CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209305Orig1s000

NON-CLINICAL REVIEW(S)

Pharmacology/Toxicology Supervisory Memorandum

NDA number:	209305
Supporting documents:	1, 14
CDER Stamp Date:	February 24, 2017, August 7, 2017
Type of submission:	Original NDA; 505(b)(2)
Applicant:	Anclaris Therapeutics, Inc.
Supervisor name:	Barbara Hill, PhD
Review Division:	Dermatology and Dental Products
Product:	Eskata (hydrogen peroxide) solution, 40%
Pharmacologic class:	Not designated
Indication:	Topical treatment of seborrheic keratosis in adult patients

General comments:

- I concur with the overall assessment and conclusions contained in Dr. Kumar Mainigi's Pharmacology/Toxicology review for this drug product, including that the conducted nonclinical studies support the safety of Eskata.
- I concur that Eskata is an ocular irritant based on the available literature data for hydrogen peroxide solution. It would be appropriate to include an ocular irritation warning in the Eskata label.
- I concur that there are no nonclinical approval issues for this drug product and that this NDA is approvable from a Pharmacology/Toxicology perspective.
- I concur that there are no nonclinical Post-Marketing Requirements recommended for this NDA.
- I do not concur with the recommended nonclinical labeling changes proposed by Dr. Mainigi for the Eskata label contained in Section 1.3.3 of his review. My recommended edits for the nonclinical portions of the Eskata label are provided below.

Proposed edits for the nonclinical sections of the Eskata label:

It is unclear what is the mechanism of action of hydrogen peroxide for the treatment of seborrheic keratosis. Therefore, the pharmacologic class for hydrogen peroxide for the treatment of seborrheic kearosis has not been determined for Eskata.

I concur with the Clinical Pharmacology review team that there is no systemic absorption of hydrogen peroxide under clinical conditions of use. Therefore, the one sentence provided under the "Risk Summary" subsection of Section 8.1 is appropriate per the PLLR labeling guidance and as recommended by the Division of Pediatric and Maternal Health reviewer, Dr. Christos Mastroyannis. Also, I concur with the one sentence proposed Dr. Christos Mastroyannis for Section 8.2 of the Eskata label based on no systemic absorption of hydrogen peroxide under clinical conditions of use.

(b) (4)

It is recommended that the underlined wording be inserted into the label and the ~~strikethrough~~ wording be deleted from the label below

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE

ESKATA™ [redacted] is indicated for the treatment of seborrheic keratosis lesions (1)

8 USES IN SPECIFIC POPULATIONS
8.1 Pregnancy
Risk Summary
Hydrogen peroxide is not absorbed systemically following topical administration, and maternal use is not expected to result in fetal exposure to the drug.

[redacted]

8.2 Lactation
Risk Summary
Hydrogen peroxide is not absorbed systemically by the mother following topical administration, and breastfeeding is not expected to result [redacted] in exposure of the child to hydrogen peroxide [redacted]

12. CLINICAL PHARMACOLOGY
12.1 Mechanism of Action

[redacted]

The mechanism of action for ESKATA for the treatment of seborrheic keratosis is unknown.

13 NONCLINICAL
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Long-term animal studies have not been performed to evaluate the carcinogenic potential of ESKATA™ [redacted] or hydrogen peroxide.

Hydrogen peroxide has been found to exhibit positive results in in vitro tests [redacted] for genotoxicity, but ~~this~~ has not exhibited positive results in in vivo tests for genotoxicity [redacted] presumably due to the rapid metabolism of hydrogen peroxide [redacted].

The effects of hydrogen peroxide on fertility have not been evaluated. Hydrogen peroxide has been associated with effects on sperm function and elevated testicular hydrogen peroxide concentration has been implicated in male infertility, although in vivo (b) (4), no effect of hydrogen peroxide on sperm function has been demonstrated. (b) (4)

(b) (4)

Clean Version of the Nonclinical Sections for the Label

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE

ESKATA™ is indicated for the treatment of seborrheic keratosis lesions (1)

8 USES IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Hydrogen peroxide is not absorbed systemically following topical administration, and maternal use is not expected to result in fetal exposure to the drug.

8.2 Lactation

Risk Summary

Hydrogen peroxide is not absorbed systemically by the mother following topical administration, and breastfeeding is not expected to result in exposure of the child to hydrogen peroxide.

12. CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism of action for ESKATA for the treatment of seborrheic keratosis is unknown.

13 NONCLINICAL

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of ESKATA™ or hydrogen peroxide.

Hydrogen peroxide has been found to exhibit positive results in in vitro tests for genotoxicity, but has not exhibited positive results in in vivo tests for genotoxicity, presumably due to the rapid metabolism of hydrogen peroxide.

The effects of hydrogen peroxide on fertility have not been evaluated. Hydrogen peroxide has been associated with effects on sperm function and elevated testicular hydrogen peroxide concentration has been implicated in male infertility, although in vivo, no effect of hydrogen peroxide on sperm function has been demonstrated.

