b.
 - c.



2. The two sentences describing the literature information on the genotoxic potential of ALA should be maintained in the label.

Reviewer Signature _____

Supervisor Signature _____

3.7. 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/

Paul Brown
10/1/03 02:33:10 PM
PHARMACOLOGIST

Abby Jacobs
10/1/03 03:37:41 PM
PHARMACOLOGIST

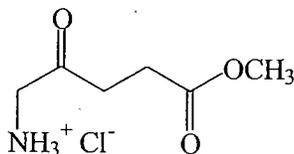
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-415
Review number: 1
Sequence number/date/type of submission: original submission; received 26 September 2001
Information to sponsor: Yes (X) No ()
Sponsor and/or agent: Photocure ASA, Oslo, Norway
U.S. agent: Clementi and Associates, Rosemont, PA
Manufacturer for drug substance: _____

Reviewer name: Amy C. Nostrandt, D.V.M., Ph.D.
Division name: Division of Dermatologic and Dental Drug Products
HFD #: 540
Review completion date: 6/12/02

Drug:

Trade name: Metvix® 168 mg/g cream / _____
Generic name (list alphabetically): methyl 5-aminolevulinate hydrochloride, 5-ALA methyl ester
Code name: P-1202
Chemical name: 5-aminolevulinic acid methyl ester hydrochloride, methyl 5-aminolevulinate hydrochloride, 5-amino-4-oxo-pentanoic acid methyl ester hydrochloride, methyl 5-amino-4-oxo-pentanoate hydrochloride, methyl 5-amino-4-oxo-valeroate hydrochloride, 5-amino-4-oxo-valeric acid methyl ester hydrochloride
CAS registry number: 79416-27-6
Mole file number: not provided
Molecular formula/molecular weight: C₆H₁₂NO₃Cl; MW=181.62
Structure:



Relevant INDs/NDAs/DMFs: both sponsored by Photocure

IND 59,756 Metvix for PDT of actinic keratoses

Drug class: photodynamic therapy agent

Indication: for the photodynamic therapy of non-hyperkeratotic actinic keratoses

Clinical formulation:

<u>ingredient</u>	<u>percent (w/w)</u>	<u>mg/g</u>
methyl 5-aminolevulinate hydrochloride equivalent to methyl 5-aminolevulinate	16.8	168
glyceryl monostearate, BP		
cetostearyl alcohol, NF		
polyoxy-10-stearate, NF		
methylparaben, NF		
propylparaben, NF		
edetate disodium, USP		
glycerin, USP		
white petrolatum, USP		
cholesterol, NF		
isopropyl myristate, NF		
peanut oil, NF		
refined almond oil, Ph. Eur.		
oleyl alcohol, NF		
Total	100	1000

Reviewer's comment: The sponsor has previously indicated that the peanut and almond oils are processed to adequately denature proteins to which patients might be allergic.

Route of administration: topical to lesion surface

Proposed use:

The proposed labeling states that after superficial preparation of the lesions, Metvix cream is applied to lesions and held under an occlusive bandage for three hours. At that time the bandage is removed and the skin is rinsed free of excess cream. The lesion is then illuminated with red light (wavelength 570-670 nm) for a total light dose of 75 J/cm² using the Curelight lamp.

Sites may be reassessed after three months

two treatments one week apart.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

The proposed drug product is a photodynamic therapy (PDT) agent that contains as its active ingredient a methyl ester of aminolevulinic acid or ALA. The sponsor proposes that the increased lipophilicity of the esterified ALA will allow better penetration into target lesions than with ALA. The sponsor claims that there is limited penetration of the drug into normal skin and that there is preferential accumulation of photoactive porphyrins in lesions. After de-esterification, ALA is used in the target cells to synthesize protoporphyrin IX (PpIX), which is the photoactive moiety. The drug is applied to target lesions and allowed to penetrate for a

period of time, after which the site is washed and illuminated with an appropriate wavelength of light to initiate the phototoxic reaction in the lesion.

A number of the studies submitted to the original IND as drafts were finally provided as final reports to the NDA. The sponsor was requested to highlight any differences in the final reports from the drafts, in order to expedite review. The sponsor states in the cover letter to the NDA that the draft and final reports did not differ substantially, and they refused to highlight any differences. This was not true in at least one report (study no. 1555/13) and necessitated re-review of all of the affected studies.

Studies reviewed within this submission:

Pharmacology studies:

1. Report FT-18: Formation of Protoporphyrin IX in murine skin after topical application of cream formulations containing different concentrations of P1202.
2. Report FT-13: Formation of Protoporphyrin IX in murine skin after topical application of P-1202 in different cream formulations.
3. Report FT-40: Comparison of protoporphyrin IX (PpIX) formation after 5-aminolevulinic acid (ALA) and 5-aminolevulinic (ALA) methyl ester addition to cell cultures.

Pharmacokinetic studies:

1. Report no. A-1.2A: ALA and ALA-esters: Skin build-up after ip or iv injection, and Report 1.2A: ALA and ALA-esters: Skin build-up after ip injection
2. Report no. FT-11: PpIX formation in mouse skin after administration of P1202. Oral vs. intraperitoneal administration.
3. Report no. FT-39: Biocalization of 5-aminolevulinic acid (ALA)- and ALA methylester-induced porphyrins in normal mouse skin.
4. Study # 1555/10: Quantitative whole body autoradiography and excretion of radioactivity following topical application of (¹⁴C)-P-1202 cream to the rat.
5. Study # 1555/13: (¹⁴C)-P-1202 cream: Rates of penetration through human and rat skin using a static *in vitro* system.

Toxicology studies:

1. Study # 1458/8: P-1202: Single dose oral toxicity study in the mouse.
2. Study # 1458/7: P-1202: Single dose oral toxicity study in the rat.
3. Study # 1555/003: P-1202: Single dose intravenous toxicity study in the mouse.
4. Study # 1555/002: P-1202: Single dose intravenous toxicity study in the rat.
5. Study # 1555/001: P-1202: Single dose dermal toxicity study in the rat with integral photoactivation procedure.
6. Study # 1555/005: P-1202: Repeated application dermal toxicity study in the rat with integral photoactivation procedure.
7. Study # 1555/7: P-1202: 7 day intravenous dose range-finding toxicity study in the rat.
8. Study # 1555/8: P-1202: 14 day intravenous toxicity study in the rat.
9. Study # 35635: P1202 cream: Repeated dose dermal toxicity study with integral photoactivation in minipigs.

Genetic toxicology studies:

1. Study # 1458/11: P-1202: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*.

2. Study # 1458/12: Reverse mutation in three histidine-requiring strains of *Salmonella typhimurium* and a tryptophan-requiring strain of *Escherichia coli* in the presence of visible light.
3. Study # 1458/13: P-1202: Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO) cells in the presence of visible light.
4. Study # 1458/24: P1201: Induction of micronuclei in the bone marrow of treated rats

Special toxicology studies:

1. Study # 1555/4: P1202: Skin sensitisation study in the guinea pig
2. Study # 1555/009: P-1202: Eye irritation study in the rabbit

Reviewer's comment: Only toxicology study no. 35635 (P1202 cream: Repeated dose dermal toxicity study with integral photoactivation in minipigs) and special toxicology study no. 1555/009 (P-1202: Eye irritation study in the rabbit) were performed using the clinical Metvix® formulation.

Studies not reviewed within this submission:

Journal articles provided in the submission are not reviewed. Validation reports and assay development reports are not reviewed.

**APPEARS THIS WAY
ON ORIGINAL**

Executive Summary

I. Recommendations

A. Recommendation on Approvability

From a pharmacology/toxicology standpoint, the application is approvable.

B. Recommendation for Nonclinical Studies

No additional nonclinical studies are recommended at this time. However, histopathological examination of tissues collected in studies already performed is recommended.

C. Recommendations on Labeling

Wording for nonclinical portions of the label is included at the end of this review. Revisions were made for consistency of statements with language in the CFR and with the approved product, Levulan® (5-ALA HCl).

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Toxicity of the drug product administered topically is primarily limited to phototoxicity at the site of application, which is the mechanism of pharmacologic action. Normal skin was susceptible, even when the only exposed to ambient light. However, serum concentrations of ALA were seen to increase after repeated dermal dosing in the rat, and clinical pathology parameters suggestive of toxicity to erythrocytes and the liver were seen in the rat and the minipig.

When administered via a systemic route, targets of toxicity included the red blood cells (decreased RBC count, hemoglobin, and hematocrit), the liver (increased serum bilirubin, AST, ALT, increased liver weights, cholangitis/pericholangitis), and increased kidney weights. Increases in clotting times were also seen.

B. Pharmacologic Activity

ALA methylester is more lipophilic than ALA HCl. The sponsor states that this property results in more efficient penetration of the drug substance into cells, where it is de-esterified to ALA and metabolized to PpIX, which is photoactive. Illumination of cells containing the excess PpIX with red light results in cellular damage and death.

C. Nonclinical Safety Issues Relevant to Clinical Use

Effects were seen in normal animal skin, indicating the potential for uptake into normal tissue surrounding target lesions in humans, rendering that normal skin susceptible to phototoxicity. Based on animal studies, there may be potential for systemic exposure to ALA after repeated treatments, which may result in adverse effects on the liver and blood. Risk for systemic exposure may be increased by the preparation of the treatment site prior to treatment. Methyl-ALA was a contact sensitizer in the guinea pig.

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

No pharmacology studies were conducted under GLP conditions.

1. Report FT-18: Formation of Protoporphyrin IX in murine skin after topical application of cream formulations containing different concentrations of P1202.

Three formulations were tested: 2%, 10%, and 20% methyl ALA in Photocure cream base (formulation not specified). Two formulations were applied to each of three Balb/c nude mice per group (0.1 g cream per site). Group 1 mice had 20% methyl-ALA cream applied to left flank and 10% methyl-ALA cream to the right flank. Group 2 mice had 20% methyl-ALA cream applied to the left flank and 2% methyl-ALA cream to the right flank. Each treatment site was covered with a dressing after application. Fluorescence at 637 nm was measured using a probe at the time of application and at 1, 2, 4, 6, 8, 10, 12 and 24 hours after application as a measure of PpIX formation.

Fluorescence in group 1 animals was almost identical between the 10% and 20 % formulations. Both increased over the first 12 hours and appeared to reach a plateau from 12-24 hours.

In group 2 mice, fluorescence increased more rapidly and to higher levels at the sites treated with the 20% formulation than at sites treated with the 2% formulation. Fluorescence at the site treated with the 2% formulation increased over the first 12 hours, but then decreased at the 24 hour time point.

(Reviewer's comment: This study report was not previously reviewed under the IND, and may not have been submitted prior to the NDA. It is unclear why the 20% formulation would have been chosen for clinical development if the same tissue fluorescence was achieved with both concentrations. It is assumed that the decision must have been based on results of clinical phase 2 dose-ranging studies.)

2. Report FT-13: Formation of Protoporphyrin IX in murine skin after topical application of P-1202 in different cream formulations.

Formulations containing 2% and 20% of P-1202 in Unguentum Merck and three developmental cream formulations were evaluated. Cream (0.5 g) was applied to the right flank over 2 cm² to female Balb/c nude mice (5/treatment group). Fluorescence measurement (excitation 407 nm, emission 550-750 nm) was performed noninvasively as a measure of PpIX formation. Higher maximal fluorescence and higher initial rates of PpIX formation were seen with 20% formulations. The PpIX level induced by the Unguentum Merck formulations decreased more rapidly after reaching a maximum than with the other formulations.

3. Report FT-40: Comparison of protoporphyrin IX (PpIX) formation after 5-aminolevulinic acid (ALA) and 5-aminolevulinic (ALA) methyl ester addition to cell cultures.

Cellular uptake was studied in human tumor WiDr cells (primary adenocarcinoma of the rectosigmoid colon), NHIK 3025 cells (carcinoma of the cervix) and V79 Chinese hamster lung fibroblasts. Cells were treated with P-1202 or ALA for 4 hours in serum free medium. Both resulted in concentration-dependent fluorescence identified as PpIX. Intracellular distribution of the two was similar. Fluorescence was emitted from defined spots in the cytoplasm, with diffuse background fluorescence in the entire cytoplasm and no fluorescence in the nuclear region of the cells. The report suggests association of some PpIX with the plasma membrane. The amount

of PpIX formed after either exposure to ALA or ALA methyl ester was similar, as was phototoxicity after light exposure at 24 hours (exposed to light from a bank of 4 fluorescent tubes emitting light around 405 nm at a fluence rate of 15 W/m²; 0.5 J/cm²). Cell survival was approximately 20%. The sponsor concluded that esterification of ALA had no impact on the fluorescing porphyrin species formed nor on their intracellular localization. The PpIX formed from ALA and ALA methyl ester was equally efficient in phototoxic effect to the cultured cells.

The following studies were reviewed under — IND 59,756. Both are comparisons of ALA and ALA methyl ester in female Balb/c nude mice.

Both ALA and ALA methyl ester produced porphyrins in normal mouse skin which were mainly localized in the epidermis, epithelial hair follicles and sebaceous glands of the skin. The formulation for both was a 20% concentration in Unguentum Merck. Cream was applied to the right flank of the animals (0.1 g cream to 2.25 cm²), and fluorescence was measured from biopsies at 1, 3, and 6 hours. No fluorescence was observed in control skin. ALA methyl ester was concluded to have been de-esterified and to have produced porphyrins in the skin. No fluorescence of ALA or its derivatives was detected in the dermis. Fluorescence intensity increased over time.

Peng Q et al. (J. Photochem Photobiol B: Biology 34:95-96, 1996) compared ALA to three esters of ALA. 20% creams were applied to the right flank of mice. Maximal fluorescence intensity was seen at 14 hours after application for all test articles, but the authors state that the fluorescence intensity was greater in skin treated with ALA esters. No fluorescence of ALA-induced porphyrins in tissues other than the site of topical application of ALA esters was seen, whereas it was induced in distant tissues in animals treated with unesterified ALA.

Pharmacology summary:

Methyl-ALA is taken up into cells and metabolized to form PpIX, a photoreactive compound that is an intermediate in the synthesis of heme. Illumination of PpIX results in fluorescence and cellular damage and death. Studies *in vivo* and *in vitro* have demonstrated this effect and a dose-related production of PpIX after topical treatment with 2% and 20% concentrations of the active ingredient in various vehicle creams. However, no difference was seen in the time courses and quantity of fluorescence following treatment with 10% and 20% concentrations. Maximum fluorescence was reported to be at 10-12 hours. *In vitro*, there was no difference in cellular uptake between ALA and P-1202. *In vivo*, PpIX localized in epidermis after topical application of P-1202.

Pharmacology conclusions:

The drug substance is a precursor to the active phototoxic compound, PpIX. The precursor is taken up into cells and metabolized to PpIX. The mechanism of action in ablation of lesions is phototoxicity.

Based on a dose-ranging pharmacology study in mice, there was no difference in the tissue fluorescence achieved with either the 10% formulation or the 20% formulation. It is unclear why the 20% formulation would have been chosen for clinical development under those circumstances.

II. SAFETY PHARMACOLOGY:

No safety pharmacology studies were performed.

III. PHARMACOKINETICS/TOXICOKINETICS:

1. Report no. A-1.2A: ALA and ALA-esters: Skin build-up after ip or iv injection.

ALA or ALA-methyl ester was injected as 100 µL/mouse to female Balb/c athymic nude mice. Groups of five animals each were treated as follows, with the following results:

Group no.	Treatment	Dose (mg/kg)	Route	Maximum fluorescence*	Time to maximum fluorescence (h)
1	5-ALA	250	ip	4.3	3.5
2	ALA-methyl ester	250	ip	2.2	1.6
3	5-ALA	250	iv	4.5	2.2
4	ALA-methyl ester	250	iv	2.0	1.6
5	5-ALA	150	ip	4.5	2.1
6	ALA-methyl ester	150	ip	1.2	0.9
7	5-ALA	50	iv	5.5	2.8
8	ALA-methyl ester	50	ip	0.7	0.5

*Units were illegible in study report.

