

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-670

PHARMACOLOGY REVIEW(S)

**Memo to File
Consultation Report**

NDA 21-670

Drug: Trypan Blue (Vision Blue 0.06%)

Sponsor: Dutch Ophthalmic Research Center, International

Reviewer: Conrad Chen

Date: November 10, 2004

Background: In original pharmacology review of NDA 21-670, dated March 1, 2004, the approval of Trypan Blue Dye 0.06% was recommended for —

On November 5, 2004, the review chemist of NDA 21-670 requested a pharmacology consultation regarding the safety of recently reported impurities in the drug substance. This memo is written in response to that request.

The request for consultation from chemistry reviewer, dated Nov. 5, 2004:

NDA 21-670 (Vision Blue, 0.06%) toxicology consultation

According to drug substance specification please evaluate the following impurities with acceptance criteria of NMT — of the drug substance. Is it safe when injected into the anterior chamber of the eye during surgery at this level?

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Evaluation:

The literature search for the Impurities

—, did not come up with any toxicology information.

The recommended clinical dose of Vision Blue 0.06% is a one-time application of 0.1 to 0.3 ml (60-180 µg) into the anterior lens capsule. Therefore, the amount of impurities in the clinical dose will be

—
According to the Guidance for Industry Q3B (Impurities in New Drug Products), the thresholds for qualification of degradation products in new drug products with a maximum daily dose of <10 mg is 1.0% or 50 µg TDI (Total Daily Intake), whichever is

lower. The daily intake of 180 μg is <10 mg. The amount of impurities in the clinical dose, —, is —.

— . Therefore, the rule of — will apply in this case.

It should be noted that the evaluation above is based on oral administration of the drug. Since this product is applied ocularly and the anterior chamber is continuously irrigated during the cataract surgery, the sponsor estimates that the only 10% of dye residue will remain. If this estimate is correct, 6-18 μg ($60\text{-}180 \mu\text{g} \times 10\%$) Trypan Blue and —, Impurities will remain in the eye.

Recommendation:

Based on the ICH Guideline Q3B, the sponsor is recommended to either qualify the safety of the impurities by collecting the toxicology information or to lower the impurity levels from —.

However, Trypan Blue 0.06% is used clinically only once by ocular route and the dose is very small, it is felt that these factors must be taken into consideration. The clinical safety data of previously marketed Trypan Blue in foreign countries should also be evaluated. The comparison of impurity profiles of the marketed product and the current product will be useful.

Conrad H. Chen, Ph.D.
Pharmacology Reviewer

Concurrence by: Josie Yang, Ph.D.
Pharmacology Team Leader

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/s/

Conrad Chen

11/12/04 01:57:35 PM

PHARMACOLOGIST

The requirements in ICH Guideline Q3B are discussed.

Josie Yang

11/12/04 05:01:02 PM

PHARMACOLOGIST

Draft Labeling for NDA 21-670, Trypan Blue

NDA 21-670

Drug: Trypan Blue

Sponsor: Dutch Ophthalmic Research Center, International

Reviewer: Conrad Chen

Date: May 11, 2004

Labeling Sections:

Carcinogenesis, Mutagenesis, Impairment of Fertility

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Trypan blue was mutagenic in the Ames test and caused DNA strand breaks *in vitro*.

Teratogenic Effects: Pregnancy Category C

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Conrad Chen
5/12/04 04:33:51 PM
PHARMACOLOGIST
Draft Labeling recommended by Pharm/Tox

Josie Yang
5/12/04 06:27:17 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-670

Review number: No.1

Sequence number/date/type of submission: SN0000/October 27, 2003/Original NDA

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Dutch Ophthalmic Research Center, International

Manufacturer for drug substance:

Reviewer name: Conrad H. Chen, Ph.D.

Division name: Anti-inflammatory, Analgesic, and Ophthalmic Drug Products

HFD#: 550

Review completion date: March 1, 2004

Drug:

Trade name: VisionBlue®

Generic name: Trypan Blue Dye 0.06%

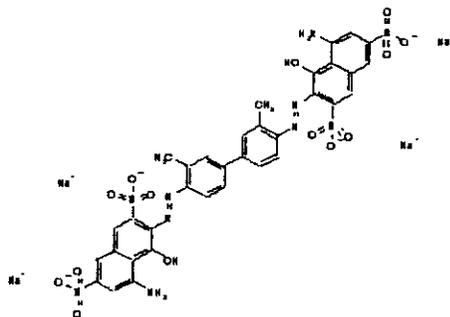
Code name: Not provided

Chemical name: 2,7-naphthalenedisulfonic acid, 3,3'-((3,3'-dimethyl(1,1'-biphenyl)-4,4'-diyl)bis(azo))bis(5-amino-4-hydroxy-, tetrasodium salt

Molecular formula/molecular weight: C₃₄H₂₄N₆O₁₄S₄Na₄, MW: 960.83

CAS registry number: 72-57-1

Structure:



Relevant INDs/NDAs/DMFs:

Drug class: Biological stain

Indication: Ocular tissue staining

Clinical formulation: Each ml VisionBlue® contains 0.6 mg trypan blue; 1.9 mg sodium mono-hydrogen orthophosphate (Na₂HPO₄·2H₂O); 0.3 mg sodium di-hydrogen orthophosphate (NaH₂PO₄·2H₂O); 8.2 mg sodium chloride, and water for injection.

