

# Chronic Intermittent Exposure to Solar-Simulated Ultraviolet and Visible Radiation Induces Ocular Changes in Albino but not Pigmented Mice

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## Abstract

Evaluating the ocular photo safety of pharmaceuticals can involve complex study designs, unfamiliar technologies and multiple variables. Acute versus chronic exposure, continuous versus discontinuous exposure, pigmentation in the eye structures, dark repair of light-induced damage and melanin binding all may affect toxicity and be must be accounted for to ensure accurate risk assessment. Female albino CD-1 and pigmented C3B6F1 mice were orally (by gavage) administered a simple vehicle formulation daily for twelve consecutive weeks. Five days per week for 12 weeks, the free-moving mice were exposed to 0, 120, 240, 360 or 720 Robertson Berger Units (RBU/day) of xenon arc solar-simulated light (SSL), including ultraviolet radiation (UVR; 290-400 nm) and visible light (400-700 nm). Evaluation of the eyes of all mice using indirect ophthalmoscopy and slit lamp observations during weeks 4, 6, 8, 10 and 12 revealed minimal corneal changes in only a small number of CD1 mice exposed to the high light dose (720 RBU/day) and no other adverse changes in either CD1 or C3B6F1 mice. Histopathologic evaluation revealed no lesions in the eyes of C3B6F1 mice at SSL doses as high as 360 RBU/day. CD-1 mice exposed to 360 RBU/day exhibited lenticular degeneration (cataracts) with minimal to mild fiber swelling, nuclear disorganization and/or nuclear debris within the area of the nuclear bow, rare morgagnian globules and occasional presence of hyperplasia, necrosis or fibrous change of the lens epithelial layer in 4 of 10 mice (8 eyes). Retinal degeneration occurred in 1 of 10 mice (1 eye). These findings demonstrate that chronic discontinuous light exposure, even at this elevated intensity and including UVR, induced only a moderate incidence of eye lesions in albino mice, these lesions were only revealed by histopathological evaluation and no lesions were induced in pigmented mice.

## Introduction

Evaluating the ocular photosafety of pharmaceuticals can involve complex study designs, unfamiliar technologies and multiple variables. Acute versus chronic exposure, continuous versus discontinuous exposure, pigmentation in the eye structures, dark repair of light-induced damage and melanin binding all may affect toxicity and must be accounted for to ensure accurate risk assessment. In this study, female albino CD-1 and pigmented C3B6F1 mice were orally (by gavage) administered a simple vehicle formulation daily for twelve consecutive weeks. Five days per week for 12 weeks, the free-moving mice were exposed to 0, 120, 240, 360 or 720 Robertson-Berger Units (RBU/day) of xenon arc solar-simulated light (SSL), including ultraviolet radiation (UVR; 290-400 nm) and visible light (400-700 nm).

## Materials and Methods

### Study Design

| Group | Mouse Strain | UVR <sup>a</sup> Exposure (RBU/Day) |
|-------|--------------|-------------------------------------|
| 1     | CD1          | 0                                   |
| 2     | CD1          | 120                                 |
| 3     | CD1          | 240                                 |
| 4     | CD1          | 360                                 |
| 5     | CD1          | 720                                 |
| 6     | B6C3F1       | 0                                   |
| 7     | B6C3F1       | 120                                 |
| 8     | B6C3F1       | 240                                 |
| 9     | B6C3F1       | 360                                 |
| 10    | B6C3F1       | 720                                 |

a. Solar-Simulated Light  
b. Robertson-Berger Unit  
10 female mice per group, vehicle administration (oral) to all mice (10 mL/kg)

### Test Systems

Female Albino Cr1:CD1(ICR) Mice - 8 weeks (21.5 - 25.2 grams)  
Female Pigmented B6C3F1/Cr1 Mice - 8 weeks (19.4 - 21.5 grams)

### Formulation Administration

Vehicle - 2% (w/v) hydroxypropylmethylcellulose (HPMC E5) with 0.2% (w/v) sodium lauryl sulfate (SLS) in R.O. deionized water  
All mice orally administered the vehicle once daily for 83 consecutive days (~12 weeks), 10 mL/kg.

### Simulated Sunlight (SSL) Exposure

Source - A 6.5 kilowatt xenon long arc, water cooled burner was horizontally suspended with a metal frame holding one optical filter (15 cm by 15 cm, 1 mm thick; Schott WG 320 doped glass). See Figures 1 through 3). During exposure, the cages holding the mice were located approximately 1.2 meters from the UVR source. Exposure Frequency - five days per week for 12 weeks  
Exposure Doses - 0, 120, 240, 360 and 720 Robertson-Berger Units (RBU/Day) [400 RBU is a Minimal Erythema Dose (MED) in skin]

### Tests, Analyses and Measurements

Viabilities, Clinical Observations, Body Weights, Ophthalmologic Evaluations and Histopathological Evaluation (Eyes Only)

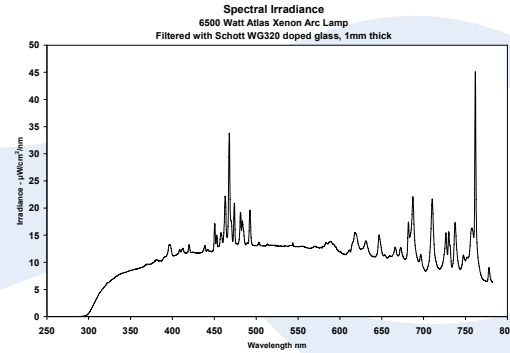


Figure 1. Spectral output of the solar simulator with emission in the UVB (280 – 315 nm), UVA (315 – 400 nm) and visible (400 – 700 nm) wavebands.