5-ALA treatment resulted in more fluorescence than did treatment with ALA methyl ester. Saturation of the effect with 5-ALA was apparent even at the lowest administered dose. The report states that ALA-methyl ester is degraded via two different pathways; only one yields ALA and represents 20% of the metabolism of that compound.

Report 1.2A: ALA and ALA-esters: Skin build-up after ip injection.

ALA and its methyl, butyl, and hexyl esters were injected intraperitoneally as 1.5 mmol/kg into female Balb/c athymic nude mice. Skin fluorescence from the flank was measured prior to injection and at 1, 2, 4, 6, and 12 hours post-injection. Peak fluorescence with ALA was seen at 2-4 hours. Fluorescence with the esters all peaked at 1 hour. The values were lower than with free ALA, but the rate of disappearance of fluorescence was similar for ALA and its methyl and butyl esters. The authors concluded that the esters localized less efficiently than free ALA in mouse skin after ip injection.

2. Report no. FT-11: PpIX formation in mouse skin after administration of P1202. Oral vs. intraperitoneal administration.

5-ALA HCl (125 mg/mL in isotonic saline) and P-1202 (methyl 5-ALA HCl, 136 mg/mL in isotonic saline) were compared. Three groups of five Balb/c nude mice each were administered oral or ip P-1202 or oral 5-ALA HCl. Each animal, weighing approximately 25 g

was administered 50 μ L of the respective treatment. Skin fluorescence at 630 nm was recorded at 1 hour intervals after dosing as a measure of PpIX formation. PpIX formation was much lower in animals administered methyl-ALA. Oral ALA yielded much higher PpIX than did IP ALA (as reported in report 1.2A). PpIX fluorescence after administration of methyl-ALA was similar from the two routes. The sponsor hypothesized slow conversion of P-1202 to ALA or an alternate pathway, since the kinetics of the two substances were not the same.

3. Report no. FT-39: Biolocalization of 5-aminolevulinic acid (ALA)- and ALA methylester-induced porphyrins in normal mouse skin.

ALA and ALA methyl ester were formulated as 20% in Unguentum Merck, a commercial oil-in-water cream. Groups of five female Balb/c nude mice per timepoint per treatment were treated with the creams painted on the right flank (0.1 g cream to 2.25 cm²) and covered by a semi-permeable dressing for 1, 3, or 6 hours before biopsy of the treatment site. Tissue samples were removed at the respective time points and frozen in liquid nitrogen. Slides were prepared and fluorescence microscopy using an excitation filter of 390-440 nm, a 460 nm beam splitter, and a >600 nm emission filter was performed. The same frozen sections were also stained with H&E for histological examination (results not provided in report).

No fluorescence was seen in control skin. Both ALA and ALA methylester produced porphyrins in normal mouse skin, mostly localized in the epidermis, epithelial hair follicles and sebaceous glands. There did not appear to be any difference in skin fluorescence between ALA and ALA methyl ester; both were converted to photoactive porphyrins. No fluorescence was detected with either treatment at any time point in the dermis. The fluorescence intensity increased with time to 6 hours (last time evaluated).

4. Study # 1555/10: Quantitative whole body autoradiography and excretion of radioactivity following topical application of (¹⁴C)-P-1202 cream to the rat.

The final report was provided in the NDA to replace the draft report submitted to the IND. Topical application of ¹⁴C-P1202 cream (200.95 mg/g, specific activity = 282.4 μ Ci/mg) was made to abraded (by tape-stripping) and non-abraded skin of male Sprague-Dawley rats. Approximately 300 mg cream was applied per animal (approx. 60 mg P-1202/animal). The excreta was collected from designated groups for 48 hours. The remaining groups were sacrificed and frozen at 3, 8 and 24 hours for whole body autoradiography. In animals with abraded skin sites, 10% of the dose was recovered in the excreta (6.5% in urine, low amounts in expired air traps, feces, and cage washings), and 3% was found in the carcass. The total absorption was 13.1%, therefore approximately 50% of the absorbed radioactivity was excreted in urine and 30% remained in the carcass. In blood and plasma collected at sacrifice, concentrations were 2.7 and 3.44 μ g-equivalents/g, respectively. In animals with nonabraded sites, 4.5% of the administered dose was found in the excreta (3% in urine) and approximately 2% was found in the carcass. Total absorption was 6.4% of dose. Again, approximately 50% of absorbed dose was found in the urine and 30% remained in the carcass. In blood and plasma collected at sacrifice, concentrations were 1.64 and 2.17 μ g-equivalents/g, respectively. Less inter-animal variability was seen than in animals with abraded skin. Distribution was widespread, but concentrations in tissues were low, often below the LOQ (μ g-equivalents/g). The highest radioactivity was found in the kidney cortex. Levels did increase with time, so that other tissues in animals with abraded treatment sites had concentrations exceeding the LOQ by 24 hours, although they were often less than twice the LOQ. In animals

with nonabraded treatment sites, only the kidney cortex had quantifiable concentrations at any time point, and these concentrations were approximately half those seen in abraded animals at corresponding time points. Peak radioactivity concentrations were seen at 24 hours.

In animals with abraded treatment sites, the only quantifiable radioactivity at 3 hours was the kidney cortex (9.76 $\mu\text{g-equiv/g}$). Many other tissues had very low levels below the LOQ. At 8 hours, distribution was similar, with radioactivity quantifiable in kidney cortex (6.67 $\mu\text{g-equiv/g}$) and Harderian gland (3.84 $\mu\text{g-equiv/g}$). At 24 hours, levels in the kidney cortex (19.79 $\mu\text{g-equiv/g}$) and Harderian gland (6.62 $\mu\text{g-equiv/g}$) were increased, and quantifiable levels were present in brown fat, exorbital lachrymal gland, kidney medulla, liver, lung, salivary glands and thyroid. Levels in those additional tissues were less than twice the LOQ. Radioactivity was present in the gastrointestinal tract at all sampling times. Levels were low at 3 and 8 hours, except for moderate amounts in the small intestine at 3 hours and in the large intestine at 8 hours. By 24 hours, levels were moderate throughout the gastrointestinal tract distal to the stomach. High levels were present in the urinary bladder at 3 hours, the only time point at which that structure was sectioned. At the application site, levels in tissues at that site increased with time while radioactivity in the residual cream decreased.

In animals with nonabraded treatment sites, the only quantifiable radioactivity at 3 hours was in the kidney cortex (4.77 $\mu\text{g-equiv/g}$). Many other tissues had very low levels below the LOQ and appeared to be lower than in abraded animals. At 8 hours, the only quantifiable radioactivity again was in the kidney cortex (3.79 $\mu\text{g-equiv/g}$). At 24 hours, tissue radioactivity had increased, but the only quantifiable radioactivity was in the kidney medulla (5.59 $\mu\text{g-equiv/g}$). Radioactivity was present in the gastrointestinal tract at all sampling times, and appeared to be comparable to findings in abraded animals. Moderate levels were present in the urinary bladder at 24 hours, the only time that organ was sectioned. At the application site, levels in tissues at that site increased with time while radioactivity in the residual cream decreased. However, levels in the residual formulation were higher in nonabraded animals than in abraded animals.

5. Study # 1555/13: (^{14}C)-P-1202 cream: Rates of penetration through human and rat skin using a static *in vitro* system.

The final report was provided in the NDA to replace the draft report submitted to the IND. To isolated full thickness (400 μm) back skin from female Sprague-Dawley rats and human cadavers, 200 mg P-1202/g (20% cream, 102 $\mu\text{Ci/g}$) was applied as 100 mg of cream/ cm^2 (1 mm thick) without occlusion for 24 hours to 2 cm^2 area. Ten replicates were included for each species. The receptor fluid in the Franz cells was PBS with 1% BSA, and it was sampled pre-dose and at 1, 2, 4, 6, 10 and 24 hours after dose application. The LOD was — of applied dose. By 24 hours, the mean cumulative absorption through rat skin was 2.1% and the mean cumulative absorption through human skin was 0.26% of the administered dose. The mean rate of penetration was 17.4 $\mu\text{g-equiv}$ of ALA/ cm^2/hour for rat skin and 1.50 $\mu\text{g-equiv}$ of ALA/ cm^2/hour for human skin (*Reviewer's comment: These values differ from those originally reported in the draft report.*). There was a lag time of approximately 2 hours for both species. Following the lag phase, the relationship between absorption of radioactivity and time was linear for skin from both species. The permeability coefficient for P-1202 through rat skin was or 1.083×10^{-4} cm/h, approximately 10 times the value for human skin (0.094×10^{-5} cm/h; *Reviewer's comment: These values differ from those originally reported in the draft report.*). Approximately 70% of the applied radioactivity could be removed from the skin surfaces by

washing at 24 hours. A portion of the radioactivity remained as a depot in the skin (9.45% for rat and 4.91% for human; *Reviewer's comment: The latter value differs from that originally reported in the draft report.*) and could not be removed by washing.

**APPEARS THIS WAY
ON ORIGINAL**

PK/TK summary:

After topical application of ALA or ALA methylester (20% in Unguentum Merck), both ALA and ALA methylester produced porphyrins in normal mouse skin, mostly localized in the epidermis, epithelial hair follicles and sebaceous glands. There did not appear to be any difference in skin fluorescence between ALA and ALA methyl ester; both were converted to photoactive porphyrins. No fluorescence was detected with either treatment at any time point in the dermis. The fluorescence intensity increased with time up to 6 hours (last time evaluated).

A study of quantitative whole body autoradiography (QWBA) and excretion of radioactivity following topical application of (¹⁴C)-P-1202 cream to abraded and non-abraded skin of male Sprague-Dawley rats was performed. In animals with abraded skin sites, the total absorption was 13.1. In animals with nonabraded sites, total absorption was 6.4% of dose. For both groups, approximately 50% of absorbed dose was found in the urine and 30% remained in the carcass. Distribution was widespread, but concentrations in tissues were low. The highest radioactivity was found in the kidney cortex. Peak radioactivity concentrations were seen at 24 hours. In animals with abraded treatment sites, radioactivity was quantifiable at 24 hours in the kidney cortex (19.79 µg-equiv/g) and Harderian gland (6.62 µg-equiv/g), brown fat, exorbital lachrymal gland, kidney medulla, liver, lung, salivary glands and thyroid. In animals with nonabraded treatment sites, the only quantifiable radioactivity at 3 and at 8 hours was in the kidney cortex. At 24 hours, tissue radioactivity had increased, but the only quantifiable radioactivity was in the kidney medulla (5.59 µg-equiv/g).

The rates of penetration through human and rat skin of (¹⁴C)-P-1202 cream were determined *in vitro*. (¹⁴C)-P-1202 20% cream was applied 1 mm thick without occlusion for 24 hours. The receptor fluid in the Franz cells was sampled pre-dose and up to 24 hours after dose application. By 24 hours, the mean cumulative absorption through rat skin was 2.1% and the mean cumulative absorption through human skin was 0.26% of the administered dose. The mean rate of penetration was 17.4 µg-equivalents of ALA/cm²/hour for rat skin and 1.50 µg-equivalents of ALA/cm²/hour for human skin. After a 2-hour lag phase, the relationship between absorption of radioactivity and time was linear for skin from both species. The permeability coefficient for P-1202 through rat skin was 1.083×10^{-4} cm/h, approximately 10 times the value for human skin (0.094×10^{-5} cm/h). A portion of the radioactivity remained as a depot in the skin (9.45% for rat and 4.91% for human) and could not be removed by washing.

PK/TK conclusions:

Topically applied ALA methylester results in photoactive porphyrin accumulation in normal skin, mostly localized in the epidermis, epithelial hair follicles and sebaceous glands. However, the clinical protocol calls for preparation of the skin surface, which may allow for deeper penetration of the drug substance, as well as enhanced systemic exposure. Penetration through rat skin was 10 times faster *in vitro* than through human skin. In an *in vivo* study in rats, more than 13% of the applied dose of (¹⁴C)-P-1202 was absorbed through abraded skin (higher than would be predicted by the *in vitro* study). Drug remaining as a depot in the skin may allow for continued systemic exposure over time. Tissue distribution in the rat included peak levels at 24 hours (last time evaluated) in the kidney, Harderian gland, brown fat, exorbital lachrymal gland, liver, lung, salivary glands and thyroid.

IV. GENERAL TOXICOLOGY:**1. Study Title:** P-1202: Single dose oral toxicity study in the mouse

Key Study Findings: The LD₅₀ of orally administered P-1202 in mice was concluded to be >2000 mg/kg.

Study No: 1458/8-1032

Vol. #, and page #: vol. 1.9, page 36-54

Conducting laboratory and location: —

Date of study initiation: 28 November 1996

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, batch/lot #914.091/2061, — pure

Formulation/vehicle: P-1202 in purified water

Methods:**Dosing:**

- species/strain: — CD-1(ICR).BR mice
- #/sex/group or time point: 5/sex, fasted before dosing, sacrificed 15 days after dosing
- age: 5-7 weeks
- weight: 29-33 g (males), 23-25 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: 2000 mg/kg bw, 20 ml/kg
- route, form, volume, and infusion rate: single oral dose by gavage

Observations and times:

- Clinical signs: Clinical signs were recorded at least once during the first 30 minutes after dosing, at least four times during the first four hours after dosing, twice daily on days 2-4, and once daily thereafter.
- Body weights: Mice were weighed on the day before dosing, and on days 1, 4, 8 and 15 of the experiment.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: not performed
- Clinical chemistry: not performed
- Urinalysis: not performed
- Organ weights: not performed
- Gross pathology: Mice were sacrificed by ip injection of sodium pentobarbitone on day 15, followed by exsanguination and full macroscopic necropsy.
- Organs weighed: none
- Histopathology: not performed
- Toxicokinetics: not performed
- Other:

Results:

- Clinical signs: Piloerection was observed in two males and three females at the 4-hour observation after treatment, and was resolved by day 2.
- Deaths: none
- Body weights: Body weight gains were recorded over the 2-week observation period, but small body weight losses were recorded in one male over the first week and in two males over the second week. One female showed no change in body weight between days 8 and 15.
- Food consumption: not applicable
- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable
- Hematology: not applicable
- Clinical chemistry: not applicable
- Urinalysis: not applicable
- Organ Weights: not applicable
- Gross pathology: No macroscopic changes were observed.
- Histopathology: not applicable
- Toxicokinetics: not applicable

Summary of individual study findings:

The LD₅₀ of orally administered P-1202 in mice was concluded to be >2000 mg/kg.

2. **Study Title:** P-1202: Single dose oral toxicity study in the rat

Key Study Findings: The LD₅₀ of orally administered P-1202 in rats was concluded to be >2000 mg/kg.

Study No: 1458/7-1032

Vol. #, and page #: volume 1.9, page 74-92

Conducting laboratory and location: —

Date of study initiation: 28 November 1996

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, batch/lot #914.091/2061, — pure

Formulation/vehicle: P-1202 in purified water

Methods:**Dosing:**

- species/strain: — CD.BR rats
- #/sex/group or time point: 5/sex, fasted before dosing, sacrificed 15 days after dosing
- age: 6-8 weeks
- weight: 208-233 g (males), 162-187 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: 2000 mg/kg, 20 mL/kg
- route, form, volume, and infusion rate: single oral dose by gavage

Observations and times:

- Clinical signs: Clinical signs were recorded at least once during the first 30 minutes after dosing, at least four times during the first four hours after dosing, twice daily on days 2-4, and once daily thereafter.
- Body weights: Rats were weighed on the day before dosing, and on days 1, 8 and 15 of the experiment.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: not performed
- Clinical chemistry: not performed
- Urinalysis: not performed
- Organ weights: not performed
- Gross pathology: Rats were sacrificed by ip injection of sodium pentobarbitone on day 15, followed by exsanguination and full macroscopic necropsy.
- Organs weighed: none
- Histopathology: not performed
- Toxicokinetics: not performed
- Other:

Results:

- Clinical signs: none
- Deaths: none
- Body weights: All animals gained weight during each week of the study.
- Food consumption: not applicable
- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable
- Hematology: not applicable
- Clinical chemistry: not applicable
- Urinalysis: not applicable
- Organ Weights: not applicable
- Gross pathology: No macroscopic changes were observed.
- Histopathology: not applicable
- Toxicokinetics: not applicable

Summary of individual study findings:

The LD₅₀ of orally administered P-1202 in rats was concluded to be >2000 mg/kg.