Route of administration: When performing the capsulorhexis in cataract surgery, VisionBlue® is introduced into the anterior chamber by placing a few drops (estimated 0.1 to 0.3 ml) directly onto the anterior lens capsule. Sufficient staining is achieved as soon as the dye contacts the lens capsule.

Proposed use:

Previous Marketing History:

VisionBlue® obtained CE approval as a medical device Class IIa in 1999. According to the sponsor, over — units have been used intraocularly during cataract surgery. VisionBlue® is currently marketed in 30 countries and has not been withdrawn from the marketing for any reason. In US, — the applications for marketing are pending.

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Executive Summary

I. Recommendations

- A. **Recommendation on Approvability:** Recommended for approval
- B. **Recommendation for Nonclinical Studies:** None
- C. **Recommendation on Labeling:**

The labeling proposed by the sponsor is too short. The following information in the literature was reviewed and incorporated into the labeling.

Carcinogenesis, Mutagenesis, Impairment of Fertility



Trypan blue was mutagenic in the Ames test and caused DNA strand breaks *in vitro*.

Teratogenic Effects: Pregnancy Category C



II. Summary of Nonclinical Findings

- A. **Brief Overview of Nonclinical Findings:** Trypan blue is identified as teratogen, mutagen, and carcinogen in non-clinical studies. Other toxicity profile has not been clearly identified.
- B. **Pharmacologic Activity:** Trypan blue has empirically been found effective in staining the anterior lens capsule in cataract surgery. It has been routinely used in the laboratories as a vital stain (trypan blue dye exclusion assay) to identify cell viability at concentrations ranged from 0.1-0.4%. It was formerly used as a therapeutic agent in the treatment of sleeping sickness, a serious prevalent disease in much of tropical Africa and caused by trypanosomes.

C. Nonclinical Safety Issues relevant to Clinical Use: The non-clinical findings of teratogenicity, mutagenicity, and carcinogenicity of trypan blue should be described in the labeling. However, since the clinical dose of trypan blue is small, the clinical benefit versus possible risk should be properly considered.

III. Administrative

A. Reviewer signature: _____ Conrad H. Chen, Ph.D.

B. Supervisor signature: Concurrency- _____

 Non-Concurrency- _____

 Josie Yang, Ph.D.

 (see memo attached)

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. Pharmacology:

Trypan blue has empirically been found effective in staining the anterior lens capsule in cataract surgery. VisionBlue® applied onto isolated lens capsule was equally effective in staining the capsule as in the living eye. The tissue that is intended to be stained is void of cells. Anatomically, there are no cells present on the anterior lens capsule. It is concluded that the mechanism of action of VisionBlue® in staining the lens capsule cannot be attributed to an active or metabolic staining mechanism.

The sponsor has cited several laboratory works to demonstrate that 1). No cells are present on the anterior lens capsule, 2). Eye from human eye bank, after removing the anterior segment (removing functional cells), was equally stained as living eye, 3). Isolated lens capsule was equally stained, 4). The anterior lens capsule consists predominantly of collagen IV and glycosaminoglycans, trypan blue is physically entangled into the three dimensional structure of collagen (no covalent bond or hydrogen bond are involved), and 5). The staining of lens capsule by trypan blue was not ionogenic in nature.

It has been routinely used in the laboratories as a vital stain (trypan blue dye exclusion assay) to identify cell viability at concentrations ranged from 0.1-0.4%. It was formerly used as a therapeutic agent in the treatment of sleeping sickness, a serious prevalent disease in much of tropical Africa and caused by trypanosomes.

II. Toxicology:

Although not directly related to toxicology, the sponsor submitted a report of cytotoxicity testing according to USP XXIII. Results showed that VisionBlue® was not cytotoxic to a monolayer of mouse lung fibroblasts in vitro.

The following toxicity information for trypan blue was obtained from the Micromedex Integrated Index.

The oral LD₅₀ in rat was reported as 6200 mg/kg. The intravenous LD₅₀ and subcutaneous LD₅₀ in the mouse were 328 mg/kg and 267 mg/kg, respectively. It appeared that detailed toxicity profile of trypan blue was not identified. However, several animal studies were conducted to evaluate the teratogenicity, mutagenicity, and carcinogenicity potentials of trypan blue.