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/s/

BARBARA A HILL
10/12/2017

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	**209-305**
Supporting document/s:	SD-1
Applicant's letter date:	02-24-2017
CDER stamp date:	02-24-2017
Product:	ESKATA™ (Hydrogen peroxide), topical solution,40%
Indication:	Treatment of seborrheic keratosis in adult patients
Applicant:	Aclaris Therapeutics, Inc Malvern, PA
Review Division:	Dermatology and Dental Products
Reviewer:	Kumar D. Mainigi, MSc. M.P.H., Ph.D. Diplomat American Board of Toxicology
Supervisor/Team Leader:	Barbara Hill, Ph.D.
Division Director:	Kendall Marcus, MD
Project Manager:	Strother Dixon

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of [NDA 209-305] are owned by [Aclaris Pharmaceuticals, Inc.] or are data for which [Aclaris Pharmaceuticals, Inc.] has obtained a written right of reference. Any information or data necessary for approval of [NDA 209-305] that [Aclaris Pharmaceuticals, Inc.] does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of [NDA 209-305].

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1 Executive Summary

1.1 Introduction

From regulatory point of view (FDA: Rx/OTC), hydrogen peroxide (H_2O_2) is classed as antiseptic and disinfectant. It is sold under various trade names such as Aplicare One Hydrogen Peroxide, Orajel Perioseptic, Peroxlyl, and Proxacol as aqueous solution in a wide range of concentrations. Based on its oxidizing properties, commercially it is often used as bleaching or cleaning agent.

Hydrogen peroxide generates reactive oxygen radicals in biological systems. In clinical settings, this ability is employed in nursing homes, home healthcare settings, blood banks, laboratories, emergency rooms, and other hospital departments. At low concentrations, it is mild antiseptic, germicidal and cleansing agent. Its major clinical uses are as a cleaning agent for suppurating wounds, inflamed mucous membranes and tracheotomies. Its oral preparations at concentrations ranging from 3 to 40% are used in tooth whitening products.

The current new drug application ESKATA™ (hydrogen peroxide) topical solution, 40% for the treatment of seborrheic keratosis (KS) lesions is submitted under the regulatory pathway 505(b) (2).

1.2 Brief Discussion of Nonclinical Findings

The non-clinical safety profile of proposed hydrogen peroxide formulation is complete and satisfactory to support its clinical use for the treatment of seborrheic keratosis. No additional studies are warranted.

Under the original IND 117,635, in 4-week dermal studies (vehicle and sham controls, 25%, 32.5% and 40% hydrogen peroxide) in rats and minipigs, animals received four weekly (on days 1, 8, 15 and 22) applications of test solutions, followed by 2- week recovery period.

Immediately following the first application, white patches were observed in most of the high-dose females and a few male rats; these lesions completely disappeared by day four. In addition, flaky skin and scabbing was observed on the dorsal anterior or posterior regions of some the same females. A few mid- and high-dose males exhibited very slight edema. No dermal reactions were observed at the end of the recovery period.

The mean average pre-dose plasma (basal) level of H_2O_2 on day 1 was 0.62μM (0.352-0.887μM). Level in females was always greater. On the same day, plasma peroxide levels after treatment with 25% and 32.5% remained within the basal range at all-time points. However, in 40% females, the concentration doubled to 1.2μM at 10 minutes returning to basal range within 30 minutes. . After the second weekly dose, the plasma level at 10 minutes was 1.3μM and 1.5μM at 1 hour, returning to basal level at 6.5 hour.

Histologically, transient skin reactions more pronounced in drug treated male minipigs included degeneration of superficial collagen fiber, vacuolation of superficial dermis, perivascular inflammatory infiltrate, epidermal bullae and attenuation, basal cell hyperplasia, and occasional epidermal necrosis. At recovery, 3/6 high-dose males still exhibited these lesions. All high-dose females had some transient lesions.

In minipigs, the pre-dose basal plasma concentration of H_2O_2 ranged between 0.05 to 3.255μM with combined mean average of 0.723 (males 1.019, females 0.436μM). The high level in male pigs was consistent throughout the study period. Reportedly, a similar pattern was observed in humans. However, irrespective of the gender, dose level, duration of treatment or blood sampling time, the plasma concentration of peroxide did not exceed the basal range.

In current submission, twelve-dose rat and minipig dermal studies were merely an extension of similar 4-week studies mentioned above. The dermal lesions were also microscopically identical.

In rats, 40% hydrogen peroxide was well tolerated locally and systemically following 12 weekly topical applications. Its dose-dependent (32.5% and 40%) reversible lesions (blanched skin, edema, scabs etc.) were confined to applications sites. Reactions were more pronounced in females. At the end of the recovery period of 28 days, histological pictures were similar in drug and vehicle treated groups.

The 12-dose minipig study was aimed to further explore the extended therapeutic potential and safety at dose greater than the one proposed (40% versus 45%) in the treatment of warts. The concentration level was condensed by applying the substance on body surface area (BSA) of only 2.5% (100cm^2) instead of 10% of BSA (400cm^2) as in the previous study.

Dose of 45% hydrogen peroxide for 12 weeks in minipigs did not cause any systemic toxicity, indicating none or negligible absorption. The completely reversible local toxicity was confined to skin irritation reactions such as minimal to slight capillary ectasia/congestion and inflammation of the superficial dermis, minimal to slight epidermal hyperplasia and epidermal vacuolation.

Local lesions were similar to those observed in minipigs receiving 40% hydrogen peroxide. In both studies, males and females exhibited similar lesions.

Absolutely no systemic toxicity was observed in rats and minipigs, and no penetration occurred through the dermatomed minipig and human skins (Franz pump). Based on these findings, it was decided not to conduct any safety pharmacology studies.