3. **Study Title:** P1202: Single dose intravenous toxicity study in the mouse

Key Study Findings: The acute minimum lethal intravenous dose of P-1202 in the mouse was approximately 840 mg/kg in a preliminary trial. The dose chosen for the definitive study, 925 mg/kg, was also lethal.

Study No: 1555/003-1032

Vol. #, and page #: vol. 1.9, page 55-73

Conducting laboratory and location:

Date of study initiation: 28 August 1997

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, batch #2086, certificate of analysis for P-1202 states purity to be 99.9%

Formulation/vehicle: P-1202 in physiological saline

Methods:

Dosing:

- species/strain: .CD(ICR)BR mice
- #/sex/group or time point: range-finding study – 1/sex/dose group, then 2/sex/dose group; definitive study – 5/sex
- age: 6-7 weeks
- weight: 37-41 g (males), 23-27 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: range-finding study – 1000, 2000 mg/kg, then 585, 700, 840 mg/kg; definitive study – 925 mg/kg
- route, form, volume, and infusion rate: intravenous, 20 mL/kg

Observations and times:

- Clinical signs: Clinical signs were recorded at least once during the first 30 minutes after dosing, at least four times during the first four hours after dosing, twice daily on days 2-4, and once daily thereafter.
- Body weights: Mice were weighed on the day before dosing, and on days 1, 8 and 15 of the experiment.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: not performed
- Clinical chemistry: not performed
- Urinalysis: not performed
- Organ weights: not performed
- Gross pathology: Surviving mice were sacrificed by ip injection of sodium pentobarbitone on day 15, followed by exsanguination and full macroscopic necropsy. Animals that died during the study were also necropsied.
- Organs weighed: none
- Histopathology: not performed
- Toxicokinetics: not performed
- Other:

Results:

- Clinical signs: In the range-finding study, no clinical signs were observed at 585 mg/kg. Lethargy and piloerection were seen in the first 15 minutes after dosing at 700 mg/kg and in the first hour after dosing at 840 mg/kg. The female at 840 mg/kg that died

was described as prone and gasping. One female at 1000mg/kg exhibited lethargy and increased respiratory rate in the first half hour after dosing, but recovered and survived treatment.

In the definitive study, lethargy and/or gasping were observed in three males and three females which resolved within the first hour.

- Deaths: In the range finding study, both animals at 2000mg/kg and the male at 1000 mg/kg died immediately after infusion. One of two females at 840 mg/kg died within 15 minutes. None died at 585 or 700 mg/kg.

In the definitive study, there were no deaths.

- Body weights: Most mice gained weight during the first and second weeks of study period. Two males lost 1-2 g at day 8, and no change in body weight was recorded for one male and one female for the week between day 8 and day 15.
- Food consumption: not applicable
- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable
- Hematology: not applicable
- Clinical chemistry: not applicable
- Urinalysis: not applicable
- Organ Weights: not applicable
- Gross pathology: no test article-related findings. One female had uterine distension that was not considered to be treatment-related.
- Histopathology: not applicable
- Toxicokinetics: not applicable

Summary of individual study findings:

The sponsor concluded that the acute minimum lethal intravenous dose of P-1202 in the mouse was approximately 925 mg/kg. (*Reviewer's comment: Since one death was seen at 840 mg/kg, it would seem more appropriate to consider this value as the acute minimum lethal intravenous dose.*) Clinical signs included transient lethargy and piloerection in the range-finding trial at doses of 700 mg/kg and above. In the definitive study, transient lethargy and gasping were observed at 925 mg/kg.

4. **Study Title:** P-1202: Single dose intravenous toxicity study in the rat

Key Study Findings: The sponsor concluded that the acute minimum lethal intravenous dose was between 1430 and 1500 mg/kg in rats.

Study No: 1555/002-1032

Vol. #, and page #: vol. 1.9, page 93

Conducting laboratory and location: —

Date of study initiation: 28 August 1997

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, batch #2086, certificate of analysis for P-1202 states content to be —

Formulation/vehicle: P-1202 in physiological saline**Methods:****Dosing:**

- species/strain: — J.D.BR rats
- #/sex/group or time point: range-finding study – 1 or 2/sex/dose; definitive study – 5/sex
- age: 7-9 weeks (males), 10-11 weeks (females)
- weight: 214-343 g (males), 151-255 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: range-finding study – 1000, 1200, 1430, 2000 mg/kg; definitive study – 1500 mg/kg
- route, form, volume, and infusion rate: intravenous via tail vein; 20 mL/kg

Observations and times:

- Clinical signs: Clinical signs were recorded at least once during the first 30 minutes after dosing, at least four times during the first four hours after dosing, twice daily on days 2-4, and once daily thereafter.
- Body weights: Rats were weighed on the day before dosing, the day of dosing (day 1) and on days 8 and 15, or at death.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: not performed
- Clinical chemistry: not performed
- Urinalysis: not performed
- Organ weights: not performed
- Gross pathology: Surviving rats were sacrificed by ip injection of sodium pentobarbitone on day 15, followed by exsanguination and full macroscopic necropsy. Animals that died during the study were also necropsied.
- Organs weighed: not performed
- Histopathology: not performed
- Toxicokinetics: not performed
- Other:

Results:

- Clinical signs: range-finding study – lethargy, salivation, isolated incidences of breathing pattern changes (tachypnea, bradypnea or dyspnea), piloerection and anogenital soiling at 1000, 1200 or 1430 mg/kg, with higher incidence at 1430 mg/kg and recovery by day 3; definitive study – lethargy and increased salivation immediately after dosing in all surviving animals, chromodacryorrhea, snout staining, and gasping in some females during the first half hour after dosing, recovery within 2 hours of dosing.
- Deaths: range-finding study – both rats at 2000 mg/kg died immediately after dose administration; definitive study – one male at 1500 mg/kg died during administration.
- Body weights: A slight body weight gain was recorded in the male that died. All surviving rats gained weight during the first and second week of the study.
- Food consumption: not applicable

- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable
- Hematology: not applicable
- Clinical chemistry: not applicable
- Urinalysis: not applicable
- Organ Weights: not applicable
- Gross pathology: range-finding study – one male at 1000 mg/kg had a slightly distended jejunum; definitive study – dark lungs and enlarged liver in the male that died; no findings were recorded for animals sacrificed on day 15.
- Histopathology: not applicable
- Toxicokinetics: not applicable

Summary of individual study findings:

The sponsor concluded that the acute minimum lethal intravenous dose was between 1430 and 1500 mg/kg in rats. Clinical signs in the range finding study included lethargy, salivation, isolated incidences of breathing pattern changes (tachypnea, bradypnea or dyspnea), piloerection and anogenital soiling at doses of 1000 mg/kg and above, with recovery by day 3. In the definitive study, clinical signs included lethargy, increased salivation, chromodacryorrhea, snout staining and gasping, all of which were resolved within 2 hours post-dosing.

5. Study Title: P-1202: Single dose dermal toxicity study in the rat with integral photoactivation procedure

Key Study Findings: Dermal reactions occurred after single treatment with photoactivation. Reactions were corrosive in some cases. The period of resolution extended beyond the end of the study. Increasing the topical exposure period or the illumination energy resulted in more severe changes at the test site. Test article-related findings were limited to the treatment site.

Study No: 1555/001-1032
Vol. #, and page #: vol. 1.10, page 216
Conducting laboratory and location:

Date of study initiation: 16 April 1997

GLP compliance: yes (UK and OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: batch/lot #914.091/2061, — pure

Formulation/vehicle: P-1202 in Unguentum Merck, 2 or 20% methyl 5-ALA HCl.

Methods:**Dosing:**

- species/strain: CD.BR rats
- #/sex/group or time point: 11/sex/group; 3/sex/group were sacrificed on day 3, 3/sex/group on day 8, and 5/sex/group on day 15.
- age: 7-9 weeks (males), 10-11 weeks (females)
- weight: 297-363 g (males), 238-294 g (females)

- satellite groups used for toxicokinetics or recovery: Main study animals were used for toxicokinetic sampling.

- dosage groups in administered units:

Group number	Test article concentration	Exposure time (hours)	Photoactivation (J/cm ²)
1	Vehicle	12	200
2	20%	12	100
3	20%	12	200
4	2%	12	100
5	20%	36	100

- route, form, volume, and infusion rate: single topical application of 3 mL/kg to a clipped 3 cm² site on the back covered with a semi-occlusive dressing. After exposure for 12 or 36 hours, the sites were irradiated with 100 or 200 J/cm².

Observations and times:

- Clinical signs: Clinical signs were recorded twice during the first 30 minutes and at least four times within the first two hours after application. Observations were also recorded immediately and at 15-minute intervals after photoactivation for the first hour, then hourly for up to four hours, twice daily from days 3-8 and at least daily from day 9 to termination. Dermal reactions were scored and graded immediately after removal of the test material at 12 or 36 hours and immediately after photoactivation. Additional scoring was performed at 15, 30, and 45 minutes and at 1, 2, 3, and 4 hours after photoactivation. Dermal reactions were then graded twice daily until day 8, then once daily to day 15. Erythema and edema were scored on a 5-point scale, with 0 being no effect, and 4 being the most severe.
- Body weights: Body weights were recorded on the day prior to dosing, the day of dosing, and at termination on day 3, 8, or 15.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: not performed
- Clinical chemistry: not performed
- Urinalysis: not performed
- Organ weights: not performed
- Gross pathology: Rats were sacrificed on day 3, 8, or 15 by intraperitoneal injection of sodium pentobarbitone, followed by exsanguination and full macroscopic necropsy.
- Organs weighed: none
- Histopathology: Skin samples from the treatment site and from an untreated area were taken from each animal.
- Toxicokinetics: Blood samples were taken immediately prior to photoactivation, and terminal blood samples were taken from animals sacrificed at days 3, 8, or 15 for analysis of serum concentrations of P-1202, ALA, and PpIX.
- Other:

Results:

- Clinical signs: No clinical signs other than dermal reactions were observed on the day of treatment. One control female and one group 3 male were observed with chromodacryorrhea on days 6 and/or 7, with resolution by day 8. Rats in groups 4 and 5 vocalized during days 6-8, but this resolved by day 9. Ocular changes were noted in a few rats, but this was attributed to trauma from the blood sampling procedure. There were no signs of overt systemic toxicity.

Dermal observations in control animals consisted of sores on the test site. Isolated incidences of brown staining and scabs were seen in four control females. Dermal observations in treated animals consisted of erythema and edema within the first few hours after photoactivation, followed by hyperkeratinization, hardening of the test site, desquamation and scab development, occasionally followed by eschar, necrosis or fissuring. Evidence of repair consisting of exfoliation to reveal new skin was seen as early as day 6 in group 2 animals. Repair continued up to the time of termination at day 15. Severity of dermal responses was dose-dependent with respect to concentration of P-1202, duration of exposure to the drug, and light dose. In general, the ranking of severity of lesions was group 3>5>2>4.

- Deaths: none

- Body weights: Most male rats killed on day 3 exhibited small weight losses. The remaining males gained weight during the observation period. Female rats had weight fluctuations during the first week of the study, with most making small weight gains during the second week.

- Food consumption: not applicable

- Ophthalmoscopy: not applicable

- Electrocardiography: not applicable

- Hematology: not applicable

- Clinical chemistry: not applicable

- Urinalysis: not applicable

- Organ Weights: not applicable

- Gross pathology: Group 1 findings were limited to sores at the treatment site and a reddened mucosal surface in one male and one female killed on day 8. In group 2, one male killed on day 3 had a small amount of red fluid in the cranial cavity, one female killed on day 3 had a few red foci on the subcutis, and two males killed on day 8 had a gelatinous substance at the treatment site. In group 3, four rats killed on day 3 had red foci or reddening of the subcutis at the dose site, four rats killed on day 8 had reddening and/or thickening of the dose site, and two females killed on day 8 had a gelatinous substance at the dose site. No macroscopic abnormalities were seen in group 4 animals. In group 5, all 6 animals killed on day 3 had red foci and/or a gelatinous substance at the treatment site, four rats killed on day 8 had treatment sites that were reddened, dark, or gelatinous, and no macroscopic abnormalities were noted in animals killed on day 15.

- Histopathology: On day 3, prominent acute inflammatory lesions were seen in the treated skin, particularly in the epidermis. Findings included epidermal necrosis with inflammatory cell infiltrate, erosion/ulcer, occasional vesicles, acanthosis, and dermal edema with inflammatory cells. Severity was dose-dependent, with group 3>5>2>4. There were no remarkable findings in untreated skin.

On day 8, focal inflammatory lesions were found in control animals, with inflammatory cells, occasional erosion/ulcer, and acanthosis. Lesions in group 2 animals

had progressed to more subacute lesions such as erosion/ulcer, and more prominent acanthosis, less dermal edema, but with widespread inflammatory cells. In group 3, severity was greater than group 2, with the addition of vesicles present. Group 4 lesions consisted mostly of acanthosis and inflammatory cell infiltration. Group 5 lesions were similar to those in group 2. There were no remarkable findings in untreated skin.

At day 15, lesions were healing. Control animals had minor to prominent focal inflammatory lesions. Group 2 lesions included acanthosis, dermal fibrosis and inflammatory cells, with occasional erosion/ulcer, but with general healing. Acanthosis and dermal fibrosis were more prominent in group 3 with healing not as far along as in group 2. Group 4 lesions were less severe and unremarkable in some animals. Group 5 findings were similar to those in group 2. There were no remarkable findings in untreated skin.

- Toxicokinetics: A separate report (FT-10) was provided. P-1202 could not be measured due to its instability in serum. Very low levels of ALA were found in most samples. Significant increases in ALA concentration were seen in one control group (vehicle for 12 hours followed by exposure to 200 J/cm² light) and one treated group (20% P-1202 for 12 hours followed by exposure to 200 J/cm² light) immediately before illumination, but were not present by 4 hours after illumination. Both groups had a high degree of variability between individuals and the sponsor attributed the increases to normal variability. *(Reviewer's comment: Four of the six values in the control group ranged from 300-600 ng/mL, or at least one order of magnitude greater than approximate background concentrations. This raises concerns about the integrity of the labeling of samples.)* Levels of PpIX (means 28-69 ng/mL) were found in all groups with no apparent change over time; this was presumed to be due to background concentrations.

Summary of individual study findings:

Dermal reactions occurred after single treatment with photoactivation. Reactions were corrosive in some cases. Severity of dermal responses was dose-dependent with respect to concentration of P-1202, duration of exposure to the drug, and light dose. The period of resolution extended beyond the end of the study. Increasing the topical exposure period or the illumination energy resulted in more severe changes at the test site. Test article-related findings were limited to the treatment site.

6. Study Title: P-1202: Repeated application dermal toxicity study in the rat with integral photoactivation procedure

Key Study Findings: Dermal reactions were found in photoactivated sites treated with 2%, 10% or 20% P-1202 creams. Reactions were somewhat more severe in the mid-dose group than in the high dose group; this reinforces the finding of no significant difference in cutaneous PpIX formation after treatment with 10% or 20% P-1202 in mice (Study FT-18). Reactions increased in duration, persistence, or incidence with repeated dosing. Repair was incomplete at the end of the recovery period. Systemic findings that may have been treatment related included increased PT or APTT in mid- and high dose males, dose-related increases in alkaline phosphatase in females and glucose in males, and decreased total protein and/or albumin in mid- and high dose animals. Spleen weights were slightly increased and livers were enlarged or increased in weight at the mid- and high doses. Splenic hematopoiesis was seen in high dose females.

Study No: 1555/005-1032

Vol. #, and page #: vol. 1.11, page 1

Conducting laboratory and location:

Date of study initiation: 31 July 1997

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, drug product lots #0161N (placebo), 0162N (2% P-1202 cream), 0163N (10% P-1202 cream), 0164N and 0129N (20% P-1202 cream), % purity

Formulation/vehicle: 2, 10, and 20% P-1202 creams, vehicle composition not specified in this report.

