Appendix G

Additional BCOP Studies Received in Response to *Federal Register* Notices (Vol. 69, No. 57, pp. 13859-13861) and (Vol. 70, No. 38, pp. 9661-9662)

[This Page Intentionally Left Blank]

Appendix G1

Dataset Received from S.C. Johnson & Son, Inc. in Support of Cuellar et al. (2004) Poster Presentation

[This Page Intentionally Left Blank]

SC Johnson
A FAMILY COMPANY

S.C. Johnson & Son, Inc.
Worldwide Consumer Products, RD & E
Global Safety Assessment and Regulatory Affairs, Product Toxicology
MS 139 1525 Howe Street, Racine WI 53403

August 27, 2004

Christina Inhof, MSPH
Senior Project Coordinator/Technical Writer ILS, Inc.
NICEATM
P.O. Box 12233
NIEHS MD EC-17
Research Triangle Park, NC 27709

Christina,

Hi! How are you? I am happy to be submitting data on solvents and simple fragrance formulations, which were discussed in the poster citation listed below:

Cuellar, N., Lloyd, P.H., Swanson J.E., Merrill, J.C., Mun, G., Harbell, J.W. and Bonnette, K.L. (2004) Phase Two: Evaluating the eye irritancy of solvents in a simple fragrance mixture with the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 78(S-1): Abstract No. 1306.

Included with this submission are the following documents:

1. Cover letter
2. Poster text
3. Poster graphs
4. Histology slides
5. Coded formula spreadsheet

Study Protocols:

Modified Draize protocol was used for the in-vivo studies. Four animals were treated per sample (3 for histopathology and 1 animal for recovery). Each animal received a 0.1 ml dose of the formula in the conjunctival sac of the right eye. The left eye served as the untreated control. All animals were scored at 1, 4, and 24 hours after dosing. Histology was conducted on 3 of the 4 animals.

Standard BCOP protocol was used for the in-vitro work at IIVS. The first BCOP study required exposure times of 1 and 3 minutes with a post exposure of 20 hours to compliment the timing in the animal. The second BCOP study utilized a 3-minute exposure time with

post exposures of 2 to 4 hours to understand effects using standard post exposure times. Histology was conducted on all corneas. Since the 1-minute exposure did not produce extensive lesions, only the 3-minute exposure was evaluated in the second study. Only the 3-minute exposure data are reported in this poster.

Formula Spreadsheet:

The formulas listed in this spreadsheet are coded similarly to formulas listed in the poster. Test material number is the unique sample number and the group name denotes formula description. Raw materials are listed followed by their percentages in each formula.

Poster:

Fragrance poster not included. John Harbell previously sent it to you.

Poster Text:

A word document consisting of poster text and tables is included in this submission for ease of reading. The poster contained a wealth of information, thus limited visibility. This document highlights where the graphs and histology slides should be inserted for ease of understanding. Please note: Table one has improved coloring/formatting on the poster.

Poster Graphs:

Poster graphs should be referenced on page 10.

Histology Slides:

Histology slides should be referenced on page 12.

Data Worksheet:

The data worksheet is not included for this submission. GHS and EPA classification of results was not possible due to lack of animals. Only 1 of the 4 animals was carried out for recovery purposes. The remaining 3 animals were used for histopathology.

Summary:

Solvents have a major impact on the ocular irritation potential of fragrance mixtures. Both the degree and the time-course of the irritation can be impacted by the solvent. Over the three harvest times, the BCOP assay was able to identify histological changes that characterize the treatment groups into severe (ethanol alone), moderate (2-stages), and mild categories. One treatment group (fragrance + DPG) was over predicted by the BCOP compared to the in vivo assay. The time course of the tissue scores in vivo was similar to

the time course of the histological changes in BCOP. The BCOP model was more consistent in its response to a given treatment than the in vivo model.

If you have any questions or comments on this data set, please feel free to contact either Judith Swanson or myself at the following:

Nicole Cuellar
(262) 260-6916
ncuellar@scj.com

Judith Swanson
(262) 260-2688
jeswanso@scj.com

Sincere regards,

Nicole Cuellar
Sr. Research Toxicologist

POSTER TEXT FOR S.C. JOHNSON SUBMISSION DATED AUGUST 27, 2004

TITLE:

PHASE TWO: EVALUATING THE EYE IRRITANCY OF SOLVENTS IN A SIMPLE FRAGRANCE MIXTURE WITH THE BOVINE CORNEAL OPACITY AND PERMEABLITY (BCOP) ASSAY

N Cuellar[1], P H Lloyd[2], J E Swanson[1], J C Merrill[3], G Mun[3], J W Harbell[3], and K L Bonnette[4]. [1]S.C. Johnson & Son, Inc. Racine, WI, USA; [2]SCJ EURAFNE Ltd. Egham, Surrey, England; [3]Institute for In Vitro Sciences, Inc. Gaithersburg, MD, USA; [4]Charles River Laboratories, Inc. Spencerville, Ohio, USA.