Because of high cytotoxicity of H_2O_2, all 93 *in vitro* genotoxicity (cf from literature) studies were positive in terms of reverse mutation assays, gene mutation, chromosomal aberration, DNA strand break, unscheduled DNA synthesis, and DNA damage and sister chromatid exchange.

On the other hand, all 9 *in vivo* genotoxicity (literature) studies were negative, presumably due to lack of translation, rapid catabolism of peroxide to its basal nontoxic level, and buffering/detoxifying systems in plasma not permitting this level to elevate above the endogenous concentration.

Consistently generated peroxide in all internal organs including gonads is rapidly disposed. The molecule is not absorbed dermally; and is instantly degraded into water and oxygen by catalases in the skin. Catalase has extremely high turnover, one enzyme moiety catabolizes over a million hydrogen peroxide molecules per second.

In addition to short duration of treatment, lack of any dermal absorption and systemic toxicity, and negative non-mutagenic and non-clastogenic behavior in *in vivo* genotoxicity studies, carcinogenicity studies with hydrogen peroxide are not warranted.

Based on the same biological rationale, there is no need to conduct reproductive and developmental studies with hydrogen peroxide.

LLNA assay in mice clearly established hydrogen peroxide formulation as a non-sensitizer.

After comprehensive evaluation of ocular studies conducted with various H_2O_2 formulations (consumer products), the European Commission opinion SCCP/1129/07 in 2007 declared hydrogen peroxide as an ocular irritant with a threshold level for human eye ranging from 0.03 to 0.1 percent.

In phototoxicity assay, MEC (molar extinction coefficient) of 0.807 L $mol^{-1}cm^{-1}$ for hydrogen peroxide was considered to be negligible compared to threshold of 1,000 MEC required to cause any cellular photo damage.

Obviously, there is no valid reason to determine the margin of safety for systemic toxicity (versus minipig) in human. Short lived local lesions were 100% reversible.

In a nut shell, available non-clinical safety profile soundly supports the clinical safety of 40% hydrogen peroxide solution used at schedule treatment intervals.

1.3 Recommendations

None

1.3.1 Approvability

Yes

1.3.2 Additional Non Clinical Recommendations

N/A

1.3.3 Labeling

It is recommended that the underlined wording be inserted into the label and the ~~strikethrough~~ wording be deleted from the label below.

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE

ESKATA™ [redacted] (b) (4) is indicated for the treatment of seborrheic keratosis lesions (1)

Reviewer's comments: The pharmacologic class for hydrogen peroxide has not been determined. It is unclear what is the mechanism of action of hydrogen peroxide for the treatment of seborrheic keratosis.

8 USES IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Hydrogen peroxide [redacted] (b) (4)

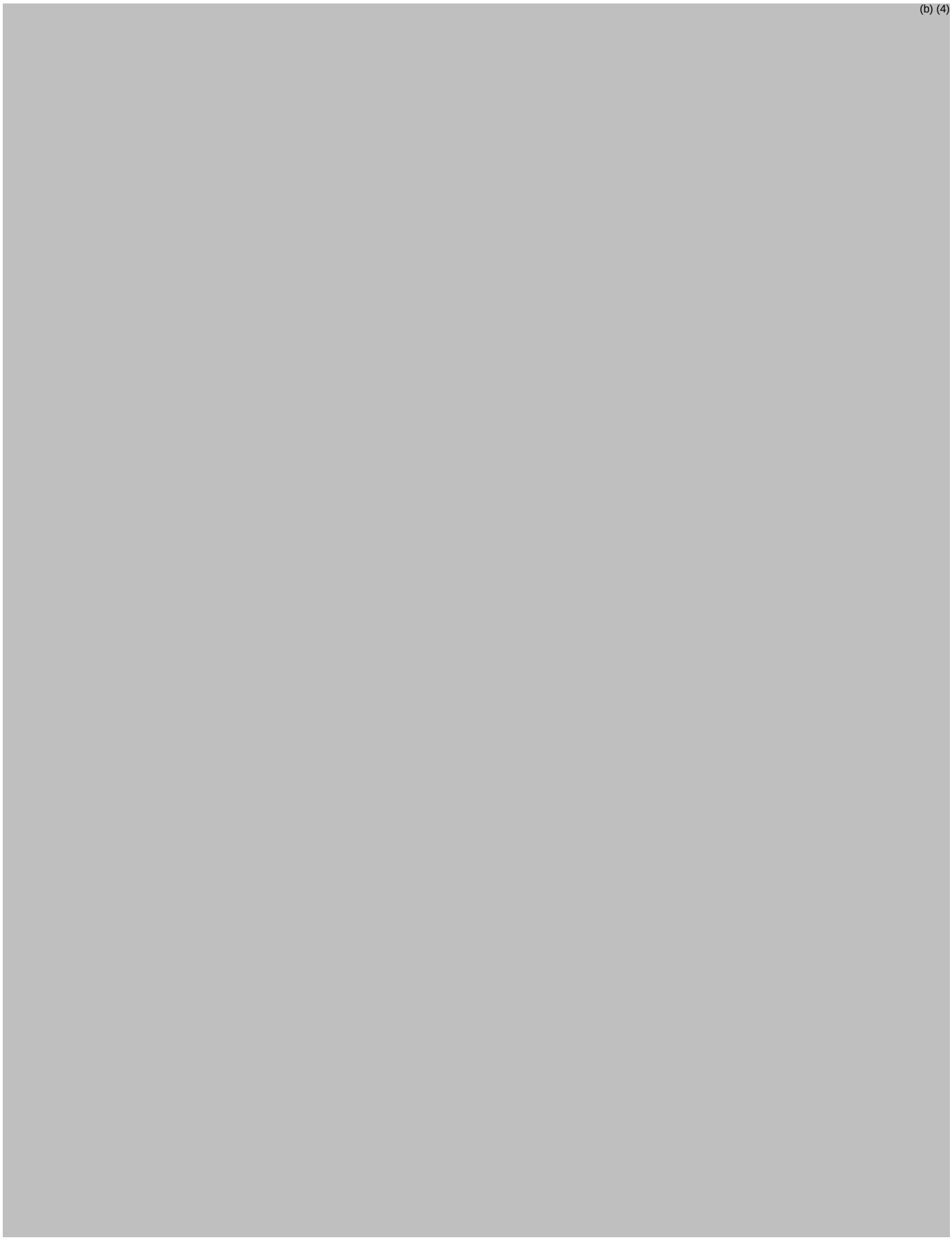
[redacted] (b) (4)