Methods:

Dosing:

- species/strain: — CD.BR rats
- #/sex/group or time point: 10/sex/group
- age: 10-11 weeks (males) and 13-15 weeks (females)
- weight: 317-437 g (males) and 244-289 g (females)
- satellite groups used for toxicokinetics or recovery: Blood samples for toxicokinetics were taken from main study animals prior to the first and fourth photoactivation treatments. Five animals per sex were terminated four days after the last dose and the remaining animals were sacrificed 12 days after the last dose.
- dosage groups in administered units: vehicle control, active control without photoactivation, cream at 16, 80, or 160 mg/g (methyl-ALA) for 24 hours followed by photoactivation with 100 J/cm²
- route, form, volume, and infusion rate: 3g cream/kg topically administered to clipped 3 cm² area on the back, repeated on days 1, 11, 29, and 43 (males) or 47 (females). The interval between doses varied to allow for resolution from the previous treatment. After application, a semi-occlusive dressing was applied to the treated skin. After 24 hours, the dressing was removed and the site wiped clean prior to photoactivation.

Observations and times:

- Clinical signs: Signs were recorded once within an hour of application of cream and at least 3 times during the exposure periods, then at 0.5, 1, 2, 3, and 4 hours after each photoactivation, and twice daily between applications. Dermal reaction scoring (erythema and edema) on a scale of 0-4 was performed at the time of dressing removal, then at 0.5, 1, 2, 3, and 4 hours after photoactivation, and twice daily between treatments.
- Body weights: recorded weekly and prior to each topical application.
- Food consumption: measured weekly and calculated as g/animal/week
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: Blood samples for hematology and clotting profiles were collected from all animals from the orbital sinus two days after removal of the dressing following the final test article application. Samples were also taken from the abdominal aorta of all

unscheduled kill animals during necropsy. Bone marrow smears were made at necropsy to be examined only if a blood disorder was suspected.

- Clinical chemistry: Blood samples were collected from all animals from the orbital sinus two days after removal of the dressing following the final test article application. Samples were also taken from the abdominal aorta of all unscheduled kill animals during necropsy.

- Urinalysis: not performed

- Organ weights: performed at necropsy

- Gross pathology: 5 rats/sex/group were killed 4 days after removal of the last dressing. The remaining animals were allowed an additional 8-day recovery period. Animals were killed by ip injection of sodium pentobarbitone after an overnight fast, exsanguinated and subjected to a full macroscopic examination.

- Organs weighed: adrenals, kidneys, spleen, liver, heart, brain, testes and epididymides, ovaries.

- Histopathology: of skin at treatment site in all treatment groups and a limited number of other tissues.

- Toxicokinetics: Samples were taken from the retro-orbital sinus from groups 1 and 2 (controls) and group 5 (20% P-1202 for 24 hours followed by illumination at 550-700nm at 100 J/cm²) prior to the dose, immediately before illumination, and at 3 and 6 hours after illumination for the first and fourth doses. Serum samples were analyzed for methyl-ALA, ALA, and PpIX.

- Other:

Results:

- Clinical signs: Transient dermal reactions were seen in placebo controls with photoactivation and in drug-treated controls without photoactivation. Effects in the latter consisted of transient erythema followed by scabs, and hyperkeratinization in a few animals. These effects were considered to be transient and mild, and likely due to photoactivation by background light. Brown staining was seen in all P-1202 treated animals, with or without photoactivation.

For animals treated with P-1202 and illumination, local reactions that varied in degree. Reactions were mildest at a concentration of 16 mg/g and most severe at 80 mg/g; dermal reaction severity was intermediate at the 160 mg/g concentration (*Reviewer's comment: There may be a rate-limiting step in hydrolysis of the drug substance to the active form or in uptake so that there was no significant difference between 80 mg/g and higher concentrations; the sponsor speculates that this may be due to differences in penetration capabilities of the two concentrations. This and evidence seen in pharmacology study FT-18 may indicate that the 10% P-1202 formulation may have been a more appropriate choice for clinical development.*). Reactions increased in duration, persistence, or incidence with repeated dosing. During the first few hours after photoactivation, erythema and edema were seen that resolved in a few days. Blanching and atonia were seen in the mid-dose group. Initial effects were followed by induration, hyperkeratinization, hardening, eschar formation, hemorrhage, and bruising at the treatment site (also blistering with discharge, some fissuring and petechiation in mid- and high dose animals and necrosis in mid-dose animals). These signs increased in incidence in the first 2-3 days after photoactivation, then progressed to the formation of scabs, which peeled off, and exfoliation to reveal new intact skin. Subsequent doses were

administered only after the test sites were near full resolution. The sites in low dose animals were nearer full resolution than were those in mid- and high dose animals.

Other signs that were seen were related to blood collection procedures.

Additional sores and skin observations were attributed to the animals being housed intermittently in groups rather than singly.

After the recovery period after the fourth dose, residual effects were present in both the mid- and high dose groups. Those effects were considered to be marked in females at the 80 mg/g concentration.

- Deaths: One high dose female (on day 12 after the second photoactivation) and one female in each of the control groups died, the latter two as a result of blood sampling or due to confinement in the photoactivation restraint.

- Body weights: no effect

- Food consumption: no effect

- Ophthalmoscopy: not applicable

- Electrocardiography: not applicable

- Hematology: Prothrombin time in high dose males and activated partial thromboplastin time in mid-dose males were significantly increased. White blood cell counts were decreased in low and mid-dose groups. The sponsor theorized that this was related to inflammatory reactions at the dosing site. (*Reviewer's comment: These changes were slight and were probably not biologically significant.*)

- Clinical chemistry: There was a significant dose-related increase in alkaline phosphatase in female rats and a dose-related increase in glucose in males. Alterations in potassium, chloride, and/or inorganic phosphorus were seen in the three treated and photoactivated groups, but without consistent trend. Decreased total protein, decreased albumin and/or a resultant shift in A/G ratio were significant in the mid- and high dose groups. (*Reviewer's comment: Deviations from control for all of these clinical chemistry parameters were not great, and values appeared to be within normal limits for the species.*)

- Urinalysis: not applicable

- Organ Weights: There was a small increase in mean spleen weight in high dose females at the end of treatment. After the recovery period, high dose females had increased absolute and relative mean liver and heart weights. A small, but not statistically significant, increase in mean spleen weights in males of the mid- and high dose groups was seen after the recovery period.

- Gross pathology: At the end of treatment, most necropsy findings were sporadic and not apparently dose-related. Widespread liver enlargement was reported in mid-dose males, with a single incidence in a mid-dose female. Small, soft testes were noted in two mid-dose males. Findings at the application site in treated nonphotoactivated and photoactivated animals included sore, thickened and flaky skin with an increased incidence in the mid- and high dose groups. Skin reddening or firmness was reported in mid- and high dose males.

After the recovery period, isolated changes without apparent dose relationship were again reported. Additionally, one low and one mid-dose male had enlarged or mottled livers, and enlarged hearts were reported in one low dose and two high dose males.

- Histopathology: At the end of the treatment period, microscopic findings in treated skin were most severe in the mid- and high dose animals, with lesser effects in the low

dose animals. In the vehicle control group, findings at the application site were limited to isolated incidences of acanthosis and serocellular crust. In treated but nonphotoactivated animals, findings of low-grade irritation included inflammatory cells, erosion/ulcer, acanthosis/epidermal hyperplasia and dermal fibroplasia/fibrosis. Treated and photoactivated groups had similar findings with increased severity and the additional findings of epidermal/dermal necrosis, epidermal vesicle, edema, and granuloma/pyogranuloma in the mid- and high dose animals.

A limited list of organs were examined for systemic effects. Unfortunately, only tissues in the control and high dose groups were examined in some instances, and in other instances the organ in question was only examined in one or more sporadic animals, presumably in order to follow up on gross findings. Systemic findings included increased splenic hematopoiesis in the high dose females. Lymphoid hyperplasia was noted in lymph nodes, but not all tissues were examined in all animals, so dose-dependency could not be determined. Inflammatory foci were found in livers across groups, with an increase in incidence at the recovery sacrifice.

At the end of the recovery period, there was evidence of incomplete repair at the treatment site. The severity of treated skin lesions in mid-dose animals was greater than in high dose animals, consistent with the in-life observations.

- Toxicokinetics: A separate report (FT-15) was provided. P-1202 was unstable in rat serum and could not be measured. ALA concentrations in all groups after exposure to the first dose, but before illumination, were near the LOQ (\sim ng/ml). ALA was slightly higher (approximately 2-fold) after the first dose in groups 2 and 5, treated with the 20% P-1202 cream (nonphotoactivated and photoactivated, respectively) relative to the vehicle control group (1). The ALA increase was much more pronounced (approximately 10-fold higher than after the first dose) after the fourth dose in groups 2 and 5. The sponsor speculates that skin irritation in the P-1202 treated groups as a result of treatment may have contributed to the higher serum concentrations of ALA. PpIX concentrations remained relatively constant between groups and time points (range \sim ng/mL). Analysis of some samples was not performed.

Summary of individual study findings:

The major findings were apparent at the treatment site. Dermal reactions of increasing severity were found in photoactivated sites treated with 2%, 10% or 20% P-1202 creams. Reactions were somewhat more severe in the mid-dose group than in the high dose group; the sponsor speculated that this was due to inherent differences in formulation. Reactions increased in duration, persistence, or incidence with repeated dosing. Repair was incomplete at the end of the recovery period. Systemic findings that may have been treatment related included increased PT or APTT in mid- and high dose males, dose-related increases in alkaline phosphatase in females and glucose in males, and decreased total protein and/or albumin in mid- and high dose animals. Spleen weights were slightly increased and livers were enlarged or increased in weight at the mid- and high doses. Splenic hematopoiesis was seen in high dose females. Serum concentrations of ALA were increased after the fourth dose.

7. Study Title: P-1202: 7 day intravenous dose range-finding toxicity study in the rat

Key Study Findings: Findings were limited to transient clinical signs and clinical pathological abnormalities (decreased red blood cell parameters, increased bilirubin and serum potassium), mostly at 750 mg/kg/day. Doses chosen for the definitive study were 50, 200, and 800 mg/kg/day.

Study No: Covance study #1555/7

Vol. #, and page #: serial #000, vol. 1.4, page 004-336

Conducting laboratory and location: —

Date of study initiation: 24 March 1999

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, lot #2159, certificate of analysis indicates content to be — w/w.

Formulation/vehicle: P-1202 in physiological saline (0.9% NaCl)

Methods:

Dosing:

- species/strain: — CD.BR rats
- #/sex/group or time point: 3/sex/group, sacrificed on day 8
- age: six weeks
- weight: 171-199 g (males), 141-182 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: vehicle, 250 or 750 mg/kg/day
- route, form, volume, and infusion rate: intravenous infusion via the tail vein, daily for 7 days, 5 mL/kg, injected at a rate of 3 mL/minute.

Ambient light was reduced due to concerns regarding photoactivation of the metabolites of the drug substance. Light was reduced to 50% of the standard 700 lux produced by the fluorescent tube lighting in the animal facilities from the beginning of the 12-hour light cycle until after completion of dosing. Light was then adjusted to 20% of standard illumination for the remainder of the 12-hour light cycle.

Observations and times:

- Clinical signs: Clinical signs were recorded immediately after dosing, at 15, 30 and 60 minutes, then at hourly intervals for the remainder of the working day on each day of dosing.
- Body weights: Body weights were recorded on day 1 prior to dosing and on day 8 prior to terminal necropsy.
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: Blood samples were taken on day 6 after fasting overnight. Additional samples were taken on day 8 from two animals (#15 and #18) under the same conditions, due to clotting of the previous samples and abnormally high potassium levels.
- Clinical chemistry: Blood samples taken for hematology were also used for serum chemistry determinations.

Reviewer's comment: Samples taken on day 8 were included in analyses as day 6 data.

- Urinalysis: not performed
- Organ weights: measured at terminal necropsy on day 8
- Gross pathology: Rats were sacrificed on day 8 by intraperitoneal injection of sodium pentobarbitone, followed by exsanguination and full macroscopic necropsy.
- Organs weighed: liver, spleen, adrenals, kidney, heart, thyroids and parathyroids (weighed together), pituitary, brain, testes and epididymides (weighed together), prostate, and ovaries.
- Histopathology: not performed
- Toxicokinetics: not performed
- Other:

Results:

- Clinical signs: A transient, dose-related incidence of red/brown staining on the nose and mouth was recorded as apparent within 15 minutes of dosing, but resolved within 1-2 hours. This effect was seen in males at 250 mg/kg on days 4 and 5, and in males and females at 750 mg/kg on days 3-7. One high dose female was seen to have been salivating on day 3 for approximately one half hour after dosing.
- Deaths: none
- Body weights: Over the treatment period, males made small body weight gains while females had small losses. This was attributed in part to fasting overnight prior to day 6 and day 8. The changes were similar in control and treated groups.
- Food consumption: not applicable
- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable
- Hematology: Reported findings included dose-related decreases in red blood cell count, hemoglobin, and packed cell volume in males and females. The differences from control were slight, and values were within normal limits for this species. Also reported were increases in white blood cell and lymphocyte counts that were more pronounced in males.
- Clinical chemistry: Bilirubin was increased in males and females at 750 mg/kg. A dose-related decrease in BUN in females and a dose-related increase in potassium ion concentration in males was found.

Reviewer's comment: The report states that the latter effect was not seen in females, but one female in each of the two test-article treated groups was re-sampled on day 8 partially because of "abnormally high potassium levels."

While significant increases for AST, ALT, and alkaline phosphatase were not seen, the values were higher than in control databases for AST in the high dose males and, for ALT in males in both treated groups. Alkaline phosphatase values were much higher than reported normal values in males and females in all groups, including control.

- Urinalysis: not applicable
- Organ Weights: Small increases in liver and kidney weights in both treated groups relative to control were not statistically significant. Testis and epididymis weights were statistically different (lower than) from controls after adjustment for body weight. Prostate weights in males appeared to be lower than in controls, but did not appear to be statistically significant.
- Gross pathology: no test article-related findings.

- Histopathology: not applicable
- Toxicokinetics: not applicable

Summary of individual study findings:

Findings were limited to transient clinical signs and slight clinical pathological abnormalities, mostly at 750 mg/kg/day. Doses chosen for the definitive study were 50, 200, and 800 mg/kg/day.

8. Study Title: P-1202: 14 day intravenous toxicity study in the rat

Key Study Findings: A NOEL was not identified. Findings at 50 mg/kg/day (HED = 8.3 mg/kg) and 200 mg/kg/day (HED = 33 mg/kg) were limited to was a small liver weight increase and a dose related trend toward reduction of RBC parameters. Findings at the high dose (800 mg/kg, reduced to 600 mg/kg) included clinical signs of labored noisy respiration, increased salivation and ataxia, decreased RBC counts and hematocrits, increased serum bilirubin and ALT, increased liver weights associated with gross and microscopic abnormalities.

Study No: 1555/8 -D6144

Vol. #, and page #: vol. 1.10, page 1

Conducting laboratory and location:

Date of study initiation: 28 April 1999

GLP compliance: yes (UK/OECD)

QA- Report Yes (X) No ()

Drug, lot #, radiolabel, and % purity: P-1202, lot #2159, — , w/w

Formulation/vehicle: P-1202 in physiological saline (0.9% NaCl)

Methods:**Dosing:**

- species/strain: —.CD (SD) IGS.BR rats
- #/sex/group or time point: 10/sex/ group, sacrificed on day 15 or 16
- age: 32-36 days
- weight: 227-285 g (males), 165-199 g (females)
- satellite groups used for toxicokinetics or recovery: no
- dosage groups in administered units: vehicle, 50, 200, 800 mg/kg/day; the high dose was reduced to 600 mg/kg/day after death of one male rat on day 2 and marked clinical signs in surviving animals in that group.
- route, form, volume, and infusion rate: intravenous infusion via the lateral tail vein, daily for a minimum of 14 days, 5 mL/kg, 3 mL/minute.