ABSTRACT:

Fragrances are complex mixtures used in many consumer products. Organic solvents, such as ethanol, are major components of fragrance formulations functioning mainly as solubilizers and fragrance delivery mechanisms. In Phase One (Cuellar et al, 2003), the BCOP assay and primary eye irritation study (EPA-OPPTS 870.2400) were conducted using simple fragrance mixtures containing six commonly used solvents. The corneal depth of injury was assessed histologically both in vitro and in vivo. In the BCOP assay, corneas were exposed for 1 and 3 minutes, rinsed and incubated for 20 hours before the opacity and permeability endpoints were assessed. In vivo, animals were scored at 1, 4, and 24 hours. Individual solvents impacted the level of irritation of these formulations. Phase Two evaluated the time course of lesion development after exposure in the BCOP assay and determined those early lesion that would be predictive of damage observed after 20+ hours in vitro and in vivo. Bovine corneas were exposed for 3 minutes, rinsed, and incubated for 2 or 4 hours before the endpoints were assessed and tissue taken for histology. In vivo, certain solvents increased the rate of lesion development but not the overall intensity or duration compared to the fragrance alone. Other solvents decreased the overall intensity and duration. The BCOP assay showed a generally similar pattern of lesion development. Those combinations that showed opacity at 4 hours in vivo, showed epithelial and stromal lesion in the BCOP by 4 hours post-exposure. Fragrance alone was slower to develop opacity in vivo and required the 20 hour post-exposure to produce appreciable lesions in vitro. These data suggest that our standard post exposure (2 hour) can be predictive of irritation potential of fragrance/solvent mixtures.

INTRODUCTION:

The Bovine Corneal Opacity and Permeability (BCOP) Assay is routinely used by S.C. Johnson and Son as a tool for evaluating air fresheners for potential ocular irritation. Depending on the type of air freshener, other components may be added to solubilize a fragrance or to facilitate fragrance delivery mechanisms. In Phase I of this study (Cuellar et al., 2003), the BCOP assay and the primary eye irritation assay (EPA OPPTS 870.2400) were conducted using simple fragrance mixtures containing six commonly used solvents. As with Cuellar et al. (2002), Cuellar et al. (2003) found that solvents had an impact on the ocular irritation potential of fragrance mixtures. Based on this information, we examined the use of the BCOP at our standard post exposure times (2 and 4 hour) in comparison with Phase I post exposure times both in vitro (20 hour) and in vivo (24 hour) for rate, degree and intensity of irritation potential of the same fragrance mixtures.

Histological examination is necessary to understand the degree and depth of injury associated with the permeability and opacity measurements with diverse chemical classes or mixtures (Curren et al., 2000). Depth of injury has been shown to be a predictor of the degree and duration (reversibility) of ocular injury by Maurer et al. (2002) and Jester et al. (1998). Histological evaluation was used in Cuellar et al. (2002) to define the degree of injury to a reference sample and also to set the upper bound limit for other formulations of that type. Cuellar et al. (2003), demonstrated that permeability and opacity scores in the BCOP assay and the MAS scores in vivo paralleled the depth of injury and cellular changes seen histologically from both BCOP and in vivo samples. In Phase II of this study, we evaluated the progression of lesion development after exposure in the BCOP assay. Additionally, we determined specific early lesions that would be predictive of damage observed after 20+ hours in vitro and in vivo in the same fragrance mixtures.

MATERIALS AND METHODS:

Phase	In – vitro (BCOP)	In-vivo
One	3 & 10 minute exposure, 20 hour post-exposure	1, 4, & 24 hour scored
Two	3 minute exposure, 2 & 4 hour post-exposure	Not repeated

IN VITRO - BCOP:

Bovine Eyes

The BCOP assay was performed following the methods of Sina et al. (1995). Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals. The eyes were grossly examined for damage and those exhibiting defects were discarded. The corneas were excised such that a 2 to 3 mm rim of sclera was present around the cornea. The corneas were mounted in the holders and the two chambers filled with Minimum Essential Medium Eagle (MEM) without phenol red, supplemented with 1% fetal bovine serum (complete MEM). The corneal holders were incubated at 32 ± 1ºC for a minimum of 1 hour.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the medium replaced in both chambers and the opacity was determined for each cornea using a Spectro Designs OP-KIT opacitometer. Three corneas, whose opacity readings were close to the median opacity for all the corneas, were selected as the negative control corneas. The medium was then removed from the anterior chamber and replaced with either the test article, positive control, or negative control.

Testing Procedure

Each test article was administered neat to the BCOP test system. An aliquot of 750 µl of either the test article, positive control (100% ethanol), or negative control (deionized water) was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. Six corneas were used for each test article (three corneas per each post-exposure incubation period of 2 or 4 hours) were exposed for three minutes at 32 ± 1ºC. Six corneas were treated with the negative control (three corneas per each post-exposure incubation period of 2 and 4 hours) were exposed for ten minutes at 32 ± 1ºC. Three corneas were treated with the positive control for 10 minutes 32 ± 1ºC. After the exposure periods, the test or control article treatments were removed. The corneal surface was washed at least three times to ensure total removal of the test or control articles. The corneas exposed were returned to the incubator for approximately 2 hours (test articles**,** positive and negative controls) and 4 hours (test articles and negative controls). After this incubation (2 or 4 hours), the final measure of opacity was obtained. Corneas cultured for 4 hours were refed immediately prior to the final measure of opacity. The values obtained at this second opacity measurement are presented in the report and were used in calculating the corneal opacity.

After the second opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was refilled with complete MEM, and 1 ml of a 4 mg/ml fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1ºC. After the incubation, an aliquot of 360 µl from each chamber was placed into the designated well on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader.

Opacity Measurement: The change in opacity for each cornea was calculated by subtracting the pre-treatment opacity readings from the final opacity readings. The corrected opacity value of each cornea was calculated by subtracting the average change in opacity of the negative control corneas from that of each treated cornea. The mean opacity values of each treatment group were then calculated.