8.2 Lactation

Risk Summary

Hydrogen peroxide [redacted] (b) (4)

(b) (4)



(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): **7722-84-1**

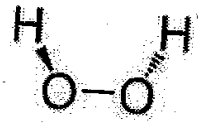
Generic Name: Hydrogen peroxide

Code Name: **A-101**

Chemical Name: Dihydrogen dioxide (IUPAC)

Molecular Formula/Molecular Weight: **H_2O_2/34.01**

Structure or Biochemical Description



Pharmacologic Class: The pharmacologic class for hydrogen peroxide for the treatment of seborrheic keratosis has not been determined. Hydrogen peroxide is a keratolytic agent. However, actual mechanism of action of hydrogen peroxide for the treatment of seborrheic keratosis has not established.

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 117,635

2.3 Drug Formulation

ESKATA™ topical solution contains 40% hydrogen peroxide in an aqueous solution containing isopropyl alcohol and water. The drug preparation is supplied in a single dose treatment package. One unit applicator delivers a maximum volume of 0.7mL to the surface of seborrheic keratosis lesion.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Adult patients with seborrheic keratosis lesions (face, neck and extremities) will receive a topical application up to 4 times, approximately 1 minute apart, during a single in-office treatment session.

2.7 Regulatory Background

From regulatory point of view (FDA: Rx/OTC), hydrogen peroxide is classed as an antiseptic and disinfectant. It is sold under various trade names such as Aplicare One Hydrogen Peroxide, Orajel Perioseptic, Peroxlyl, and Proxacol as aqueous solution over a wide range of concentrations. Based on its oxidizing properties, it is often used as bleaching or cleaning agent.

FDA has also granted 510(k) clearance for H_2O_2 in individual device manufacturing applications.

3 Studies Submitted

3.1 Studies Reviewed

1. 12-dose dermal application of up to 40% H_2O_2 formulation in rats
2. 12-dose dermal application of 45% H_2O_2 formulation in minipigs
3. Local lymph node assay in mice
4. Ocular irritation of hydrogen peroxide (compiled from publications)
5. Phototoxicity of hydrogen peroxide

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

Primary review of IND 117,635

4 Pharmacology

4.1 Primary Pharmacology

Endogenous toxicants-stressors H_2O_2 and reactive oxygen species (ROS) such as anion $O_{2^{\cdot}}^{-}$ are continuously generated in all cells. Enhanced levels of these radicals are implicated in the pathophysiology of conditions such as ischemia/reperfusion injury, and inflammatory bowel disease. Studies in humans and rats have shown that the normal (basal) plasma level of below 1μM was elevated by 10 times in hypertension.

There are efficient cellular defense mechanisms to maintain the cellular concentration of these radicals at steady state (basal) level much below the threshold for systemic toxicity. Peroxisomes in mammalian eukaryotic cells contain enzymes that oxidize several endogenous products generating ROS. Superoxide dismutase (SOD) acts as a free radical scavenger to catalyze the dismutation of $O_{2^{\cdot}}^{-}$ to hydrogen peroxide.

$$O_{2^{\cdot}}^{-}+O_{2^{\cdot}}^{-}+2H^{+}=H_2O_2+O_2$$

Next catalase, here H_2O_2 serves both as a donor and acceptor to detoxify itself.

$$H_2O_2+H_2O_2=O_2+2H_2O$$

Catalases found in all cells exposed to oxygen have one of the highest turnover numbers of all enzymes; one enzyme molecule can oxidize millions of H_2O_2 molecules each second. The exact half-life of H_2O_2 is not possible to determine; however, from its metabolic turnover, it is assumed to be in seconds. The half-life of large molecular weight benzoyl peroxide (another karyatolytic agent) runs between 1 to 60 minutes depending on the temperature. Degradation products of H_2O_2 are scavenged by glutathione peroxidase. By having both the peroxide producing and utilizing enzymes in one cellular compartment peroxisome, the cell protects itself from the toxicity of hydrogen peroxide.