Ambient light was reduced due to concerns regarding photoactivation of the metabolites of the drug substance. Light was reduced to 50% of the standard 700 lux produced by the fluorescent tube lighting in the animal facilities from the beginning of the 12-hour light cycle until after completion of dosing. Light was then adjusted to 20% of standard illumination for the remainder of the 12-hour light cycle.

Observations and times:

- Clinical signs: Clinical signs were recorded immediately after dosing, at 30 and 60 minutes, then at hourly intervals for the next three hours and at the end of the working day on each day of dosing.
- Body weights: Rats were weighed on days 1, 4, 8, 11, and 15, and prior to necropsy.
- Food consumption: not measured
- Ophthalmoscopy: Examinations were conducted on all animals pre-treatment and on control and high dose animals in week 2.
- EKG: not performed
- Hematology: Blood samples were taken from males on day 13 and females on day 14 after fasting overnight. Repeat samples were taken at necropsy for some animals, again after fasting overnight, to replace samples lost to clotting.
- Clinical chemistry: Blood samples taken for hematology were also used for clinical chemistries.
- Urinalysis: Urinalysis was performed on samples collected from surviving animals overnight between days 8 and 9. Food and water was withheld during the collection period.
- Organ weights: measured at necropsy
- Gross pathology: Male rats were killed on day 15, and female rats were killed on day 16 following overnight fasting. Rats were killed by intraperitoneal injection of sodium pentobarbitone, followed by exsanguination and full macroscopic necropsy.
- Organs weighed: liver, spleen, adrenals, kidney, heart, thyroids and parathyroids (weighed together), pituitary, brain, testes and epididymides (weighed together), prostate, and ovaries.
- Histopathology: Microscopic examination was performed on all protocol listed tissues from all control and high dose animals and decedents from all groups and on macroscopic abnormalities from all groups.
- Toxicokinetics: not performed
- Other: Parameters were analyzed by one-way ANOVA. Pairwise comparisons were made using Dunnett's test. Regression testing was performed to determine dose-response. In all cases, Levene's test for homogeneity of variances showed no heterogeneity. Nonparametric methods were used of clinical chemistry parameters with values above or below the limit of detection. Organ weights were analyzed using ANCOVA.

Results:

- Clinical signs: No clinical signs were noted in any of the groups on day 1. On days 2 and 3 in the high dose group only, signs were apparent in the first 30 minutes after administration, including labored noisy respiration, increased salivation and ataxia. There were also sporadic incidences of protruding eyes, red/brown staining of the nose and mouth, and piloerection in the high dose group. In high dose males staining of the ventral surface and dorsal sores were reported on clinical examination. After dose reduction, no signs were seen on days 4-6. Signs in the high dose animals reappeared on day 7 and were present for the duration of the treatment period in that group.
- Deaths: One male in the 800 mg/kg group died of unknown causes immediately after dosing on day 2.

- Body weights: Body weights and weight gains were similar between groups
- Food consumption: not applicable
- Ophthalmoscopy: No treatment-related ocular abnormalities were noted.
- Electrocardiography: not applicable
- Hematology: In high dose males and females, hemoglobin, RBC counts and hematocrits were decreased. A dose-related trend for the same effects was noted in the low and mid-dose groups. Mean prothrombin time was significantly decreased in mid-dose male rats. Low dose female rats had a significantly decreased WBC count, apparently due to a decrease in lymphocyte count. Similarly reduced WBC and lymphocyte counts in low dose males were not significantly different from control. In high dose female rats there was a statistically significant decrease in the percent lymphocytes with a corresponding increase in the percent neutrophils.

Reviewer's comment: Of these findings, only the decreases in red blood cell parameters appear to be toxicologically significant.

- Clinical chemistry: Serum bilirubin was significantly increased in high dose males and females. Significantly increased ALT was seen in males at the high dose. AST was increased in high dose males, but was not statistically significant due to high variability. Alkaline phosphatase was decreased in high dose males and females. Additional findings in high dose males include lower creatinine, and increased total protein, albumin, and globulin relative to control. Total cholesterol was increased for high dose males and females. Small increases in inorganic phosphorus and calcium in high dose males were statistically significant.
- Urinalysis: Urine volume in high dose males was decreased relative to controls. There appeared to be a treatment-associated decrease in urine pH in males.
- Organ Weights: There was a dose-related increase in absolute and body weight-adjusted liver weights in both males and females. The adjusted liver weights were significantly different for mid- and high dose males and females and for low dose males. The mean adjusted spleen weight in low dose females and the mean adjusted pituitary weight in mid-dose males were significantly different from their respective controls (lower and higher, respectively). These latter findings do not appear to be toxicologically significant.
- Gross pathology: Enlarged livers were noted in high dose males.
- Histopathology: There was treatment-associated cholangitis/peri-cholangitis in high dose males and females. This finding was not present in any control animals. Periductal inflammatory cell accumulation and intraductal brown pigmentation were seen. Phlebitis/periphlebitis and fasciitis/fibrosis were reported at the injection sites in control and high dose animals, and were considered to be related to the dosing procedure.
- Toxicokinetics: not applicable

Summary of individual study findings:

In animals treated with the high dose of 800 mg/kg, reduced to 600 mg/kg after three days of dosing, clinical signs of labored noisy respiration, increased salivation and ataxia were seen. Also in high dose animals, RBC counts and hematocrits were decreased. A dose-related trend for these findings was also seen at 50 and 200 mg/kg. Serum bilirubin and ALT were increased at the high dose. Slight liver weight increases were seen in mid-dose animals and in low dose males. Statistically significant liver weight increases were seen in high dose animals and were accompanied by the gross finding of liver enlargement and the histopathologic finding of

cholangitis/pericholangitis. Due to effects on liver weights at all doses and a dose related trend toward reduction of RBC parameters, there was no NOEL determined. The LOAEL was 50 mg/kg/day (HED = 8.3 mg/kg).

9. Study Title: Repeated dose dermal toxicity study with integral photoactivation in minipigs

Key study findings: Findings were limited to phototoxic tissue damage at the site of application. Clinical pathology changes suggestive of toxicity to erythrocytes and liver were seen, but the values were within the range of historical control values for the laboratory.

Study no: 35635

Volume #, and page #: volume 1.12, page 1-329

Conducting laboratory and location: —

Date of study initiation: 05 July 2000

GLP compliance: OECD

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: P-1202 (Metvix®) cream, batch no. 0080S

Formulation/vehicle: clinical Metvix® formulation

Methods (unique aspects):

Dosing:

Species/strain:	SPF minipigs
#/sex/group or time point (main study):	2/sex/group
Satellite groups used for toxicokinetics or recovery:	an additional 2/sex/group for recovery
Age:	3-4 months
Weight:	5.5-7.5 kg
Doses in administered units:	Group 1 – placebo control Group 2 – P-1202 cream
Route, form, volume, and infusion rate:	topical dose to shaved 50 mm diameter sites on the back.

Each animal had two treatment sites, one on each side of the back. The right side was not illuminated, and the left side was illuminated. Two g of placebo or test article was applied to each of the two sites, covered with Tegaderm® dressing and Vêtflex® bandaging, for three hours. The material was then wiped off of both sites, and the site on the right side of the back was again covered with the light-proof dressing. The site on the left was exposed to 75 J/cm² (570-670 nm) light, then recovered. Both dressings were removed after 24 hours.

It was intended that the total of four treatments were to be given at eight day intervals, but those intervals were extended to 12, 23, and 26 days, respectively, due to the severity of the reactions at the treatment sites.

The animals were anesthetized with an intramuscular cocktail of tiletamine, zolazepam, xylazine, ketamine, and methadon for these procedures. Buprenorphine was administered for pain management.

Observations and times:

- Clinical signs: daily. Skin reactions were observed and scored at 3 and 6 hours after treatment and daily thereafter. Erythema and edema were be scored on a scale of 0-4 (OECD guidelines).
- Body weights: one week before the start of dosing (day -7), first day of dosing (day 1), weekly thereafter, and at necropsy
- Food consumption: not measured
- Ophthalmoscopy: not performed
- EKG: not performed
- Hematology: Blood samples were taken prior to treatment and before the termination of treatment from overnight fasted animals for hematology and clotting function.
- Clinical chemistry: Blood samples were taken prior to treatment and before the termination of treatment from overnight fasted animals for clinical chemistry determinations.
- Urinalysis: Urine samples were collected prior to the start and prior to the termination of treatment overnight in clean stainless steel trays under cages.
- Gross pathology: Animals were fasted prior to necropsy. After anesthesia with an intraperitoneal injection of pentobarbital, the animals were killed by exsanguination and were subjected to a full macroscopic examination. Main study animals were sacrificed three days after the last treatment, and recovery animals were sacrificed 15 days after the last treatment.
- Organs weighed: see table
- Histopathology: Microscopic examination was limited to untreated skin and skin treatment sites.
- Toxicokinetics: none
- Other: Statistical evaluation was performed by Student's T-test or Wilcoxon Rank-Sum test, as appropriate.

Results:

- Mortality: none
- Clinical signs: none
- Treatment site observations in the placebo group noted no effect on the non-illuminated site. At the illuminated site, erythema and, in a few animals, edema and/or wound formation were observed.
- In the group treated with P-1202 cream, erythema was seen after treatment that became more severe in grade with repeated treatments, with edema in some animals at the non-illuminated site. At the illuminated site, erythema and edema were more severe in grade (statistically significant), and wound formation with necrosis or crusting was observed in most animals.
- Body weights: All animals gained weight during the study. There were no treatment-related effects on body weight.
- Food consumption: not applicable
- Ophthalmoscopy: not applicable
- Electrocardiography: not applicable

- Hematology:** After the last treatment, thrombin time was higher in treated males than in control males. The value was within the range of historical controls for the laboratory and was considered an incidental finding.
- Clinical chemistry:** After the last treatment, serum ALT was higher and blood glucose was lower in treated males than in control males. These values were within the range of historical controls for the laboratory and were considered incidental findings.
- Urinalysis:** There were no treatment-related effects.
- Organ weights:** No treatment-related effects were reported. There was no statistical treatment of the data. Values were somewhat variable, especially at the sacrifice at the end of treatment; this was likely due to the small sample size and the young age of the animals. Uterine weights were increased in one of two females in the treated group at the end of treatment and in one of two control and one of two treated females at the end of the recovery period. Ovarian weight was also increased in that treated recovery female. These findings are likely to be due to the estrous status of the animals, but there are no remarks in the clinical observations or necropsy findings to that effect. The thymus weight was somewhat greater in one of the recovery treated females than in the other recovery females; this may be related to the age of the animal.
- Gross pathology:** There were no treatment-related macroscopic findings other than effects at the treatment site. In the placebo group, there were no findings at either treatment site. In the P-1202-treated group, two animals had reddening at the non-illuminated treatment site, and all four had wounds at the illuminated treatment site.
- In the recovery animals, one placebo animal had a wound at the illuminated site. One P-1202-treated animal had red discoloration at the non-illuminated site. Three animals had wounds at the illuminated treatment site; one of these also had red discoloration at the illuminated site.
- Histopathology:** In the placebo main study group, minimal to slight epidermal crusts and mononuclear cell infiltration and edema in the epidermis and dermis were noted in both illuminated and non-illuminated sites. One illuminated site had minimal dermal hemorrhage.
- In the P-1202 cream-treated main study group, minimal to slight epidermal crusts, epidermal hyperplasia and dermal/epidermal inflammation were recorded at the non-illuminated site. The incidence and grades of these findings were significantly different from control. (*Reviewer's comment: This may represent some degree of phototoxicity from ambient lighting after removal of the dressings from the non-illuminated site.*) Minimal to slight dermal hemorrhage was recorded in three of the main study animals.
- In the P-1202 cream-treated main study group, at the illuminated treatment site, the incidence and severity of epidermal crusts, epidermal hyperplasia, dermal/epidermal inflammation, and dermal hemorrhage were increased, relative to non-illuminated sites in both groups. Three animals had acute wounds (epidermal/dermal coagulation necrosis); two of these also had subacute or healed wounds. The fourth animal had a chronic partially healed wound.

In recovery animals, one placebo-treated female had slight to marked epidermal crusts, epidermal hyperplasia and dermal/epidermal inflammation in both the illuminated and non-illuminated sites. At the non-illuminated site in P-1202 treated animals, there were epidermal crusts, dermal inflammation and hemorrhage. The incidence and severity of these were low and not significantly different from placebo. At the illuminated site in P-1202 treated animals, the incidence and severity of epidermal crusts, epidermal hyperplasia, dermal/epidermal inflammation, dermal hemorrhage, chronic partly healed wounds and healed wounds were significantly greater than at the illuminated site in placebo-treated animals.

Toxicokinetics: not applicable

Summary of individual study findings:

Four sequential treatments with P-1202 cream followed by photoactivation resulted in marked chronic dermatitis that persisted through the 15-day recovery period. (*Reviewer's comment: Since the interval between treatments had to be extended to up to 26 days to allow sufficient recovery for re-treatment, perhaps a longer recovery period would have been more appropriate.*) Photoactivation of the areas treated with the drug product resulted in acute wounds. Areas treated with the drug product but not illuminated exhibited slight chronic dermatitis that was mostly reversed in the recovery period. After the last treatment, thrombin time was higher in treated males than in control males, and serum ALT was higher and blood glucose was lower in treated males than in control males. These values were within the range of historical controls for the laboratory and were considered incidental findings by the sponsor. However, they are consistent with signs of systemic exposure observed in the sponsor's studies in other species.

Reviewer's comment: This study was requested of the sponsor to support clinical phase 3 studies. It should have been submitted for review prior to initiation of those studies, but was withheld until the NDA submission.

Toxicology summary:

Single dose oral studies of P-1202 were conducted in mice and rats with a 14-day observation period. At doses of 2000 mg/kg, piloerection was observed in mice. No clinical signs were observed in rats, and no deaths occurred in either species.

Single dose studies were conducted in mice and rats with intravenous administration of P-1202. In mice at doses of 700 mg/kg and above, signs included lethargy, piloerection, and gasping. Deaths were seen at doses of 840 mg/kg and above. In rats, observations after doses of 1000 mg/kg and above included lethargy, salivation, chromodacryorrhea, snout staining, breathing pattern changes, piloerection, and anogenital soiling. Two of two animals administered 2000 mg/kg died. At 1500 mg/kg, one of five males died during drug administration. The remaining nine animals at that dose recovered, showing no clinical signs at two hours after dosing. The acute minimal lethal doses were 840 mg/kg iv in mice and 1500 mg/kg iv in rats.

A single dose dermal toxicity study with photoactivation was performed in rats. Shaved skin sites were treated with vehicle (Unguentum Merck®), 2, or 20% P-1202 for 12 or 36 hours, then illuminated with 100 or 200 J/cm². Signs and significant necropsy findings were limited to dermal reactions at the treatment site and some initial weight losses. Dermal observations in treated animals consisted of erythema and edema within the first few hours after photoactivation,

followed by hyperkeratinization, hardening of the test site, desquamation and scab development, occasionally followed by eschar, necrosis or fissuring. Evidence of repair consisting of exfoliation to reveal new skin followed. Effects were dose-dependent in severity with respect to P-1202 concentration, duration of skin exposure, and light dose. Histological examination revealed acute and progressive inflammatory lesions in the treated skin with healing underway by day 15.

A repeated dose dermal toxicity study in rats with photoactivation was also performed. Vehicle, 2, 10, or 20% P-1202 in what may be the clinical formulation was applied to clipped sites for 24-hours and illuminated with 100 J/cm². Dermal reactions increased in duration, persistence, or incidence with repeated dosing. During the first few hours after photoactivation, erythema and edema were seen that resolved in a few days. Blanching and atonia were seen in the mid-dose group. Initial effects were followed by induration, hyperkeratinization, hardening, eschar formation, hemorrhage, and bruising at the treatment site (also blistering with discharge, some fissuring and petechiation in mid- and high dose animals and necrosis in mid-dose animals). These signs increased in incidence in the first 2-3 days after photoactivation, then progressed to the formation of scabs which peeled off, and exfoliation to reveal new intact skin. Subsequent doses were administered only after the test sites were near full resolution. After recovery period after fourth dose, residual effects were present in both the mid- and high dose groups. Those effects were considered to be marked in females at the 10% concentration. Systemic findings that may have been treatment-related included increased PT or APTT in mid- and high dose males, dose-related increases in alkaline phosphatase in females and glucose in males, and decreased total protein and/or albumin in mid- and high dose animals. Spleen weights were slightly increased and livers were enlarged or increased in weight at the mid- and high doses. Splenic hematopoiesis was seen in high dose females. Serum concentrations of ALA were increased relative to control after the fourth dose.