Permeability Measurement: The corrected OD_{490} was calculated by subtracting the mean OD_{490} of the negative control corneas from the OD_{490} value of each treated cornea. The mean OD_{490} values of each treatment group were then calculated.

Histology

The corneas were placed in individual, prelabelled cassettes and fixed for at least 24 hours in 10% buffered formalin. The fixed corneas were transferred to Pathology Associates - A Charles River Company (Frederick, MD) for embedding, sectioning and staining. Each slide was then stained with hematoxylin and eosin. Slides were returned to the Institute for In Vitro Sciences, Inc. for evaluation. Photomicrographs and thickness measurements were prepared using a Spot Insight (Spot Diagnostic Instruments) digital camera and associated software.

IN VIVO:

The acute eye irritation study was conducted in accordance with the US EPA, Health Effects Test Guidelines (OPPTS 870.2400). Four New Zealand White rabbits were treated per sample (three animals for histopathology and one animal for recovery). Each animal received a 0.1mL dose of the appropriate test article in the conjunctival sac of the right eye. The left eye of each animal remained untreated and served as the control. Eyes were macroscopically scored at 1, 4, and 24 hours after dosing for both histopathology and recovery animals according to the Ocular Grading System based on Draize (1959). The group mean irritation score was then calculated for each scoring interval based on the number of animals intially dosed in each group. The calculated group mean ocular irritation scores for each interval were used to classify the test article according to the Ocular Evaluation Criteria of Kay and Calandra (1962).

Histology:

The test and control eyes were collected, identified, and placed in 10% neutral buffered formalin for fixation. The sections were processed histologically (embedded in paraffin, cut, and stained with hematoxylin and eosin). The histology was conducted by HistoTechniques (Powell, Ohio). The resulting slides were examined by a board certified pathologist (Dr. J. Dale Thurman, Senior Director of Pathology). No tissues were retained or examined for the recovery animals. Subsequently, slides were scored for cellular changes (paralleling those scored for bovine corneas) and photographed by one of us (JHW). These observations are reported in Table 1.

RESULTS:

The histological results of the BCOP and acute eye irritation assay for thirteen treatment groups are presented in Tables 1-3. The numerical scores of the BCOP and acute eye irritation assay for the thirteen treatment groups are graphically presented in Figures 1-6. The thirteen treatment groups include fragrance only, six solvents only, and six solvent/fragrance mixtures. The simple fragrance alone consists of 25% of each of the following fragrance components: benzyl acetate, linalool, dihydroxymyrcenol, and Verdox. The solvents consist of 100% of each of the following solvents: ethanol (ETOH), Dowanol DPM (DPM), Isopar M, dipropylene glycol (DPG), carbitol, and benzyl benzoate (BB). The solvent/fragrance mixtures (solvent + F, e.g. ETOH+F) consist of 80% fragrance mixture (20% of each fragrance component) and 20% of each solvent. Fragrance components and solvents were chosen because they are more frequently used in fragrance formulations.

Table 1 shows the depth of injury, cellular change and opacity score x area score for each animal for the thirteen treatment groups. Characteristic lesions are grouped together by severity of injury and cellular change from Group I (least irritating) to Group IV (most irritating). Days to clear for the recovery animal is listed below.

Table 1. In-Vivo Histological Summary

		Opacity Score x Area Score for each animal					
Group	Characteristic Lesions	2x4	2x3	2x2	2x1	1x1	0x0
IV	1) Complete or nearly complete loss of epithelium over the cornea 2) Marked inflammatory infiltrate extending well into the corneal stroma 3) Loss of keratocytes in the upper stroma 4) Increased frequency of enlarged keratocytes in the mid stroma	ETOH ETOH ETOH					
III	1) Focal, full thickness loss of epithelium 2) Marked inflammatory infiltrate restricted to the edge of the cornea 3) Increased frequency of enlarged keratocytes in the upper stroma under the epithelial lesion	**Frag-rance** ETOH+F DPM+F	DPG+F Carbitol +F	**Frag-rance** Carbitol+ F BB+F	**Frag-rance** Carbitol+F DPM DPM		
II	1) Small focus of epithelial loss or thinning 2) Limited inflammatory infiltrate 3) Slight increase in enlarged keratocytes		DPM Carbitol	Carbitol	ETOH+F DPM+F Carbitol	Isopar M+F BB+F	DPG+F ? DPG
I	1) Epithelium was intact or just slightly thinned 2) Little or no inflammatory infiltrate 3) No enlarged keratocytes						Isopar M Isopar M DPG DPG BB BB BB ETOH+F DPM+F Isopar M+F Isopar M+F DPG+F BB+F ETOH Isopar M

Days to clear (for the remaining animal in the treatment group)
>28 days - ETOH
7 Days - Fragrance, ETOH+F, DPM+F, Carbitol +F
3 Days – DPG+F, DPM, DPG, Carbitol
2 Days – Isopar+F, BB+F
1 Day – Isopar M, BB

Table 2 demonstrates the description of the characteristic lesions seen in the epithelial layer of the corneas of the 13 treatment samples in the BCOP assay at three different post-exposure times (2, 4, and 20 hours). Corneas are grouped by severity of the depth of injury and cellular changes from Group E (most irritating) to Group A (least irritating).