Figure 3. Photograph of CD1 and B6C3F1 mice during solar-simulated light exposure.



Figure 2. Solar simulator.

Table 1. A 12-WEEK INVESTIGATIVE PHOTOTOXICITY STUDY TO EVALUATE EFFECTS OF REPEATED SOLAR-SIMULATED LIGHT EXPOSURE ON EYES IN FEMALE ALBINO Cr1:CD1(ICR) AND PIGMENTED B6C3F1/Cr1 MICE

|                               |                                     | OPHTHALMOLOGICAL OBSERVATIONS |       |       |       |       |
|-------------------------------|-------------------------------------|-------------------------------|-------|-------|-------|-------|
|                               |                                     | CD1 MICE (ALBINO MICE)        |       |       |       |       |
| GROUP                         | UVR EXPOSURE (RBU/DAT) <sup>a</sup> | 1                             | 2     | 3     | 4     | 5     |
|                               |                                     | 0                             | 120   | 240   | 360   | 720   |
| <b>DAY 44 OF STUDY</b>        |                                     |                               |       |       |       |       |
| ANIMALS EXAMINED              |                                     | 9b                            | 9b    | 10    | 10    | 10    |
| EYES EXAMINED                 |                                     | 18b                           | 18b   | 20    | 20    | 20    |
| CORNEAL DYSTROPHY             | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 2 (4) |
| CORNEAL EDEMA                 | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 1 (2) |
| PIGMENT DEPOSIT IN EPITHELIUM | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 1 (2) |
| <b>DAY 83 OF STUDY</b>        |                                     |                               |       |       |       |       |
| ANIMALS EXAMINED              |                                     | 9b                            | 9b    | 10    | 10    | 9b    |
| EYES EXAMINED                 |                                     | 18b                           | 18b   | 20    | 20    | 18b   |
| CORNEAL DYSTROPHY             | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 3 (5) |
| CORNEAL EDEMA                 | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 2 (3) |
| INTERSTITIAL KERATITIS        | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 1 (1) |
| PIGMENT DEPOSIT IN EPITHELIUM | N (N2)                              | 0 (0)                         | 0 (0) | 0 (0) | 0 (0) | 1 (2) |

N1 = NUMBER OF ANIMALS AFFECTED  
N2 = NUMBER OF EYES AFFECTED

a. Formulation administration occurred on Days 1 through 83 of study. UVR exposure occurred 5 times per week (Monday through Friday), approximately 2 hours after formulation administration except during weeks in which ocular evaluations were performed.  
b. Exclude values for animals that were found dead or euthanized due to adverse clinical observations. These animals did not have adverse eye findings.

Table 2. A 12-WEEK INVESTIGATIVE PHOTOTOXICITY STUDY TO EVALUATE EFFECTS OF REPEATED SOLAR-SIMULATED LIGHT EXPOSURE ON EYES IN FEMALE ALBINO Cr1:CD1(ICR) AND PIGMENTED B6C3F1/Cr1 MICE

|                            |                                     | OPHTHALMOLOGICAL OBSERVATIONS |     |     |     |     |
|----------------------------|-------------------------------------|-------------------------------|-----|-----|-----|-----|
|                            |                                     | B6C3F1 MICE (PIGMENTED MICE)  |     |     |     |     |
| GROUP                      | UVR EXPOSURE (RBU/DAT) <sup>a</sup> | 1                             | 2   | 3   | 4   | 5   |
|                            |                                     | 0                             | 120 | 240 | 360 | 720 |
| <b>NO ADVERSE FINDINGS</b> |                                     |                               |     |     |     |     |
| ANIMALS EXAMINED           |                                     | 10                            | 10  | 10  | 10  | 10  |
| EYES EXAMINED              |                                     | 20                            | 20  | 20  | 20  | 20  |

a. Formulation administration occurred on Days 1 through 83 of study. UVR exposure occurred 5 times per week (Monday through Friday), approximately 2 hours after formulation administration except during weeks in which ocular evaluations were performed.  
b. Animal 7733 had an accidental death on Day 38 of study. All eye observations were normal.