A seven-day repeated dose intravenous study was performed in rats at doses of 0, 250 and 750 mg/kg/day. Signs observed included transient dose-related red-brown staining of the nose and mouth and one incidence of salivation at the high dose. Dose-related decreases were seen in red blood cell count, hemoglobin and hematocrit. Bilirubin was increased in high dose animals. There were apparent increases in AST and ALT but they were not reported to be statistically significant. There were nonsignificant increases in liver and kidney weights in treated animals, a significant decrease in relative testis and epididymis weights and an apparent decrease in prostate weights.

14-day repeated dose intravenous study was performed in rats at doses of 50, 200, and 800 mg/kg/day. The high dose was decreased to 600 mg/kg/day after day 2. At the high dose, observations included labored noisy respiration, salivation, ataxia, protruding eyes, red/brown staining of the nose and mouth, and piloerection. Red blood cell count, hemoglobin, and hematocrit were decreased. Bilirubin and ALT were increased (AST appeared to be as well). Livers were enlarged and liver weight was increased. Histological examination revealed cholangitis and peri-cholangitis in high dose animals. The only finding at 200 mg/kg/day was a small increase in liver weight. The NOEL was considered to be 50 mg/kg/day (HED=8.3 mg/kg/day).

A dermal study in minipigs was performed, consisting of four sequential treatments with P-1202 cream followed by photoactivation. Results were limited to marked chronic dermatitis that persisted through the 15-day recovery period. Photoactivation of the areas treated with the drug product resulted in acute wounds. Areas treated with the drug product but not illuminated exhibited slight chronic dermatitis that was mostly reversed in the recovery period. Clinical

pathology findings consistent with systemic ALA exposure in the sponsor's studies included increased clotting times, and increased serum ALT, although the values were within the range of historical controls for the laboratory.

Toxicology conclusions:

The primary toxicity of the topically applied drug product is phototoxicity, which is also the mechanism of action of the drug. Lesions produced in the normal skin of laboratory animals included acute wounds accompanied by signs of inflammation and necrosis that persisted up to 15 days following the last treatment. The systemic targets of toxicity are the red blood cells and the liver. Toxicokinetic evaluation in the repeated dose study of topically applied drug in rats indicated increased serum concentrations of ALA after four doses. Similar evaluation was not performed in the minipig, but clinical pathology findings consistent with systemic exposure to ALA were seen.

**APPEARS THIS WAY
ON ORIGINAL**

Histopathology Inventory for NDA # 21-415

Study	1555/001	1555/005	1555/8	35635
Species	rat	rat	rat	minipig
Adrenals			X*	C*
Aorta				
Bone Marrow smear				
Bone (femur)				
Brain			X*	C*
Cecum				
Cervix				
Colon				
Duodenum				
Epididymis			X*	
Esophagus				
Eye				
Fallopian tube				
Gall bladder				
Gross lesions				
Harderian gland				
Heart			X*	C*
Ileum				
Injection site				
Jejunum				
Kidneys			X*	C*
Lachrymal gland				
Larynx				
Liver			X*	C*
Lungs				C*
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland				
Nasal cavity				
Optic nerves				
Ovaries			X*	C*
Pancreas				
Parathyroid			X*	
Peripheral nerve				
Pharynx				

Pituitary			X*	C*
Prostate			X*	C*
Rectum				
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin	X	X		
Spinal cord				
Spleen			X*	C*
Sternum				
Stomach				
Testes			X*	C*
Thymus				C*
Thyroid			X*	C*
Tongue				
Trachea				
Urinary bladder				
Uterus				C*
Vagina				
Zymbal gland				

C, collected
 X, histopathology performed
 *, organ weight obtained

V. GENETIC TOXICOLOGY:

1. Study Title: P-1202: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli*.

Study No: 1458/11-1052

Study Type: Ames assay

Volume # and Page #: vol. 1.13, page 100

Conducting Laboratory: —

Date of Study Initiation/completion: 20 December 1996 / 24 September 1997

GLP Compliance: yes (UK/OECD)

QA- Reports Yes (X) No ():

Drug Lot Number: batch #2061, puritv —

Study Endpoint: mutation; reversion of histidine or tryptophan-requiring bacterial strains to competence to grow on histidine or tryptophan-free medium.

Methodology:

- Strains/Species/Cell line: *S. typhimurium* strains TA98, TA100, TA1535 and TA1537
E. coli strains Wp2 pKM101 and WP2 uvrA pKM101
- Dose Selection Criteria:
 - Basis of dose selection: ICH recommendation of 5000 µg/plate maximum

- Range finding studies: The initial toxicity range-finding study was performed in strain TA100 only at P-1202 concentrations of 8-5000 µg/plate.
- Test Agent Stability: P-1202 was soluble in water at all concentrations tested. Stability data was not provided.
- Metabolic Activation System: P-1202 was tested in the presence and absence of S9 (Aroclor 1254-induced rat liver post-mitochondrial fraction). Findings in the first experiment in the presence of S9 were negative, so the second experiment included a pre-incubation step with S9.
- Controls:
 - Vehicle: Positive controls were in sterile anhydrous analytical grade DMSO, except for NaN₃, which was in water. Test article was in sterile purified water and filter-sterilized after admixture.
 - Negative Controls: sterile purified water
 - Positive Controls: 2-nitrofluorene (2-NF, 5 µg/plate, for TA98), sodium azide (NaN₃, 2 µg/plate, for TA100 and TA1535), 9-aminoacridine (AAC, 50 µg/plate, for TA1537), 4-nitroquinoline-1-oxide (NQO, 10 and 2 µg/plate, for E. coli strains), all without S9. 2-aminoanthracene (AAN, 5 µg/plate) was used for "at least one strain" with S9 to verify the activity of the S9 mixture.
 - Comments: AAN in the presence of S9 was generally tested in two bacterial strains to confirm S9 activity.
- Exposure Conditions:
 - Incubation and sampling times: Plates were inverted and incubated in the dark at 37°C for 3 days. In the experiment 2, test article or control, bacteria, and S-9 mix were incubated for 1 hour at 37°C prior to addition of molten agar and plating.
 - Doses used in definitive study: 8, 40, 200, 1000, and 5000 µg/plate in experiment 1; 1000, 2000, 3000, 4000, and 5000 µg/plate in experiment 2.
 - Study design: Positive controls and test article concentrations were tested in triplicate in each strain. Test article was tested with and without S9 in two separate experiments. Solvent controls were tested in quintuplicate with and without S9.
- Analysis:
 - No. slides/plates/replicates/animals analyzed: 3/treatment/experiment with and without S9
 - Counting method: electronic, using — Colony Counter
 - Cytotoxic endpoints: thinning of background bacterial lawn, or marked decrease in number of revertant colonies
 - Genetic toxicity endpoints/results: statistically significant increase in number of revertant colonies
 - Statistical methods: Dunnett's test and linear regression analysis
- Other:
- Criteria for Positive Results:
 1. significance in Dunnett's test and a significant dose correlation
 2. reproducible positive responses

Results:

- Study Validity:

The study was considered valid, as the following criteria were met:

1. Mean negative control counts fell within range of historical values.
 2. Positive control chemicals induced clear and large increases in revertant numbers confirming discrimination between different strains and an active S9 preparation.
 3. No more than 5% of plates were lost through contamination or other unforeseen event.
- Study Outcome: TA100 in the absence of S9 showed an increase in the number of revertant colonies in the range-finding study (at 1000 µg/plate) and in experiment 1 (at 5000 µg/plate), but not in experiment 2. Counts were ≤1.5 times the concurrent solvent control counts, and there was no clear dose relationship. It was concluded that there was no evidence of toxicity in the range-finding study or in experiment 1.

In experiment 2, a narrower range of concentrations, 1000-5000 µg/plate, was used. No clear evidence of toxicity was observed, and the increase in TA100 revertant colonies was not seen. Increases (1.15- and 1.14-fold) in revertants in WP2 uvrA pKM101 in the presence of S9 at 2000 and 3000 µg/plate were statistically significant, but appeared to be due to variability rather than to a genotoxic event.

The study was considered negative for mutagenicity in the presence or absence of metabolic activation.

2. Study Title: Reverse mutation in three histidine-requiring strains of *Salmonella typhimurium* and a tryptophan-requiring strain of *Escherichia coli* in the presence of visible light.

Study No: 1458/12-1052

Study Type: Ames assay with light exposure

Volume # and Page #: vol. 1.13, page 161

Conducting Laboratory:

Date of Study Initiation/completion: 23 December 1996 / 25 September 1997

GLP Compliance: yes (UK/OECD)

QA- Reports Yes (X) No ():

Drug Lot Number: batch #2061, purity

Study Endpoint: mutation; reversion of histidine or tryptophan-requiring bacterial strains to competence to grow on histidine or tryptophan-free medium.

Methodology:

- Strains/Species/Cell line: *S. typhimurium* strains TA98, TA100, and TA1537
E. coli strain WP2 pKM101
- Dose Selection Criteria:
 - Basis of dose selection: phototoxicity results of range-finding study
 - Range finding studies: all strains, irradiation at 0, 5, and 50 J/cm²,
treatment concentrations of 0, 5, 50, 500, and 5000 µg/mL
- Test Agent Stability: data not provided
- Metabolic Activation System: none
- Controls:
 - Vehicle: sterile purified water for P-1202 and MNNG; other control materials were prepared in anhydrous analytical grade DMSO

- Negative Controls: sterile purified water, with and without irradiation for range-finding and mutation experiments; 8-methoxypsoralen (8-MOP) in the absence of light treatment for mutation experiments only
- Positive Controls: 2-nitrofluorene (2NF, 25 µg/mL, for TA98), 4-nitroquinoline-1-oxide (NQO, 1 µg/mL, for TA100), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 7.5 µg/mL, for WP2 pKM101) and ICR-191 (1 µg/mL, for TA1537), all in the absence of light; 8-methoxypsoralen (8-MOP, 500 µg/mL, for WP2 pKM101), in the presence of light.
- Comments: Solutions were filter-sterilized.
- Exposure Conditions:
 - Incubation and sampling times: Suspensions of bacteria were dispensed into treatment wells to which test or control article solution was added. The mixtures were incubated in the dark for 3 hours at 37°C. Mixes were then exposed to light as required. Suspensions were then centrifuged and the bacterial pellet resuspended and plated on soft agar. Plates were inverted and incubated in the dark at 37°C for 3 days.
 - Doses used in definitive study: For TA98, TA100, and TA1537: 0, 15.8, 50, 158, 500 µg/mL at 0 and 5 J/cm²; 0, 1.58, 5, 15.8, 50 µg/mL at 50 J/cm². For WP2 pKM101: 0, 158, 500 1580, and 5000 µg/mL at 0 and 5 J/cm²; 0, 15.8, 50, 158, 500 at 50 J/cm² (mutation experiment 1).

In mutation experiment 2, TA100 was treated with 0, 100, 178, 316, 562, 1000 µg/mL at 0 and 5 J/cm², and with 0, 5, 15.8, 50, 158, 500 at 50 and 100 J/cm².
 - Study design: Treatments were performed under reduced lighting. P-1202 was tested for toxicity in all tester strains in the presence and absence of visible light. All test treatments and negative controls were conducted in duplicate platings for range-finding. Test treatments and positive controls were conducted in triplicate, and negative controls were conducted in quintuplicate for mutation experiments. Experiment 2 for mutation used only strain TA100 and no 8-MOP controls were included.
- Analysis:
 - No. slides/plates/replicates/animals analyzed: for range-finding, 2; for mutation experiments, 5 for negative controls and 3 for treatments and positive controls.
 - Counting method: Counting for the range-finding study was manual; the number of colonies in a defined optical field of each of three areas per plate were counted. The mean plate count was calculated. For the mutation experiments, counting was electronic. A Colony Counter was used and the background lawn was inspected for signs of toxicity.
 - Cytotoxic endpoints: The mean plate count was compared to a similarly irradiated solvent control plate counts as a measure of phototoxicity.
 - Genetic toxicity endpoints/results: statistically significant increase in number of revertant colonies
 - Statistical methods: Dunnett's test and linear regression analysis
- Other:
- Criteria for Positive Results:

1. significance in Dunnett's test after irradiation and a significant dose correlation
2. significant responses were induced in the presence of visible light but not its absence
3. the induction of revertants occurred at significantly higher frequencies, or at lower doses, in the presence of visible light.

Results:**- Study Validity:**

Criteria for study validity were:

1. Mean negative control counts fell within range of historical values.
2. Positive control chemicals induced clear and large increases in revertant numbers confirming discrimination between different strains and an active S9 preparation.
3. No more than 5% of plates were lost through contamination or other unforeseen event.

Initial mutation experiments using TA98 were considered invalid due to contamination of test plates and those using TA1537 were considered invalid due to thinning of the background bacterial lawn in all control and test plates.

- Study Outcome

The report indicates that there was phototoxicity in all tester strains in the range-finding study. Data tables indicate that no TA98 colonies grew from the suspension incubated with 5000 µg/mL and that the colony count was decreased at 500 µg/mL with 50 J/cm² visible light. For TA100, colonies were decreased at 5000 µg/mL with or without irradiation and at 500 µg/mL with 50 J/cm² visible light. For TA1537, colonies were decreased in all groups, and the range-finding study was considered invalid for that strain. For WP2 pKM101, colony counts did not indicate toxicity, with the exception of plates of bacteria treated with 5000 µg/mL with 50 J/cm² visible light.

In mutation experiment 1, thinning of the bacterial lawn in was seen only at the highest test dose in TA100 non-irradiated samples and in WP2 pKM101 plates irradiated at 50 J/cm². A 1.2-1.3 fold increase in revertant numbers in TA100 at the highest dose (500 µg/mL) at 5 or (50 µg/mL) at 50 J/cm² was statistically significant, and regression analysis showed dose-related trend at the two highest doses.

In mutation experiment 2, TA100 was examined further with maximal concentrations and light doses. Thinning of the bacterial lawn was seen at the highest non-irradiated dose and in plates irradiated at 100 J/cm². Small significant increases in the number of revertants were observed at the next to the highest dose at 0 and 100 J/cm², and significance for linear trend was shown at the two highest doses at 100 J/cm². However, the increases were slight and appeared to be most likely due to normal variability.

Mean solvent control counts in the absence of irradiation fell within historical control values. No increase in revertants was seen with 8-MOP treatment; this was considered to be due to use of an inappropriate wavelength of light. Other positive controls did demonstrate the ability of the assay to detect mutations.

The study was considered negative for photomutagenicity.