Table 2. Summary of the epithelial layer changes in the BCOP

In Vitro	Characteristic Lesions	2-hour post-exposure	4-hour post-exposure	20-hour post-exposure
Group E	Full thickness loss or separation on >50% of the corneal surface	ETOH	ETOH	ETOH DPM + F (2) Carbitol + F
Group D	Full thickness loss/separation on <50% of the corneal surface but substantial damage to wing and basal cell layers	ETOH+F(2) DPG+F Carbitol+F	ETOH+F Carbitol+F(2)	Fragrance ETOH+F DPG+F (2) DPM+F (1)
Group C	Little or no full thickness loss but damage well into the wing and basal cell layers (may include nuclear changes and cytoplasmic vacuolization)	ETOH+F(1) DPM+F DPM Carbitol	DPM+F DPG+F Carbitol+ F(1) DPM Carbitol	DPG+F(1) BB+F DPM Carbitol
Group B	Loss of the full squamous cell layer with occasional damage into the wing cell layer. No full thickness loss	Fragrance Isopar M+F	Fragrance	Isopar M+F
Group A	Similar to the time-matched negative control epithelium or with some loss/loosening of the surface squamous epithelial layers	BB+F Isopar M DPG BB	Isopar M+F BB+F Isopar M DPG BB	Isopar M DPG BB

Table 3 demonstrates the description of the characteristic lesions seen in the stroma of the corneas of the 13 treatment samples in the BCOP assay at three different post-exposure times (2, 4, and 20 hours). Corneas are grouped by severity of the depth of injury and cellular changes from Group E (most irritating) to Group A (least irritating).

Table 3. Summary of the Stromal Lesions in the BCOP.

In Vitro	Characteristic Lesions	2-hour post-exposure	4-hour post-exposure	20-hour post-exposure
Group E	1) Thickness: Appreciably thicker than the time-matched controls 2) Stromal matrix vacuolization: Marked to 50% depth 3) Keratocytes: a. Upper stroma: Marked cell loss and/or increase in nuclear changes (degeneration, pyknosis, vacuolization or abnormal chromatin condensation) to ≤ 50% depth b. Nuclear enlargement/ cytoplasmic eosinophilia: Marked or Moderate to ≥ 50% depth		ETOH	ETOH
Group D	1) Thickness: Thicker than the time-matched controls 2) Stromal matrix vacuolization: Some marked but moderate to 50% depth 3) Keratocytes: a. Upper stroma: Moderate cell loss and/or increase in nuclear changes (degeneration, pyknosis, vacuolization or abnormal chromatin condensation) to ≤ 25% depth b. Nuclear enlargement/ cytoplasmic eosinophilia: Moderate/Marked to 50% depth		ETOH+F DPG+F Carbitol+F(2)	Fragrance ETOH+F DPM+F Carbitol+F
Group C	1) Thickness: Thicker than the time-matched controls 2) Stromal matrix vacuolization: Moderate to ≤ 50% depth 3) Keratocytes: a. Upper stroma: Slight cell loss and/or increase in nuclear changes (degeneration, pyknosis, vacuolization or abnormal chromatin condensation) to ≤ 25% depth b. Nuclear enlargement/ cytoplasmic eosinophilia: Moderate to ≤ 50% depth	Carbitol+F DPM Carbitol	DPM+F DPM Carbitol	DPG+F Carbitol DPM
Group B	1) Thickness: Slightly thicker than the time-matched controls 2) Stromal matrix vacuolization: Moderate to ≤ 30% depth 3) Keratocytes: a. Upper stroma: Normal (no cell loss or nuclear degeneration) b. Nuclear changes (enlargement)/ cytoplasmic eosinophilia: Moderate increase to ≤ 30% depth	ETOH+F ETOH		BB+F
Group A	1) Thickness: Similar to the time-matched controls 2) Stromal matrix vacuolization: Slight or less increase to ≤ 30% depth 3) Keratocytes:	Fragrance DPM+F Isopar M+F DPG+F	Fragrance Isopar M+F DPG+F Carbitol+F(1)	Isopar M + F Isopar M DPG BB

In Vitro	Characteristic Lesions	2-hour post-exposure	4-hour post-exposure	20-hour post-exposure
	a. Upper stroma: Normal (no cell loss or nuclear degeneration) b. Nuclear changes (enlargement)/ cytoplasmic eosinophilia: Slight or less increase to ≤ 20% depth	BB+F Isopar M DPG BB	BB+F Isopar M DPG BB	

GRAPHICAL RESULTS: Appended Below

RESULTS:

- Alone, the fragrance induced only slight corneal changes until 24 hours after treatment in vivo. In the BCOP assay, the similar time course was observed.

- Impact of Solvent:

    1. The addition of ethanol or DPM to the fragrance increased the in-vivo corneal scores (opacity and area) at 4 hours compared to the fragrance alone.
    2. The addition of ethanol, DPM, DPG, or carbitol to the fragrance increased the BCOP opacity and permeability scores (and histological changes) at 2 and 4 hours compared to fragrance alone.
    3. The addition of IsoPar M to the fragrance showed a strong mitigating effect on the overall irritancy of the fragrance in-vivo and BCOP assays.
    4. The addition of BB to the fragrance slowed the onset and reduced the overall irritation (in vivo) and BCOP opacity and permeability scores (and histological changes).

- The rapid onset of irritancy to the cornea (in-vivo) of ethanol, DPM, and carbitol was correctly predicted by the BCOP at 2 hours based on both the scores (opacity and permeability) and histological changes in the epithelium. The full expression of stromal changes took 4 hours in the BCOP (e.g., ethanol).