In a published clinical study, subjects (n=16) were treated with 10% H_2O_2 bleaching gel on strips. At 5 minutes post-application, median concentration of peroxide in gingiva was declined to 0.7%, and at 30 minutes to 0.1%. However, at any time point, peroxide concentration in salivary samples never exceeded the basal level of 0.014% (Gerlach et. el; *J. Clin Dent*. 2008; 19(2), 59-63).

Seborrheic keratosis (SK) is one of the most common types of epidermal noncancerous growths in older adults; these sharply-marked, brownish, smooth surface tumors on face and trunk measure a few millimeters in diameter. Histologically, the pathognomonic feature is an exophtic tumor overlying a straight line from the normal epidermis at one end of the tumor to the normal epidermis at the other end.

Recent investigations into the molecular pathogenesis of SK have identified a somatic mutation in a membrane-bound tyrosine kinase receptor- the Fibroblast Growth Factor Receptor 3 (FGr3), and second mutation in the P1K3CA gene encoding a p110 subunit of class phosphatidylinositol 3-kinase.

The generation of $O^{\cdot -}$ and H_2O_2 in minute amounts in the skin cells is instantly disposed by the built-in complex antioxidant defense system comprised of catalases and superoxide dismutase; their degradants are scavenged by glutathione peroxidase. Thus, the nontoxic intracellular (basal) concentration of peroxide is tightly controlled under the normal physiological conditions.

The major cellular toxicity of peroxide results from its conversion to reactive hydroxyl (OH) radicals either due to UV exposure or in presence of (b) (4) metals (b) (4) in biological systems. Extreme local stress overpowers the antioxidant defense system in the skin, and shifts it to pro-oxidant state, allowing peroxide to act through direct oxidation of organic tissues, and through generation of ROS resulting in increased concentration of oxygen damaging to lesions such as seborrheic keratosis.

It is anticipated that the topical applications of superphysilogical concentrations of H_2O_2 in ESKATA™ will create a pro-oxidant state destroying growths by direct therapy (chemical excision) causing damage to membrane of SK cells without affecting the floor (newly generating epidermis), and the neighboring normal epidermis.

4.2 Secondary Pharmacology

No studies were conducted

4.3 Safety Pharmacology

Penetration studies using dermatomed human and minipig skins, and a 4-week minipig dermal study with 40% H_2O_2 formulation did not raise the plasma level of H_2O_2 indicating lack of any systemic absorption. Based on these findings, safety pharmacology studies were not warranted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

N/A

5.2 Toxicokinetics

N/A

6 General Toxicology

6.1 Single-Dose Toxicity

Not required

6.2 Repeat-Dose Toxicity

Study title: A-101: Cyclical dermal (semi-occluded) toxicity study of 12 applications in the rat with a 28 day treatment-free period.

Study no.:	FAQ0009
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08-22-2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrogen peroxide, #7189/001 &7188/001 and 99.1-104.7%

Key Study Findings

A-101 solution at dose level up to 40% hydrogen peroxide was well tolerated after 12 approximately weekly topical applications without causing any systemic toxicity, and its dose-dependent reversible local toxicity (blanched skin, edema, scabs etc.) was restricted to the application sites. These lesions were more pronounced in females. At the end of recovery period, microscopic findings were similar in drug and vehicle treated rats.

Methods

Doses:	Groups: 1. Vehicle (control) 2. 32.5% hydrogen peroxide 3. 40% hydrogen peroxide
Frequency of dosing:	A-101 solution was topically applied on 12 occasions each separated by at least 1 week. Each application site involved approximately 10% (400 cm^2) of the total body surface area (BSA)
Route of administration:	Dermal
Dose volume:	0.17mL (6µL/cm^2)
Formulation/Vehicle:	Drug in aqueous solution containing 5% isopropyl alcohol
Species/Strain:	Male and female rats/Hsd:Sprague Dawley (CD)
Number/Sex/Group:	Groups: 1 (sham control) 15/sex; group 2 (low-dose) 10/sex; group 3 (high-dose) 15/sex
Age:	8-9 weeks
Weight:	Males 291-330 , females 163-244g
Satellite groups:	5 animals/sex in groups 1 and 3 were maintained for 28 treatment-free days (recovery period)
Unique study design:	No
Deviation from study protocol:	The initial plan of weekly treatment schedule did not work because of severity and extent of skin reactions observed in most of the high-dose and some low-dose rats by 6th dose. As a result, the interval for next application was extended up to 16 days to allow the application sites of all animals to improve before the next dose.

Observations and Results

Mortality/Clinical Signs

One high-dose male was found dead on treatment day 50 and another from the same group died on treatment-free day 128. Reportedly, both animals were in good clinical condition before death. The cause(s) of deaths remained unexplained as there were no correlated histological findings. On day 108, one high-dose female was euthanized due to declining health condition; the major cause of death labored breathing was microscopically correlated to purulent Broncho-pneumonia.

Dermal reactions

Application sites were visually assessed prior to the first dose, and 3 times daily during rest of the study period. Dermal lesions were scored (Draize score).

Skin blanching (white raised areas) developed immediately after the drug application and more pronounced in high dose rats, was followed by slight to moderate erythema, scabbing and edema. Additional local lesions included matting of fur and wrinkles.