3. Study Title: P-1202: Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO) cells in the presence of visible light

Study No: 1458/13-D5140

Study Type: in vitro chromosomal aberration assay in mammalian cells

Volume # and Page #: vol. 1.13, page 225

Conducting Laboratory: —

Date of Study Initiation/completion: 14 January 1997 / 29 April 1997

GLP Compliance: yes (UK, OECD)

QA- Reports Yes (X) No ()

Drug Lot Number: batch #2061, purity —

Study Endpoint: structural chromosomal aberrations

Methodology:

- Strains/Species/Cell line: CHO cells
- Dose Selection Criteria: sufficient cell numbers and metaphases for counting
- Basis of dose selection: P-1202 was soluble in water up to 183 mg/mL. 100-fold dilution achieved by adding the test article solution to culture medium did not result in precipitation. 10 mM (1816 µg/mL final concentration) was used as the highest test concentration. Results of the range-finding study determined the doses used in the main study. If no phototoxicity was seen after test article treatment, it was tested in the main study only at 50 J/cm² visible light exposure.
- Range finding studies: light doses of 5 and 50 J/cm² with concentrations of 56.75, 113.5, 227, 454, 908, and 1816 µg/ml (10 mM).
- Test Agent Stability: not provided
- Metabolic Activation System: none
- Controls:
 - Vehicle: sterile purified water
 - Negative Controls: solvent, both irradiated and non-irradiated, and 8-methoxypsoralen (8-MOP) in the absence of visible light
 - Positive Controls: 4-nitroquinoline-1-oxide (NQO, 0.0625, 0.125, and 0.25 µg/mL) in the absence of visible light and 8-methoxypsoralen (8-MOP, 5, 7.5, and 10 µg/mL) in the presence of visible light
 - Comments: Test article stock solutions were filter-sterilized.
- Exposure Conditions:
 - Incubation and sampling times: Treatments were added (0.025 mL per culture for range-finding, 0.04 mL for the main study) to cultures suspended in 2.25 mL of medium. Cultures were incubated in the dark for 3.25 hours (0.25 hours for positive controls), then irradiated. At four hours, the cells were washed and re-fed. Cultures were incubated for an additional 16 hours and then were harvested.
 - Doses used in definitive study: 89.02, 137, 210.7, 324.2, 498.7, 767.3, 1180, and 1816 µg/mL (10 mM) with 5 J/cm² of visible light (570-700 nm) and 24.45, 37.61, 57.86, 89.02, 137, 210.7, 324.2 and 498.7 µg/mL with 50 J/cm² of visible light; all concentrations were also examined in the absence of visible light.
 - Study design: At 1.5 hours prior to harvest, colchicine was added to the cultures at a final concentration of 1 µg/mL to arrest cells in metaphase. A 500 µL

sample was taken for determination of cell counts and the remainder was centrifuged, resuspended, fixed and mounted for microscopic examination.

- Analysis:

- No. slides/plates/replicates/animals analyzed: Range-finding experiment – duplicates for the solvent controls and a single set of cultures were treated with test article. Definitive study – positive and negative controls and test article treated cultures were performed in quadruplicate; duplicates were irradiated or non-irradiated.
- Counting method: Coulter counter
- Cytotoxic endpoints: cell number and number of metaphases in irradiated cultures; this determined which dose levels were subjected to chromosome analysis (i.e. doses that induced no reduction in cell number).
- Genetic toxicity endpoints/results: Chromosome aberrations were analyzed at 3 consecutive dose levels from each radiation level, and the equivalent doses were analyzed in cultures that were not irradiated. The highest dose for chromosome analysis in the main study was either the maximum dose as described or that dose that resulted in no more than 50% reduction in cell number while providing an adequate number of metaphases for scoring. Slides from that dose and two lower doses were taken for microscopic analysis.
- Statistical methods: The proportions of aberrant cells in each replicate were used to establish acceptable heterogeneity between replicates by a binomial dispersion test. Counts for: 1) cells with structural aberrations including gaps, 2) cells with structural aberrations excluding gaps, and 3) cells with numerical aberrations were compared to concurrent and historical negative controls using Fisher's exact test.

- Other:

- Criteria for Positive Results: Results for the test article were considered to be positive if: 1) a statistically significant increase in the proportion of structural aberrations, excluding gaps, occurred at one or more concentrations and that proportion exceeded the normal historical range, or if 2) there were statistically significant increases in the cells with chromosome aberrations induced in the presence of light and not its absence, and cells with chromosomal aberrations occurred at lower doses in the presence of light or with higher frequencies than the total aberration frequencies seen in the irradiated solvent control plus the aberration frequency in the non-irradiated sample at the same concentration.

Results:

- Study Validity: The study was to be considered valid if: 1) the binomial dispersion test demonstrated acceptable heterogeneity between replicates, 2) the proportion of cells with structural aberrations, excluding gaps, in the negative control cultures fell within the range of historical controls, 3) at least 160 cells were analyzable at each dose level, and 4) the positive control chemicals induced statistically significant increases in the number of cells with structural aberrations. Chromosome aberrations in solvent control cultures were within the range of historical controls. Positive controls resulted in increases in the proportion of cells with structural aberrations. Those increases were statistically significant for NQO. 8-MOP treatment of irradiated cells resulted in increases in aberrations that were not statistically significant, but this was attributed to the lack of inclusion of UV wavelengths in the light dose. The study was considered to be valid.

- Study Outcome: In the range-finding experiment, marked phototoxicity was seen at 50 J/cm². At test article concentrations ≥ 227 $\mu\text{g/mL}$, cell counts were greatly reduced and there were insufficient metaphases for analysis.

In the main study, metaphase analysis was performed at the three highest doses irradiated at 5 J/cm² and at 57.86, 89.02, and 137 $\mu\text{g/mL}$ at 50 J/cm². Cell counts were reduced at higher concentrations irradiated with 50 J/cm² as they were in the range-finding study. At the highest analyzed dose, the proportion of chromosomal aberrations was not statistically different from concurrent solvent controls in the presence or absence of visible light. A small statistically significant increase was seen in the lowest dose irradiated at 5 J/cm², but the value was within the range of historical controls, as were the values for all other doses and irradiation levels. Numerical aberrations at all exposures were considered to have occurred at a normal frequency.

It was concluded that P-1202 did not induce chromosomal aberrations in CHO cells when tested up to cytotoxic levels in the absence or presence of visible light.

4. Study Title: P1202: Induction of micronuclei in the bone marrow of treated rats

Study No: 1458/24-D5140

Study Type: in vivo micronucleus assay

Volume # and Page #: vol. 1.13, page 275

Conducting Laboratory:

Date of Study Initiation/completion: 24 February 1999 / 21 May 1999

GLP Compliance: yes (UK/OECD)

QA- Reports Yes (X) No ():

Drug Lot Number: batch # FKH99.ALA-M07 2159 w/w

Study Endpoint: frequency of micronucleated polychromatic erythrocytes

Methodology:

- Strains/Species/Cell line: male HanWist (Glaxo:BRL) BR rats, 7 weeks old, 200-241 g
- Dose Selection Criteria:
 - Basis of dose selection: clinical signs of toxicity in range-finding study
 - Range finding studies: 1000 and 1500 mg/kg/day iv for 2 consecutive days followed by observation for clinical signs of toxicity
- Test Agent Stability: not provided
- Metabolic Activation System: in vivo study
- Controls:
 - Vehicle: for test article, water for injection; for positive control, saline
 - Negative Controls: saline
 - Positive Controls: cyclophosphamide – single injection of 40 mg/kg
 - Comments: Test article was administered under subdued lighting immediately prior to a 12-hour dark period.
- Exposure Conditions:
 - Incubation and sampling times: not applicable
 - Doses used in definitive study: 250, 500, and 1000 mg/kg/day injected iv for 2 consecutive days.
 - Study design: Animals were killed 24 hours after the final injection.
- Analysis:

- No. slides/plates/replicates/animals analyzed: 3 male rats/group for range-finding, 8/group for the main study
- Counting method: manual. The relative proportions of polychromatic erythrocytes to normochromatic erythrocytes (PCE:NCE) were determined until a total of at least 1000 cells had been counted. Counting then continued until at least 2000 PCE had been observed; PCE containing micronuclei were recorded throughout the counting process.
- Cytotoxic endpoints: see below
- Genetic toxicity endpoints/results: numbers of polychromatic erythrocytes (PCE) with micronuclei
- Statistical methods: The ratios of PCE:NCE for each animal and group mean were determined. The individual and group mean frequencies of micronucleated PCE/1000 were determined. PCE/NCE ratios were used to determine if any bone marrow toxicity had taken place. The interindividual variation in the numbers of micronucleated PCE was determined by a heterogeneity χ^2 test. Comparison of the numbers of micronucleated PCE in treated groups to that in the vehicle control group was performed using a χ^2 test. If there was significant evidence of heterogeneity in at least one group, then the Wilcoxon rank sum test was performed instead. A value of $p \leq 0.05$ was considered significant. Further testing was conducted for linear trend to determine if a dose-response relationship existed.
- Other:
- Criteria for Positive Results:

The test article was considered as positive in the assay if:

 1. a statistically significant increase in the frequency of micronucleated PCE occurred at at least one dose; and
 2. the frequency of micronucleated PCE at such a dose exceeded the historical vehicle control range.

Results:

- Study Validity:

The criteria for validity were:

 1. the incidence of micronucleated PCE in the vehicle control group should fall within the range of historical controls, and
 2. at least seven males from each group are available for analysis, and
 3. the positive control induces a statistically significant increase in the frequency of micronucleated PCE.

All of the above conditions were met.
- Study Outcome

In the range-finding study, animals at 1000 mg/kg/day showed signs of irregular breathing, prostration, protruding eyes, piloerection, salivation and lethargy. At 1500 mg/kg/day, death occurred immediately after administration in 2 of 3 animals. The remaining animal showed convulsions and unsteady gait in addition to the observations seen at the lower dose. 1000 mg/kg/day was chosen as the top dose for the study.

In the main study, no clinical signs were noted at 250 mg/kg. At 500 mg/kg, irregular breathing, piloerection, and unsteady gait were observed. At 1000 mg/kg, additional signs included prostration, convulsion, protruding eyes, salivation,

vocalization, eye secretion, and hunched posture. There were no deaths. PCE/NCE ratios in P-1202 treated groups were similar to the vehicle control group. Numbers of PCE with micronuclei in P-1202 treated groups were not statistically different from negative controls. There was a significant dose-related trend, but all values were within the range of historical control values.

It was concluded that P-1202 did not induce micronuclei in PCE of bone marrow of rats treated with up to 1000 mg/kg/day for 2 days, in the presence of overt toxicity.

Genetic toxicology summary:

P-1202 was studied in the Ames assay with and without photoactivation, in CHO cells in the presence and absence of visible light, and in an *in vivo* micronucleus assay in the rat. All studies were negative for genotoxic effects.

Genetic toxicology conclusions:

The sponsor's studies of genetic toxicity of methyl aminolevulinate were all negative. However, there are studies in the literature of 5-aminolevulinic acid (5-ALA), an intermediate metabolite of the drug substance that indicate that 5-ALA may be positive in photo-genotoxicity tests. Reference to these studies (Fiedler et al., *J. Photochem. Photobiol. B: Biol.* 33:39-44, 1996; Onuki et al., *Biochimica et Biophysica Acta* 1225:259-263, 1994; Fraga et al., *Carcinogenesis* 15:2241-2244, 1994) should be included in the label, for consistency with the label for Levulan® (5-ALA HCl).

Labeling recommendations:

Under "Carcinogenesis, Mutagenesis, Impairment to Fertility, the following wording is recommended:

Methyl aminolevulinate was negative for genetic toxicity in the Ames assay and the chromosomal aberration assay in Chinese hamster ovary cells, tested with and without metabolic activation and in the presence and absence of light.

Methyl aminolevulinate was also negative in the *in vivo* micronucleus assay in the rat. 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.

VI. CARCINOGENICITY:

No carcinogenicity studies were performed.

Carcinogenicity conclusions:

The drug product is proposed for short-term use, and does not warrant carcinogenicity testing.

Labeling Recommendations:

Under "Carcinogenesis, Mutagenesis, Impairment to Fertility", the following wording is recommended:

Long-term studies to evaluate the carcinogenic potential of Metvix® have not been performed.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

No reproductive or developmental toxicity studies were performed.

Reproductive and developmental toxicology conclusions:

The sponsor has claimed insignificant systemic bioavailability of the parent drug in human patients. Because of this and the lack of historical data regarding adverse reproductive or developmental effects in patients with porphyrias (who would have elevated systemic levels of ALA), reproductive and developmental toxicity studies do not appear to be warranted.

Labeling recommendations:

Under "Carcinogenesis, Mutagenesis, Impairment to Fertility", the following is recommended:

No animal fertility have been performed.

Under "Pregnancy," the following wording is recommended, as outlined in 21CFR201.57:

Pregnancy: Teratogenic effects: Pregnancy Category C: Animal reproduction studies have not been conducted with Metvix®. It is also not known whether Metvix® can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Metvix® should be given to a pregnant woman only if clearly needed.

VIII. SPECIAL TOXICOLOGY STUDIES:

1. Study title: P1202: Skin sensitisation study in the guinea pig

Key study findings: The drug substance, P-1202, was a positive sensitizer in the guinea pig.

Study no: 1555/4-1032

Volume #, and page #: vol. 1.13, page 53

Conducting laboratory and location:

Date of study initiation: 19 August 1977

GLP compliance: yes (UK, OECD)

QA reports: yes (X) no ():

Drug, lot #, radiolabel, and % purity: P-1202, batch #2186, assay results

Formulation/vehicle: formulated in purified water for injection and in Vaseline for topical application

Methods: method of Magnusson and Kligman

Dosing: Doses were selected on the basis of results of screening studies and maximum practical concentrations in the systemic and topical vehicles. In the main study,

intradermal induction phase, three paired injections were made on the clipped backs of female Dunkin-Hartley guinea pigs (20 in the treated group and 10 in the control group) as follows on day 1:

<u>Site</u>	<u>test group</u>	<u>control group</u>
anterior	FCA emulsion	FCA emulsion
middle	P-1202 80 mg/g P-1202 (10% m/v) in purified water	purified water
posterior	P-1202 80 mg/g P-1202 (10% m/v) in FCA emulsion	50% v/v purified water in FCA emulsion

(FCA emulsion consisted of Freund's Complete Adjuvant diluted with an equal volume of water.)

On day 7, the area was re-clipped and 0.4 ml of 10% sodium lauryl sulfate solution was applied to induce mild irritation. On day 8, the area was shaved and topical induction was performed by applying 480 mg/g P-1202 (60% m/m) in Vaseline to treatment animals and Vaseline alone to control animals. The treated skin was occluded for 48 hours, then washed with arachis oil. Dermal observations were recorded on day 11.

The flanks of all test animals were clipped on day 21, and shaved on day 22. The sites were challenged with 320 mg/g and 160 mg/g P-1202 (40% and 20% m/m) in Vaseline in Finn chambers or to Vaseline alone.

Observations and times: All animals were observed daily for clinical signs. Body weights were recorded on day 1 before induction and on day 25.

Twenty-four hours after challenge application, the chambers were removed, and the sites were washed with water. The challenge sites were re-shaved 21 hours after chamber removal. Dermal responses to challenge were recorded at 24 and 48 hours after chamber removal.

Results: Initial screening studies showed no significant irritation when P-1202 was administered as an intradermal injection or by topical application.

During the induction phase, erythema was seen at injection sites to which material containing FCA was administered. After topical application of induction materials, slight erythema was seen only at the middle site in test article-treated animals.

Positive response to challenge was seen in 13 of 20 animals. Results were considered to be equivocal in 5 of 20 animals. Negative responses were recorded for the remaining two animals.

Summary of individual study findings: P-1202 was a positive contact sensitizer in this study. P-1202 did not result in skin irritation in screening studies.

Conclusions: Positive results in the guinea pig sensitization study indicate that there is potential for this drug substance to cause delayed contact hypersensitivity.

Reviewer's comment: Testing was not performed using the clinical vehicle, which contains peanut oil and almond oil. Peanut oil is known to be a sensitizer if not thoroughly refined, and other nuts may induce allergies in susceptible individuals. The sponsor has previously indicated that _____ if the peanut oil is performed, so that excipient may not present a clinical problem with sensitization. Additionally, a positive control group was not included in this study, but historical positive controls are summarized in an appendix.

2. Study Title: P-1202: Eye irritation study in the rabbit

Key study findings: Reversible conjunctival injection was observed in animals exposed to drug product or the base cream. The sponsor concluded that the vehicle and P-1202 were not primary ocular irritants.

Study No: Covance study #1555/009-D6144

Vol. #, and page #: vol. 1.13, page 1

Conducting laboratory and location: _____

Date of study initiation: 15 March 1999

GLP compliance: yes (UK/OECD)

QA- Reports Yes (X) No ():

Drug, lot #, radiolabel, and % purity: P-1202 cream, batch #0041R
placebo cream, batch #0300P/P2

Formulation/vehicle: P-1202 cream clinical formulation

Methods: Three groups of three NZW rabbits were treated with vehicle (base cream), P-1202 cream with housing under reduced lighting conditions, and P-1202 cream with housing under normal lighting.