- Irritation Levels:

    1. Severe irritation was defined by sustained high Draize scores, high in-vitro scores, significant tissue damage (Group IV and E) and no recovery.
    2. Moderate irritation was defined at two levels with moderate tissue damage (Group III, II & D-B):
        - one level defined by moderate Draize and in-vitro scores, and seven days to clear and
        - second level defined by moderate Draize and in-vitro scores and three days to clear.
    3. Mild irritation was defined by mild Draize and in-vitro scores, minimal tissue damage (Group 1 & A) and rapid recovery (<3 days).

- Based on the histological changes over the three harvest times, the BCOP was able to distinguish a) the severe irritation potential of ethanol, b) the moderate irritation potentials of fragrance alone, fragrance + ethanol, fragrance + DPM, and fragrance + carbitol, c) the moderate but more rapidly clearing irritation of DPM and carbitol alone, d) the mild irritation of fragrance + Isopar M and fragrance + BB, and e) the very mild Isopar M, DPG, and BB alone. The exception is fragrance + DPG where the in vitro response was much more pronounced that the in vivo response.

In Vivo Histology : Appended below

Figures 7-8 show animal corneas treated with test substance.

Figure 7. In Vivo Group 1: BB & Isopar M

(A) Center of the cornea, no changes observed (magnification 230x)
(B) Area, away from limbus, showing separation of squamous epithelium (magnification 430x)

Figure 8. In Vivo Group 4: ETOH.

(A) Central cornea showing loss of epithelium, inflammation, and marked increase in larger dark staining keratocyte nuclei in area of inflammatory infiltrate. Note - the cells were not in the upper 20% of the stroma (magnification 170x)
(B) Area in denuded area showing keratocyte changes and swelling (magnification 430x)

BCOP Histology

Figures 9-12 show corneas treated for 3 minutes, at varying post exposure times with test substance in the BCOP.

Figure 9. BCOP Fragrance alone: 3-minute exposure, 2-hour post-exposure

(A) Epithelium – Loss of surface squamous epithelium and some necrotic cells within the wing and basal layers (magnification 230x)(Epithelial Group B)
(B) Stroma – Very similar to the time-matched negative control-treated corneas (magnification 430x)(Stromal Group A)

Figure 10. BCOP Fragrance alone: 3-minute exposure, 20-hour post-exposure

(A) Epithelium – Loss of the squamous epithelium and marked nuclear pyknosis and cytoplasmic eosinophilia in the wing cell layer (magnification 230x) (Epithelial Group D)
(B) Stroma – Marked collagen matrix vacuolization to 20% depth and keratocyte nuclear swelling and cytoplasmic eosinophilia (magnification 430x) (Stromal Group D)

Figure 11. BCOP ETOH alone: 3-minute exposure, 2-hour post-exposure

(A) Epithelium – Marked cellular damage and separation between the basal cells and basal lamina (magnification 230x) (Epithelial Group E)
(B) Stroma – Moderate collagen matrix vacuolization to mid depth and moderate increase in keratinocytes with nuclear pyknosis in the upper 25% of the stroma (magnification 430x) (Stromal Group B)

Figure 12. BCOP ETOH alone: 3-minute exposure, 4-hour post-exposure

(A) Epithelium - Marked cellular damage and separation between the basal cells and basal lamina (magnification 230x) (Epithelial Group E)
(B) Stroma – Marked collagen matrix vacuolization and a decrease in viable keratocytes extended to 30% depth. Marked keratocyte nuclear enlargement cytoplasmic eosinophilia was present at mid depth but is not shown in this micrograph (magnification 430x) (Stromal Group E)

CONCLUSIONS:

- Over the three harvest times, the BCOP assay was able to identify histological changes that characterize the treatment groups into severe (ethanol alone), moderate (2-stages), and mild categories. One treatment group (fragrance + DPG) was over predicted by the BCOP compared to the in vivo assay.
- Solvents have a major impact on the ocular irritation potential of fragrance mixtures. Both the degree and the time-course of the irritation can be impacted by the solvent.
- The time course of the tissue scores in vivo was similar to the time course of the histological changes in BCOP.
- When injury was significant enough, morphological changes in the keratocytes, specifically keratocyte nuclear enlargement (activation), were detectable in both the BCOP and in-vivo-treated corneas. Fini (1999) and collaborators have reported that certain morphological changes in keratocytes are associated with phenotypic changes (activation) and subsequent undesirable fibrotic scaring.
- BCOP model was more consistent in its response to a given treatment than the in vivo model (Table 1-3).

REFERENCES

Cuellar, N., Lloyd, P.H., Swanson J.E., Merrill, J.C., Clear, M.L., Mun, G., Harbell, J.W. and Bonnette, K.L. (2003) Evaluating the eye irritancy of solvents in a simple fragrance mixture with the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist 72(S-1): 312-313.

Cuellar, N., Merrill, J.C., Clear, M.L. Mun, G., and Harbell, J.W. (2002) The application of benchmarks for the evaluation of the potential ocular irritancy of aerosol fragrances. The Toxicologist 66(1-S): 243-244.

Curren, R.., Evans, M.., Raabe, H., Ruppalt, R., and Harbell, J. (2000) Correlation of histopathology, opacity, and permeability of bovine corneas exposed in vitro to known ocular irritants. Veterinary Pathology 37(5):557.

Draize, J.H. (1959) Appraisal of the safety of chemicals in foods, drugs, and cosmetics. The Association of Food and Drug Officials of the United States: 49-51.

Fini, E.M. (1999) Keratocyte and fibroblast phenotypes in the repairing cornea. Progress in Retinal and Eye Research 18(4):529-551.