Trypan blue was teratogenic in rats, mice, rabbits, hamsters, dogs, guinea pigs, pigs, and chickens. The largest number of teratogenicity studies involves intravenous, intraperitoneal, or subcutaneous administration in the rat. The teratogenic dose appeared to be 50 mg/kg as a single dose or 25 mg/kg/day by parenteral administration during embryogenesis (mostly on Day 8 of pregnancy) in the rat. It caused external, skeletal, and internal malformations. Characteristic

anomalies included neural tube, cardiovascular, tail and eye defects. In the monkey, trypan blue caused abortions with single or two daily doses of 50 mg/kg between 20th to 25th days of pregnancy but no apparent increase in birth defects. Trypan blue was mutagenic in Ames test.

According to the Human Assessment Group in EPA's Office of Health and Environmental Assessment, the weight-of-evidence for trypan blue was group B2 and was considered to be probably carcinogenic to humans based on the animal data.

In humans, the injection of 0.2 ml of 1% solution subconjunctivally did not cause any injury. Application of a small drop of this solution to the surface of eye of patients did not cause irritation.

In the NDA, the sponsor also cited other toxicology information from the literature. Mostly, it regards the teratogenicity and carcinogenicity findings of trypan blue in animals. Intraperitoneally injected trypan blue (at about 100 mg/kg on Day 8 of pregnancy) produced ocular defects, spina bifida and other malformations in rats and mice. Lymphomas were induced in rats after subcutaneous injection of 1% (10 mg/ml) trypan blue at 0.5 ml/100g (i.e. 5 mg/100 mg or 50 mg/kg) body weight every other week for 52 weeks.

III Absorption, distribution, metabolism, and excretion of the drug in animals:

In cataract surgery, a maximum volume of 0.5 ml trypan blue 0.06% is injected into the anterior chamber of the eye, to stain a portion of the anterior lens capsule, approximately 7-9 mm in diameter. Therefore, the amount of trypan blue injected is approximately 300 µg in 0.5 ml. All excess dye is irrigated from the anterior chamber within seconds thereafter. A circular portion of the stained anterior lens capsule, approximately 5 mm in diameter, is then excised and removed from the eye. During the surgery, the anterior chamber is continuously irrigated. At the end of surgery, most of the dye has been washed out. The sponsor estimated that a 10% dye residue (30 µg) would remain in anterior chamber for systemic absorption. The average systemic circulation and extracellular space in a person is 6 liters. Therefore, the hypothetical maximum peak concentration of trypan blue is, therefore, only about 30 µg/6 liter (or 5 µg/liter).

Evaluation and Comment:

VisionBlue® is made of trypan blue (0.06% or 300 µg in 0.5 ml) for providing contrast to aid visualization of the anterior lens capsule when performing the capsulorhexis in cataract surgery. VisionBlue® obtained CE approval as a medical device Class IIa in 1999. According to the sponsor, over — units have been consumed for intraocular use during cataract surgery. VisionBlue® has currently been marketed in 30 countries. The sponsor stated that in the past four years of clinical use, VisionBlue® has been found safe and effective. No ocular or systemic side effect and/or adverse effect have been described in any published or presented clinical reports.

The non-clinical pharmacology and toxicology information for trypan blue was obtained from the published literature. Trypan blue is one of several biological stains recommended for use in dye exclusion procedures for viable cell counting. In summary, it appeared that detailed toxicity profile of trypan blue was not identified. However, trypan blue has been identified as a teratogen, mutagen, and carcinogen in non-clinical studies. The proposed clinical dose of VisionBlue® is relatively small. A one time topical dose of 0.3 mg (in 0.5 ml) is proposed. The sponsor stated that the systematically absorbed trypan blue will be smaller than 0.3 mg after wash-out by irrigation during the cataract surgery. It appears that the possible systemic toxic effect of VisionBlue® is small.

The approval of this NDA is recommended. However, this reviewer recommends that the proposed labeling (FDA version) to be conveyed to the sponsor for their input.

Proposed Labeling:

The sponsor's proposed labeling needs to be expanded following the current standard. Our preliminary recommendation asking sponsor to re-write the labeling was not conveyed. At filing meeting, the review team proposed that the Division would write the labeling for the NDA.

Most non-clinical toxicity studies from the literature do not match the designs required for the labeling purpose. The following information in the literature was reviewed and incorporated into the labeling.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Trypan blue was mutagenic in the Ames test and caused DNA strand breaks *in vitro*.

Teratogenic Effects: Pregnancy Category C

Recommendation: The approval of this NDA is recommended.

Message to the PM: The proposed labeling (FDA version) should be conveyed to the sponsor for their input.

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/s/

Conrad Chen
3/5/04 04:53:40 PM
PHARMACOLOGIST
Recommend approval

Josie Yang
3/5/04 04:55:09 PM
PHARMACOLOGIST