Following extension of days between drug applications, most females exhibited reduced severity and shorter time-life of lesions; 15 days after the last application, all lesions had subsided. A similar pattern was observed in males where no lesions were observed after 7 days of the last application.

Dermal lesions were more prevalent in females, and collectively, severity was much greater in high dose rats.

Body Weights/Feed Consumption

Both parameters were recorded weekly throughout the study period.

None of the parameters were affected with drug treatment.

Ophthalmoscopy

Eye examinations were conducted on all rats prior to study initiation, and controls and high-dose animals were again examined after the 11th dose.

Eye morphology and functions were not affected by treatment with A-101 solution.

ECG

No electrocardiograms were recorded.

Hematology/Coagulation/Clinical Chemistry

Blood samples for clinical pathology were collected from 10rats/sex in each group after the 12th (last) dose.

A number of analyzed parameters indicated statistically significant changes between controls and drug-treated rats. However, none of changes were considered to be of any toxicological significance, because numerically differences were minor and within the range of biological variation, not consistent between the sexes, and did not exhibit any dose-related trend.

Urinalysis

Overnight fasting urine samples were collected after the 11th dose.

There were no inter-group differences in urinary parameters.

Gross Pathology

All survivors at the end of treatment and recovery periods were subjected to comprehensive necropsy examination of organs/tissues for macroscopic abnormalities.

At recovery, only the treatment related external lesion was scabbing at the application site in one high-dose male and four low-dose females.

Organ Weights

A total of 12 major organs were weighed to determine their absolute and relative to body weights

Organ weights in drug treated and control rats were similar.

Histopathology

Adequate Battery
Yes

A total of 44 tissues/organs were processed for microscopic examination.

Peer Review
Yes

Histological Findings

The incidence of slight to minimal hyperplasia was observed in most of the A-101 solution treated rats. The severity of lesions was similar in both sexes. At recovery, no histopathological differences were observed at the application sites of drug treated and control animals.

Special Evaluation

None

Toxicokinetics

No attempt was made to investigate the pharmacokinetic behavior of A-101 solution. In several previous topical (non-clinical/clinical) studies, applications of hydrogen peroxide caused an extremely short-lived insignificant increase in its endogenous pool, not sufficient to inflict any systemic injuries.

Dosing Solution Analysis

Not conducted due to extremely short half-life of the active agent.

Study title: A-101: Cyclical Dermal (semi-occluded) 12 dose toxicity study in the minipig with a 28-day treatment-free period

Study no.:	FAQ0012
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04-21-2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Hydrogen peroxide, #7259/001 and 100.2%

Key Study Findings

Twelve (9/10 days apart) topical applications of 45% hydrogen peroxide in minipigs absolutely caused no systemic toxicity. The completely reversible local toxicity was confined to skin irritation reactions such as minimal to slight capillary ectasia/congestion and inflammation of the superficial dermis, minimal to slight epidermal hyperplasia and epidermal vacuolation. Local lesions observed in this study were similar to those in previous study where minipigs received 40% hydrogen peroxide. In both studies, lesions were similar in both sexes.

Methods

Doses:	Sham-control (un-dosed) and 45% A-101 solution
Frequency of dosing:	Once daily. Animals received 12 applications with 9-10 days between doses (days 1-118). Each pre-shaved application site involved approximately 2.5% (100cm^2) of BSA. Semi-occluded sites were exposed to the test formulation for 6-7 hours. Following removal of dressing sites were washed gently with water.
Route of administration:	Topical
Dose volume:	0.6 mL(0.6µL/cm^2)
Formulation/Vehicle:	Hydrogen peroxide in 5% aqueous isopropyl alcohol
Species/Strain:	Minipig/Gottingen
Number/Sex/Group:	6
Age:	4-6 months
Weight:	Males 9.8-12.3 kg; females 10.3-12.1 kg
Satellite groups:	Two animals/sex from each group were maintained treatment free for 28 days
Unique study design:	Nothing significant
Deviation from study protocol:	Nothing significant

Observations and Results

Mortality/Clinical Signs

Animals were observed daily for clinical signs of toxicity, morbidity and mortality. Dermal reactions were visually assessed three times a day at one minute before application, immediately post-application and after 6 hours exposure under semi-occlusive dressing. At the end of dosing period, application sites were examined daily including the autopsy day.

Dermal reactions: No skin lesions were observed in sham controls, indicating that lesions developed in hydrogen peroxide group were caused by the active ingredient.

Local reactions appeared immediately after drug application included abnormal pale/purple patches indicating a very slight to slight to well defined erythema. The intensity of lesions persisting for a short time regressed prior to the next dose.

In a few animals (20%), dermal lesions were not fully resolved prior to the next application; persistent lesions included flaky skin, slight erythema, or scabbing.

A few animals exhibited very slight to slight edema, flaky skin and scabby and white raised patches.

Visually, in all hydrogen peroxide treated pig skin appeared normal at the end of recovery period.

Body Weights/Feed Consumption

Body weights were recorded prior to initiation of study, and weekly thereafter throughout the study period. Food consumption was visually assessed to ascertain that animals were eating properly.

No intergroup differences in gain in body weight and food consumption were observed.

Ophthalmoscopy

Both eyes of all pigs were examined prior to study initiation and during 16 week of treatment period. Tests included direct and indirect ophthalmoscopy and slit lamp biomicroscopy.

No drug related changes in eye morphology or any other signs of ocular toxicity were observed.