Jester, J.V., Li, H.F., Petroll, W.M., Parker, R.D., Cavanaugh, H.D., Carr, G.J., Smith, B., and Maurer, J.K. (1998) Area and depth of surfactant-induced corneal injury correlates with cell death. Invest Ophthalmol Vis Sci 39:922-936.

Kay, J.H. and Calandra, J.C. (1962) Interpretation of eye irritation tests. J Soc Cosmetics Chemists 13: 281-289.

Maurer, J.K., Parker, R.D., and Jester, J.V., (2002) Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. Regulatory Toxicology and Pharmacology 36(1):106-117.

Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. Fundamental and Applied Toxicology 26:20-31.

ACKNOWLEDGEMENTS

The authors wish to thank the general staffs at both the Institute for In Vitro Sciences, Inc. and the Charles River Laboratories, Inc. for their assistance on this project.

Figure 1. In Vivo Opacity Scores

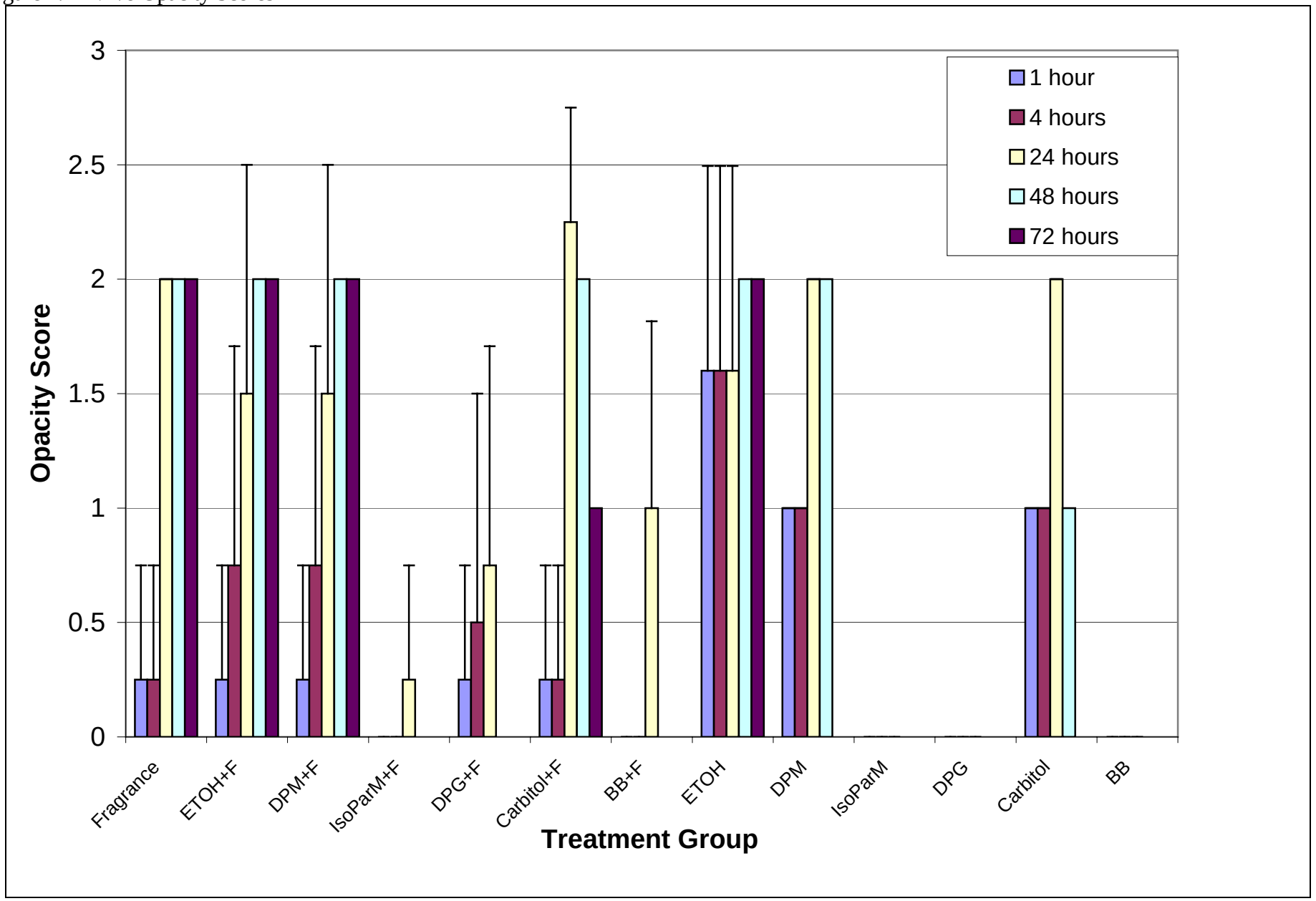


Figure 2. In Vivo Corneal Scores (Opacity x Area)