Dosing: A single instillation of 0.1 mL of undiluted test article was made into the left conjunctival sac of each rabbit.

Observations and times: Observations were made at 0.5, 1, 4, 24, 48, and 72 hours, and finally on day 5. The rabbits underwent fluorescein staining of the treated eyes at 24 hours after instillation to inspect for corneal disruption.

Results: There was no initial response to vehicle, but a slight "sting response" was seen after P-1202 cream instillation under reduced light conditions. A moderate sting response was seen in the first rabbit treated under normal lighting conditions; the remaining two animals were pre-treated with Ophthaine.

A slight discharge was seen from one eye in the vehicle-treated group in the first hour after instillation, with conjunctival injection at 4 and 24 hours. Similar changes were seen in another placebo-treated eye in the first hour after dosing.

In treated rabbits housed under reduced light, conjunctival injection was noted in 2 of 3 eyes, but resolved by four hours. In the first rabbit treated in that group (the sentinel rabbit) some ocular discharge was noted in the first hour after dosing and conjunctival injection was described as deep crimson in color at the four-hour assessment, but had resolved by day 2.

In treated rabbits housed under normal light, the first treated rabbit (no local anesthetic) developed conjunctival injection that was described as deep crimson at 30 minutes. The other two animals also developed conjunctival injection. In all three animals resolution was complete by day 2.

No changes to iris or cornea were seen in any treatment groups.

Summary of individual study findings: Reversible conjunctival injection was observed in animals exposed to drug product or the base cream. The sponsor concluded that the vehicle and P-1202 were not primary ocular irritants.

Conclusions:

The drug substance, methyl-ALA (P-1202) is a contact sensitizer to guinea pig skin. While P-1202 in a cream vehicle did cause an initial sting response and conjunctival injection in rabbit eyes, these effects were transient, and the drug product was not considered to be a primary ocular irritant.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

Toxicology studies in rodents have been performed by the oral, intravenous and dermal routes. Topical application with subsequent illumination resulted transient dermal reactions related to phototoxicity. Severity increased with increased P-1202 concentration, duration of exposure, light dose and repeated treatments. Systemic evaluation in rodents revealed effects primarily on red blood cell parameters and the liver. Clinical pathology findings suggestive of similar effects were seen in repeated dose topical studies in rats and minipigs. In the rat study, serum ALA was seen to increase after the final dose. Toxicokinetic evaluation was not performed in the minipig study, nor was microscopic examination of target tissues. P-1202 was negative in a battery of tests for genotoxicity. The drug product was negative for ocular irritation. The drug substance was negative in dermal irritation screening, but was a positive sensitizer in guinea pig skin.

General Toxicology Issues:

There may be potential for sensitization in clinical patients. Additionally, an *in vivo* radiolabeled pharmacokinetics study in the rat demonstrated 13.1% absorption of the drug substance across abraded skin. An *in vitro* study in rat skin vastly underestimated the degree of absorption *in vivo*. It is possible that absorption across curretted human skin may be greater than the sponsor theorizes.

Recommendations:

From a pharmacology/toxicology standpoint, the application is approvable. The label should be revised as described below.

2 Page(s) Withheld

 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 § 552(b)(5) Draft Labeling

X. APPENDIX/ATTACHMENTS:

Addendum to review:

Other relevant materials (Studies not reviewed, appended consults, etc.):

Any compliance issues:

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this page is the manifestation of the electronic signature.**

/s/

Amy Nostrandt
6/24/02 10:26:41 AM
PHARMACOLOGIST

At the end of the review are label revisions
and a request for additional data (not
an approvability issue) to be included in the
action letter.

Abby Jacobs
6/24/02 02:03:13 PM
PHARMACOLOGIST

Jonathan Wilkin
7/21/02 06:35:06 PM
MEDICAL OFFICER
see addendum no. 1 to review no. 1

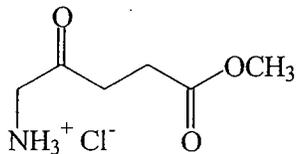
Addendum

PHARMACOLOGY/TOXICOLOGY COVER SHEET
Addendum to Review

NDA number: 21-415
Review number: addendum no. 1 to review no. 1
Sequence number/date/type of submission: original submission; received 26 September 2001
Sponsor and/or agent: Photocure ASA, Oslo, Norway
U.S. agent: Clementi and Associates, Rosemont, PA
Manufacturer for drug substance: _____

Reviewer name: Amy C. Nostrandt, D.V.M., Ph.D.
Division name: Division of Dermatologic and Dental Drug Products
HFD #: 540
Date: 7/19/02

Drug:
Trade name: Metvix® 168 mg/g cream
Generic name (list alphabetically): methyl 5-aminolevulinate hydrochloride, 5-ALA methyl ester
Code name: P-1202
Chemical name: 5-aminolevulinic acid methyl ester hydrochloride, methyl 5-aminolevulinate hydrochloride, 5-amino-4-oxo-pentanoic acid methyl ester hydrochloride, methyl 5-amino-4-oxo-pentanoate hydrochloride, methyl 5-amino-4-oxo-valeroate hydrochloride, 5-amino-4-oxo-valeric acid methyl ester hydrochloride
CAS registry number: 79416-27-6
Mole file number: not provided
Molecular formula/molecular weight: $C_6H_{12}NO_3Cl$; MW=181.62
Structure:



Relevant INDs/NDAs/DMFs: both sponsored by Photocure
IND 59,756 Metvix for PDT of actinic keratoses

Drug class: photodynamic therapy agent

Indication: for the photodynamic therapy of non-hyperkeratotic actinic keratoses

Clinical formulation:

<u>ingredient</u>	<u>percent (w/w)</u>	<u>mg/g</u>
methyl 5-aminolevulinate hydrochloride equivalent to methyl 5-aminolevulinate	16.8	168
self-emulsifying glyceryl monostearate, BP		
cetostearyl alcohol, NF		
polyoxyl stearate, NF		
methylparaben, NF		
propylparaben, NF		
edetate disodium, USP		
glycerin, USP		
white petrolatum, USP		
cholesterol, NF		
isopropyl myristate, NF		
peanut oil, NF		
refined almond oil, Ph. Eur.		
oleyl alcohol, NF		
Total	100	1000

Route of administration: topical to lesion surface

Proposed use:

The proposed labeling states that after superficial preparation of the lesions, Metvix cream is applied to lesions and held under an occlusive bandage for three hours. At that time the bandage is removed and the skin is rinsed free of excess cream. The lesion is then illuminated with red light (wavelength 570-670 nm) for a total light dose of 75 J/cm² using the Curelight lamp. — two treatments one week apart.

Sites may be reassessed after three months —

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

The proposed drug product is a photodynamic therapy (PDT) agent that contains as its active ingredient a methyl ester of aminolevulinic acid or ALA. The sponsor proposes that the increased lipophilicity of the esterified ALA will allow better penetration into target lesions than with ALA. The sponsor claims that there is limited penetration of the drug into normal skin and that there is preferential accumulation of photoactive porphyrins in lesions. After de-esterification, ALA is used in the target cells to synthesize protoporphyrin IX (PpIX), which is the photoactive moiety. The drug is applied to target lesions and allowed to penetrate for a period of time, after which the site is washed and illuminated with an appropriate wavelength of light to initiate the phototoxic reaction in the lesion.

Several studies in animals either demonstrated hepatotoxicity or were suggestive of hepatotoxicity as a result of exposure to methyl aminolevulinate. The following tables summarize the data as it pertains to potential hepatotoxicity signals in pivotal animal studies.

1. In a 14-day study in rats, doses of methyl ALA were administered daily intravenously. The findings were as follows:

Dose (nominal):	50 mg/kg/day	200 mg/kg/day	600 mg/kg/day*
Dose based on TBSA	300 mg/m ²	1200 mg/m ²	3600 mg/m ²
Human Equivalent Dose (HED)	8.3 mg/kg/day	33.3 mg/kg/day	100 mg/kg/day
multiple of clinical dose**	1.2	5	15
hepatotoxicity findings	increased liver weight	increased liver weight	increased liver weight, grossly enlarged liver, increased bilirubin, increased serum ALT, cholangitis/pericholangitis

*reduced from 800 mg/kg/day on day 2

**based on clinical dose of 2 g Metvix/60 kg = 33 mg/kg * 20% = 6.7 mg/kg methyl ALA

2. In a 4-dose dermal study in rats (study no. 1555/005-1032), doses of 3 g/kg were applied of the 2%, 10%, and 20% formulations. The interval between subsequent doses ranged from 10 to 18 days (Doses in clinical trials were up to a total of two with a 7-day interval in between them). The following was found:

Dose (nominal):	60 mg/kg	300 mg/kg	600 mg/kg
Dose based on TBSA	360 mg/m ²	1800 mg/m ²	3600 mg/m ²
Human Equivalent Dose (HED)	10 mg/kg	50 mg/kg	100 mg/kg
multiple of clinical dose**	1.5	7.5	15
hepatotoxicity findings	increased serum alkaline phosphatase, inflammatory foci in liver	increased serum alkaline phosphatase, decreased serum protein, increased clotting times, grossly enlarged liver in males, inflammatory foci in liver	increased serum alkaline phosphatase, decreased serum protein, increased liver weight after the recovery period, inflammatory foci in liver, 10-fold increase in serum ALA relative to concentration post-1 st dose

**based on clinical dose of 2 g Metvix/60 kg = 33 mg/kg * 20% = 6.7 mg/kg methyl ALA

3. In a 4-dose dermal study in minipigs (study no. 35635), doses of 2 g of the 20% formulation were applied to pigs weighing 5.5-7.5 kg. The 5.5 weight is used below for calculations (values for the 7.5 kg weight are in parentheses). The intervals between doses were 12, 23, and 26 days, respectively (Doses in clinical trials were up to a total of two with a 7-day interval in between them). The following was found:

Dose (nominal):	80 mg/kg (53 mg/kg)
Dose based on TBSA	2160 mg/m ² (1440 mg/m ²)
Human Equivalent Dose (HED)	57 mg/kg (38 mg/kg)
multiple of clinical dose**	8.5 (5.6)
hepatotoxicity findings	<p>increased serum ALT (statistically significant in males, but within laboratory range of historical controls; not statistically significant in females because of variability, which was due to the value for one female that was more than twice the upper limit of historical controls), increased thrombin time</p> <p>potential deficiencies: sample size was small (2/sex/group/sacrifice), organ weights were too variable to draw conclusions, no monitoring for serum ALA, no histopathology</p>

**based on clinical dose of 2 g Metvix/60 kg = 33 mg/kg * 20% = 6.7 mg/kg methyl ALA

4. A quantitative whole body autoradiography study was performed in rats with application of a single 300 mg dose of 20% cream (60 mg methyl ALA; approx. 400 mg/kg = 2400 mg/m²; HED = 67 mg/kg). Absorption across abraded sites was 13.1% of the applied radioactivity (52.4 mg/kg; HED = 8.7 mg/kg), and absorption across intact skin was 6.4% of applied radioactivity, over a 48-hour period.

Reviewer signature: _____

Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

cc:

NDA 21-415

HFD-540

HFD-540/DivDirector/Wilkin

HFD-540/SupPHARM/Jacobs

HFD-540/ PHARM/Nostrandt

HFD-540/MO/Vaughan

HFD-540/CHEM/Vidra

HFD-540/PMS/Lutwak

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ON ORIGINAL**

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this page is the manifestation of the electronic signature.**

/s/

Amy Nostrandt
7/19/02 02:53:07 PM
PHARMACOLOGIST

This addendum contains a tabular summary of pivotal animal
studies with possible hepatotoxicity signals with dose extrapolations.

Abby Jacobs
7/19/02 03:03:59 PM
PHARMACOLOGIST

Jonathan Wilkin
7/21/02 07:46:45 PM
MEDICAL OFFICER

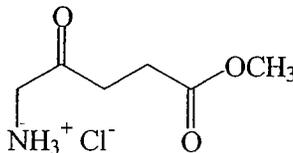
Addendum #2

PHARMACOLOGY/TOXICOLOGY COVER SHEET
Addendum to Review

NDA number: 21-415
Review number: addendum no. 2 to review no. 1
Sequence number/date/type of submission: original submission; received 26 September 2001
Sponsor and/or agent: Photocure ASA, Oslo, Norway
U.S. agent: Clementi and Associates, Rosemont, PA
Manufacturer for drug substance:

Reviewer name: Amy C. Nostrandt, D.V.M., Ph.D.
Division name: Division of Dermatologic and Dental Drug Products
HFD #: 540
Date: 7/24/02

Drug:
Trade name: Metvix® 168 mg/g cream
Generic name (list alphabetically): methyl 5-aminolevulinate hydrochloride, 5-ALA methyl ester
Code name: P-1202
Chemical name: 5-aminolevulinic acid methyl ester hydrochloride, methyl 5-aminolevulinate hydrochloride, 5-amino-4-oxo-pentanoic acid methyl ester hydrochloride, methyl 5-amino-4-oxo-pentanoate hydrochloride, methyl 5-amino-4-oxo-valeroate hydrochloride, 5-amino-4-oxo-valeric acid methyl ester hydrochloride
CAS registry number: 79416-27-6
Mole file number: not provided
Molecular formula/molecular weight: C₆H₁₂NO₃Cl; MW=181.62
Structure:



Relevant INDs/NDAs/DMFs: both sponsored by Photocure
IND 59,756 Metvix for PDT of actinic keratoses

Drug class: photodynamic therapy agent

Indication: for the photodynamic therapy of non-hyperkeratotic actinic keratoses

Clinical formulation:

<u>ingredient</u>	<u>percent (w/w)</u>	<u>mg/g</u>
methyl 5-aminolevulinate hydrochloride equivalent to methyl 5-aminolevulinate	16.8	168
glyceryl monostearate, BP		
cetostearyl alcohol, NF		
polyoxyl - stearate, NF		
methylparaben, NF		
propylparaben, NF		
edetate disodium, USP		
glycerin, USP		
white petrolatum, USP		
cholesterol, NF		
isopropyl myristate, NF		
peanut oil, NF		
refined almond oil, Ph. Eur.		
oleyl alcohol, NF		
Total	100	1000

Route of administration: topical to lesion surface

Proposed use:

The proposed labeling states that after superficial preparation of the lesions, Metvix cream is applied to lesions and held under an occlusive bandage for three hours. At that time the bandage is removed and the skin is rinsed free of excess cream. The lesion is then illuminated with red light (wavelength 570-670 nm) for a total light dose of 75 J/cm² using the Curelight lamp. two treatments one week apart.

Sites may be reassessed after three months

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:

The proposed drug product is a photodynamic therapy (PDT) agent that contains as its active ingredient a methyl ester of aminolevulinic acid or ALA. The sponsor proposes that the increased lipophilicity of the esterified ALA will allow better penetration into target lesions than with ALA. The sponsor claims that there is limited penetration of the drug into normal skin and that there is preferential accumulation of photoactive porphyrins in lesions. After de-esterification, ALA is used in the target cells to synthesize protoporphyrin IX (PpIX), which is the photoactive moiety. The drug is applied to target lesions and allowed to penetrate for a period of time, after which the site is washed and illuminated with an appropriate wavelength of light to initiate the phototoxic reaction in the lesion.

Several studies in animals either demonstrated hepatotoxicity or were suggestive of hepatotoxicity as a result of exposure to methyl aminolevulinate. The medical officer has proposed wording in the label

Handwritten marks consisting of several curved lines and a horizontal line, possibly representing a signature or initials.

Reviewer signature: _____

Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

cc:
NDA 21-415
HFD-540
HFD-540/DivDirector/Wilkin
HFD-540/SupPHARM/Jacobs
HFD-540/ PHARM/Nostrandt
HFD-540/MO/Vaughan
HFD-540/CHEM/Vidra
HFD-540/PMS/Lutwak
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/s/

Amy Nostrandt

7/29/02 12:39:47 PM

PHARMACOLOGIST

This is an addendum to my review to reflect
label changes added during the last label meeting.

Abby Jacobs

7/29/02 03:16:32 PM

PHARMACOLOGIST

Jonathan Wilkin

8/4/02 04:44:46 PM

MEDICAL OFFICER