ECG

No electrocardiographs were recorded.

Hematology/Clinical Chemistry/Coagulation/Urinalysis

Blood samples for clinical pathology analyses were collected prior to study initiation (acclimation period), and during treatment week 17.

Urine samples were collected where possible by cytokinesis at necropsy of all animals sacrificed at the end of treatment period.

No inter-group differences in spectrum of analyzed parameters were observed.

Gross Pathology

All animals were subjected to necropsy examination at study termination. The cranial, thoracic and abdominal cavities were opened to look for gross lesions.

At the end of treatment period, reddening of application sites was observed in 3/4 females and all 4 males. Reportedly, incidence was correlated with the capillary eclasia and congestion seen in the superficial dermis.

Fully reversible skin lesions graded minimal to slight in severity were similar in prevalence in both sexes; and in comparison to previous short-term study, significant decrease in severity of lesions was correlated to longer periods between dosing.

Organ Weights

Eight major internal organs trimmed free of loose fat were weighed to determine the absolute and relative to body organ weights (ratios).

Absolute and relative organ weights were similar in the treated and untreated animals.

Histopathology

Adequate Battery

Yes

A total of 45 tissues/organs were processed for microscopic examination.
In recovery animals, only tissue samples from treated and untreated application sites were examined.

Peer Review

Yes

Histological Findings

At the end of recovery period, minimal epithelial hyperplasia was evident at the treated sites of most animals.

Special Evaluation

None

Toxicokinetics

Not conducted

Dosing Solution Analysis

Not conducted

7 Genetic Toxicology

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

7.2 *In Vitro* Assays in Mammalian Cells

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

Because of high cytotoxicity of H_2O_2, all 93 *in vitro* genotoxicity literature studies were positive in terms of reverse mutation assays, gene mutation, chromosomal aberration, DNA strand break, unscheduled DNA synthesis, and DNA damage and sister chromatid exchange. On the other hand, all 9 *in vivo* genotoxicity literature studies were negative, presumably due to lack of translation, rapid catabolism of peroxide to its basal nontoxic level, and buffering/detoxifying systems in plasma not permitting levels to elevate above the endogenous concentration.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

In addition to short duration of treatment, lack of any dermal absorption and systemic toxicity, and negative non-mutagenic and non-clastogenic behavior in *in vivo* genotoxicity studies, carcinogenicity studies with hydrogen peroxide were not warranted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

9.2 Embryonic Fetal Development

9.3 Prenatal and Postnatal Development

Consistently generating hydrogen peroxide in all internal organs including gonads is rapidly disposed. The molecule is not absorbed dermally; and is instantly degraded into water and oxygen by catalases in the skin. Due to unique biochemical behavior of hydrogen peroxide, no reproductive and developmental toxicology studies can be conclusively conducted.

10 Special Toxicology Studies

Local Tolerance

Study title: **A101: Local Lymph Node Assay (LLNA) in Mice**

Study number: FAQ0010
Study initiation date: 11-12-2014
Facility: (b) (4)

Materials and Methods

Test substance: 40% hydrogen peroxide in 95% water/ 5% (w/w) isopropyl alcohol
Concentrations tested: 2.5%, 5.6%, and 10% (v/v)
Negative control: 95% water plus 5% isopropyl alcohol
Positive control: Alpha-hexyl cinnamaldehyde in acetone/olive oil (4+1, v/v)
Radio-labelled thymidine: ^{3}H-methyl thymidine (^{3}HTdR)
Test species/strain: Female mice (nulliparous and non-pregnant)/CBA/CaOlaHsd
Age: 8-9 weeks
GLP compliance: Yes
QA: Yes

The dose selection for the main study was based on a 10-11 weeks preliminary study. The highest concentration tested was the highest level that could be achieved whilst avoiding systemic toxicity and excessive local skin irritation during study period, also avoiding any mortality.

Three groups each of 5 mice received three different concentrations of the test substance daily on 3 consecutive days on dorsum of each ear. Two groups of 5 mice were treated similarly with negative (vehicle) and positive controls, respectively.

Five days after the first topical application, mice received 19.8µCi ^{3}HTdR intravenously through the tail vein. Five hours later, mice were sacrificed and draining auricular lymph nodes excised and pooled were subsequently washed and incubated overnight with trichloroacetic acid.

The proliferative capacity of pooled lymph node cells was assessed by determining the incorporated ^{3}HTdR using scintillation counter.

The Stimulation Indices (S.I.) were determined for all the test groups and compared with S.I. of positive control. In LLNA, a test substance is regarded as a sensitizer if the exposure to one or more concentration resulted in a 3-fold or greater increase in incorporation of ^{3}HTdR compared with concurrent controls as indicated by S.I. score.

Results and Conclusion

All animals survived to scheduled necropsy without exhibiting any sign of dermal irritation or systemic toxicity.

The stimulation Indices of 0.96, 1.24 and 1.49 at three increasing concentrations indicated that hydrogen peroxide was not a sensitizer. On the other hand, S.I of 10.65 for positive control also established the validity of the LLNA assay. In addition, 3- 5 mice treated with positive control also exhibited irritation (erythema) on the application sites.