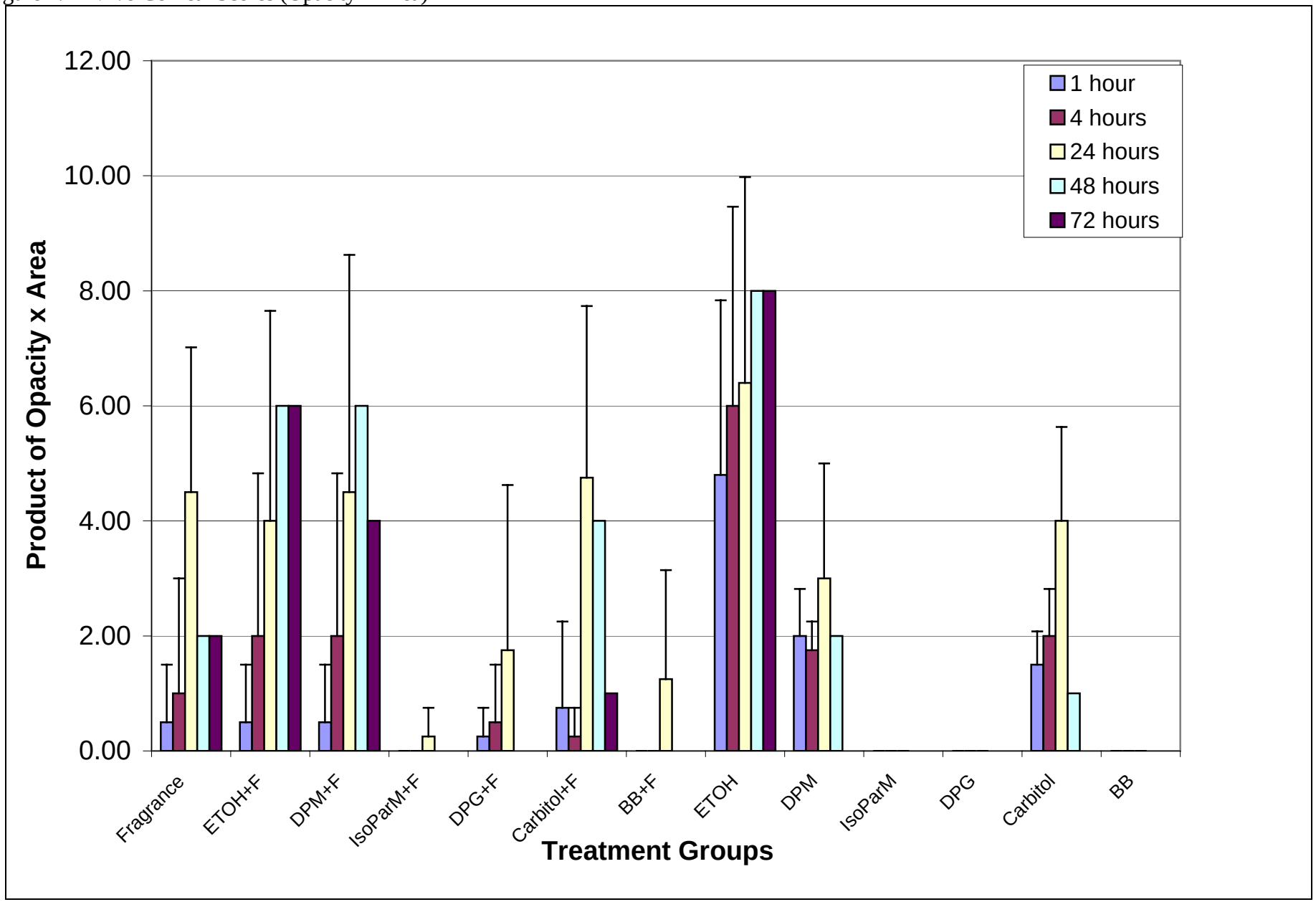


Figure 3. In Vivo MAS Scores

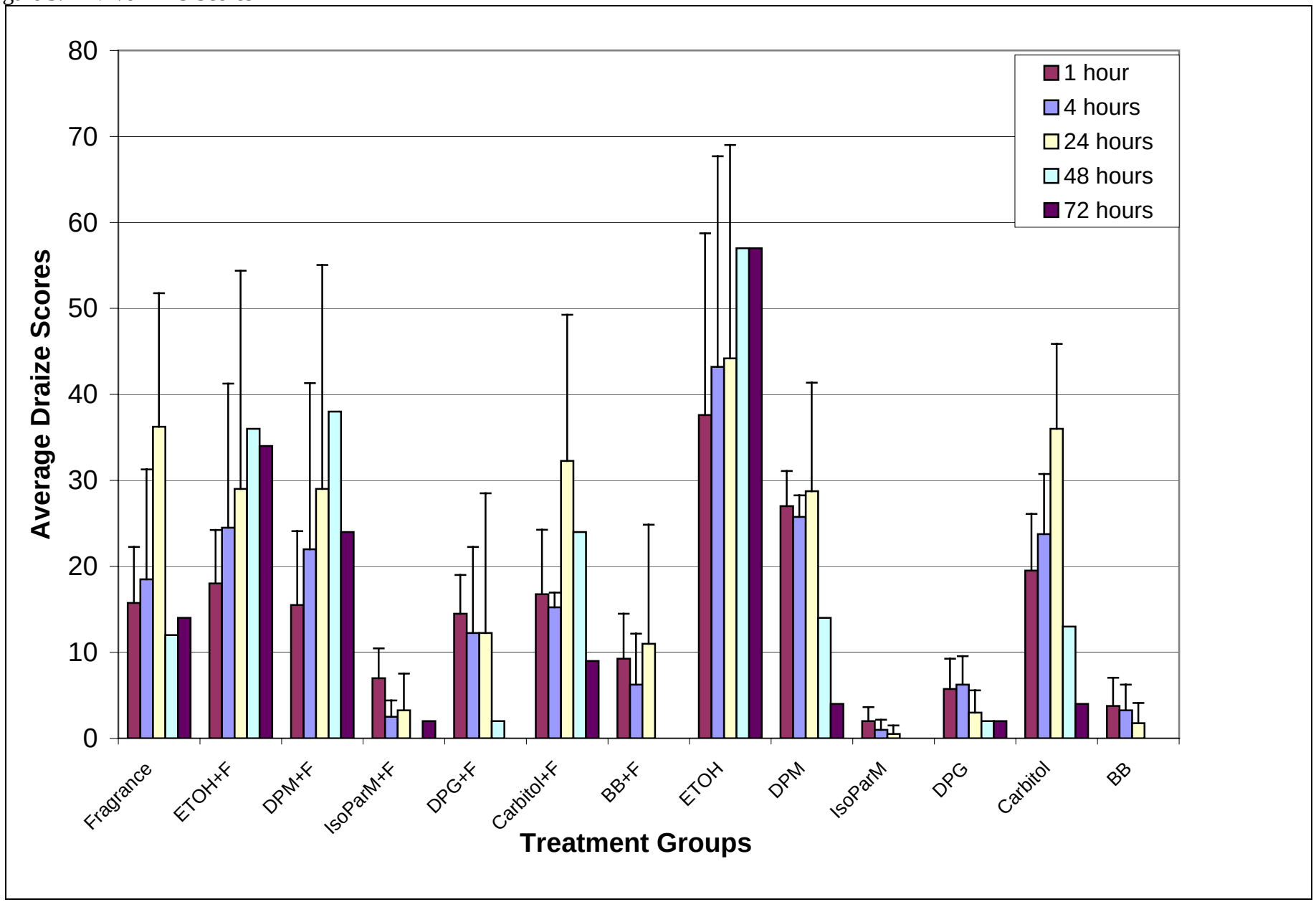