Table 3. A 12-WEEK INVESTIGATIVE PHOTOTOXICITY STUDY TO EVALUATE EFFECTS OF REPEATED SOLAR-SIMULATED LIGHT EXPOSURE ON EYES IN FEMALE ALBINO Cr1:CD1(ICR) AND PIGMENTED B6C3F1/Cr1 MICE

|  |                                     | HISTOPATHOLOGIC EXAMINATION |       |       |       |     |
|--|-------------------------------------|-----------------------------|-------|-------|-------|-----|
|  |                                     | CD1 MICE (ALBINO MICE)      |       |       |       |     |
| GROUP                                      | UVR EXPOSURE (RBU/DAT) <sup>a</sup> | 1                           | 2     | 3     | 4     | 5   |
|  |                                     | 0                           | 120   | 240   | 360   | 720 |
| <b>NO ADVERSE FINDINGS</b>                 |                                     |                             |       |       |       |     |
| ANIMALS EXAMINED                           |                                     | 10                          | 9     | 10    | 10    | 0   |
| EYES EXAMINED                              |                                     | 20                          | 18    | 20    | 20    | 0   |
| INFLAMMATORY CELL INFILTRATE (CONJUNCTIVA) | N (N2)                              | 1 (1)                       | 0 (0) | 0 (0) | 1 (1) | 0   |
| LENS DEGENERATION (CENTRAL OR HELIX)       | N (N2)                              | 0 (0)                       | 0 (0) | 0 (0) | 4 (8) | 0   |
| ARTERIAL DEGENERATION (N2)                 | N (N2)                              | 0 (0)                       | 0 (0) | 0 (0) | 1 (1) | 0   |

N1 = NUMBER OF ANIMALS AFFECTED  
N2 = NUMBER OF EYES AFFECTED

a. Formulation administration occurred on Days 1 through 83 of study. UVR exposure occurred 5 times per week (Monday through Friday), approximately 2 hours after formulation administration except during weeks in which ocular evaluations were performed.  
b. Exclude values for animals that were found dead or euthanized due to adverse clinical observations. These animals did not have adverse eye findings.

Table 4. A 12-WEEK INVESTIGATIVE PHOTOTOXICITY STUDY TO EVALUATE EFFECTS OF REPEATED SOLAR-SIMULATED LIGHT EXPOSURE ON EYES IN FEMALE ALBINO Cr1:CD1(ICR) AND PIGMENTED B6C3F1/Cr1 MICE

|                            |                                     | HISTOPATHOLOGIC EXAMINATION  |     |     |     |     |
|----------------------------|-------------------------------------|------------------------------|-----|-----|-----|-----|
|                            |                                     | B6C3F1 MICE (PIGMENTED MICE) |     |     |     |     |
| GROUP                      | UVR EXPOSURE (RBU/DAT) <sup>a</sup> | 1                            | 2   | 3   | 4   | 5   |
|                            |                                     | 0                            | 120 | 240 | 360 | 720 |
| <b>NO ADVERSE FINDINGS</b> |                                     |                              |     |     |     |     |
| ANIMALS EXAMINED           |                                     | 10                           | 7   | 9   | 7   | 0   |
| EYES EXAMINED              |                                     | 19                           | 14  | 18  | 14  | 0   |

## Conclusion

Repeated solar-simulated light exposure over a 12 week period at light doses as high as 720 RBU/Day produced no ocular changes in female pigmented B6C3F1/Cr1 mice.

In Cr1:CD1(ICR) albino mice exposed to a light dose of 720 RBU/Day, corneal dystrophy in 3 mice and corneal edema in 2 mice were revealed by ophthalmologic evaluations.

In CD1 albino mice exposed to a light dose of 360 RBU/Day, lenticular degeneration in 4 mice and retinal degeneration in 1 mouse were revealed by histopathological evaluation.

In CD1 albino mice exposed to a light dose as high as 240 RBU/Day, there were no findings throughout the study.

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## Results and Discussion

Clinical Observations - no clinical observations were attributed to light exposure in either albino (CD1) or pigmented (B6C3F1) mice

Body weights and body weight changes were unremarkable in both albino (CD1) and pigmented (B6C3F1) mice

Ophthalmological evaluations (Tables 1 and 2)

In B6C3F1 pigmented mice exposed to light doses as high as 720 RBU/Day, there were no findings throughout the study.

In CD1 albino mice exposed to a light dose of 720 RBU/Day, corneal dystrophy, corneal edema, pigment deposit in the epithelium and/or interstitial keratitis occurred in one or more mice in the latter portion of the study (Days 64 and 83).

In CD1 albino mice exposed to a light dose as high as 360 RBU/Day, there were no findings throughout the study.

Histopathological Evaluation (Tables 3 and 4)

In B6C3F1 pigmented mice exposed to light doses as high as 360 RBU/Day, there were no findings.

In CD1 albino mice exposed to a light dose of 360 RBU/Day, lenticular degeneration characterized by minimal to mild fiber swelling, nuclear disorganization and/or nuclear debris within the area of the nuclear bow, rare morgagnian globules and occasional presence of hyperplasia, necrosis or fibrous change of the lens epithelial layer occurred in 4 mice and retinal degeneration occurred in 1 mouse. Inflammatory cell infiltration of the conjunctiva occurred in a single mouse in each of the groups exposed to the 0 and 360 RBU/Day light doses.

In CD1 albino mice exposed to a light dose as high as 240 RBU/Day, there were no findings attributed to light exposure.