Eye Irritation

Ocular toxicity studies of hydrogen peroxide from various consumer products (e.g. oral hygiene and tooth whitening) were evaluated by European Commission in 2007. Reportedly, 5 and 8% solutions were slight, and moderate ocular irritants, respectively. A 10% solution caused a serious damage to the eye. A threshold for irritation in human eye was 0.03 to 0.1 percent.

A warning about ocular irritancy and corrosive action of hydrogen peroxide is included in the label.

Phototoxicity

Study number: SAI-ALX-101
Facility: (b) (4)

Materials and Methods

The light absorbance by hydrogen peroxide was determined at concentrations of 17.35 and 34.7mM at wave lengths between 200 and 700 nm.

Hydrogen peroxide exhibited some absorbance with a major peak below 200 nm. Low absorbance was recorded at 290 nm. The absorbance at 290nm, the highest in the range, was used to calculate the molar extinction coefficient (MEC).

Results and conclusion

MEC at both test concentrations of hydrogen peroxide was (b) (4) This value is far below the threshold of 1000 $Lmol^{-1}cm^{-1}$ for phototoxicity (ICH S10).

In conclusion, A-101 solution did not exhibit any potential to induce cellular phototoxicity.

11 Integrated Summary and Safety Evaluation

Twelve-dose (total treatment period 90 days) dermal studies (rat and minipig) are merely an extension of similar 4-week studies reviewed under original IND 117,635. In both set of studies, associated dermal lesions were microscopically identical.

In the current rat subchronic study, 40% hydrogen peroxide formulation was well tolerated locally and systemically following 12 weekly topical applications. Its dose-dependent (32.5% and 40%) reversible lesions (e.g. blanched skin, edema, scabs) were confined to the application sites. Dermal reactions were more pronounced in females. At the end of the recovery period of 28 days, histological pictures were similar in drug and vehicle treated groups.

The 12-dose minipig study was aimed to further explore the possible extention of therapeutic potential and safety at dose greater (40% versus 45%) than recommended in the treatment of warts. The dose was further condensed by applying the substance on total body surface area of only 2.5% ($100cm^2$) instead of 10% ($400cm^2$) as in the previous study.

Twelve (9/10 days apart) topical applications of 45% hydrogen peroxide in minipigs absolutely caused no systemic toxicity, indicating none or negligible absorption. The completely reversible local toxicity was confined to skin irritation reactions including minimal to slight capillary ectasia/congestion and inflammation of the superficial dermis, minimal to slight epidermal hyperplasia and epidermal vacuolation. Local lesions were similar to those observed in minipigs receiving 40% hydrogen peroxide. In both studies, males and females exhibited similar lesions.

In mice lymph node assay (LLNA), A-101 solution did not exhibit any sensitization.

After comprehensive evaluation of ocular studies with H_2O_2 in consumer products, the European Commission opinion SCCP/1129 in 2007 declared hydrogen peroxide as an ocular irritant with a threshold level for human eye ranging from 0.03 to 0.1 percent.

In phototoxicity assay, MEC (molar extinction coefficient) of (b) (4) for hydrogen peroxide was negligible compared to threshold of 1,000 MEC required to cause any cellular photo damage.

In vitro assays with fresh mounted skins (Franz pump) of minipig and human, no peroxide was detected in the receptor fluid, indicating an absolute absence of absorption in both species.

At all dose levels in all test species, very low amount of peroxide (0.006% of the applied dose (~11.69ng) was found in the skin matrices. This value was well within the range of endogenous levels of H_2O_2 detected in the negative control skin.

Furthermore, this amount was far below the standardized quantitation limit of 23.66ng, indicating no impact on endogenous tissue levels of H_2O_2 after topical applications.

Because of high cytotoxicity of H_2O_2, all 93 *in vitro* genotoxicity literature studies were positive in terms of reverse mutation assays, gene mutation, chromosomal aberration, DNA strand break, unscheduled DNA synthesis, and DNA damage and sister chromatid exchange.

On the other hand, all 9 *in vivo* genotoxicity literature studies were negative, presumably due to lack of translation, rapid catabolism of peroxide to its nontoxic catabolites, and buffering/detoxifying systems in plasma not letting hydrogen peroxide level to elevate above the endogenous pool concentration.

Consistently generating hydrogen peroxide in all internal organs including gonads is rapidly biodisposed and reduced to below the nontoxic level. The molecule is not absorbed dermally; and is instantly degraded into water and oxygen by catalases in the skin. Catalase has one of the highest turnovers of all enzymes; one enzyme molecule can convert millions of substrate molecules per second.

In addition to short duration of treatment, lack of any dermal absorption causing any systemic toxicity, and negative nonmutagenic and nonclastogenic behavior in *in vivo* genotoxicity studies, carcinogenicity studies with hydrogen peroxide were not warranted, and or possible to conduct conclusively.

Based on the same biological rationale, reproductive and developmental studies with hydrogen peroxide were also not possible to conduct.

Obviously, there is no valid reason to determine the margin of systemic toxicity in human (versus minipig); short lived local toxicity was 100 percent reversible.

On the whole, presented non-clinical safety profile of hydrogen peroxide soundly supports the safe topical clinical use of 40% hydrogen peroxide.

12 Appendix/Attachments

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

/s/

DAIVENDER K MAINIGI
10/11/2017

BARBARA A HILL
10/12/2017

Please refer to the secondary nonclinical review for Pharmacology/Toxicology recommended edits for the nonclinical sections of the label.