Figure 4. BCOP Opacity Scores

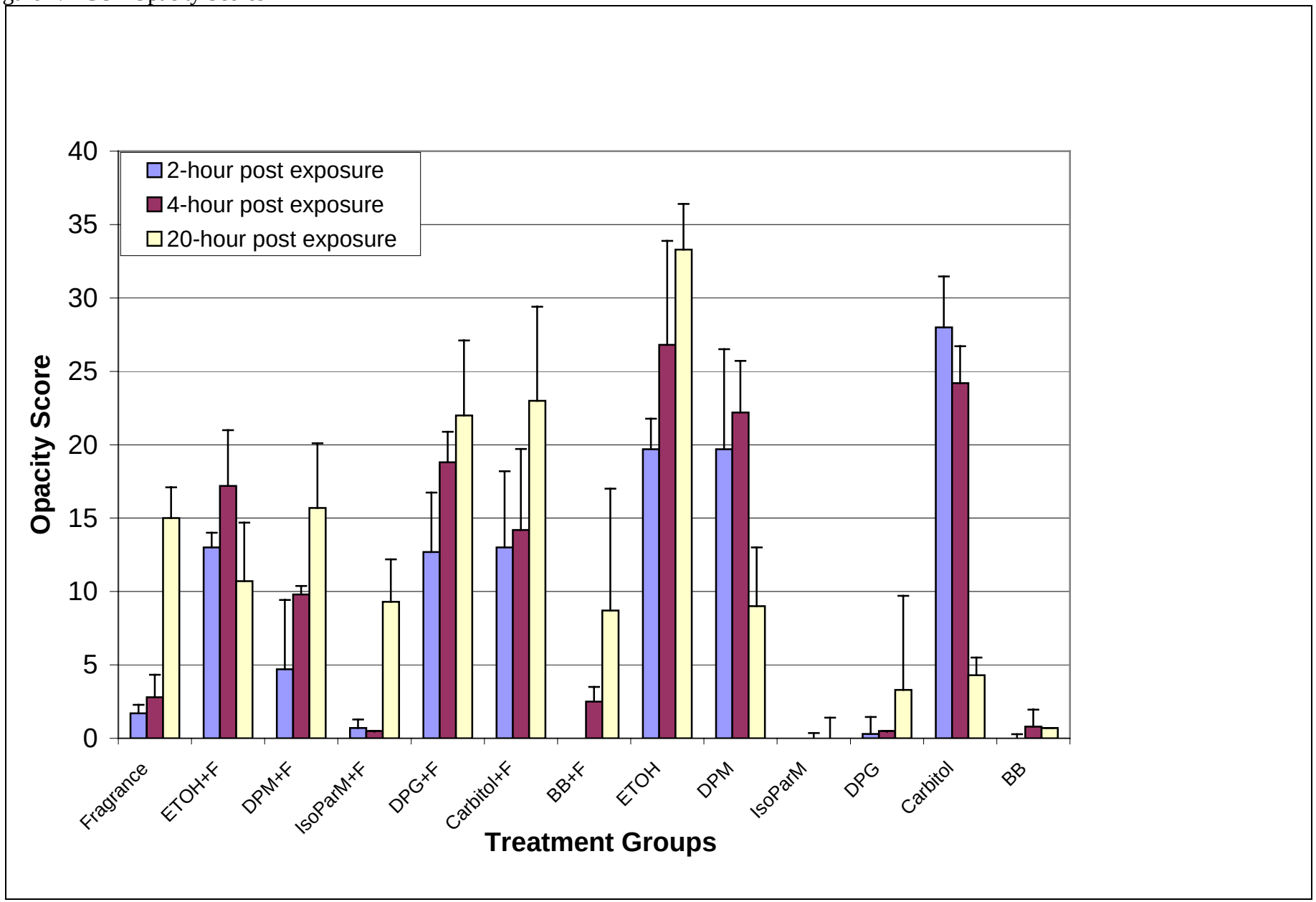


Figure 5. BCOP Permeability Scores

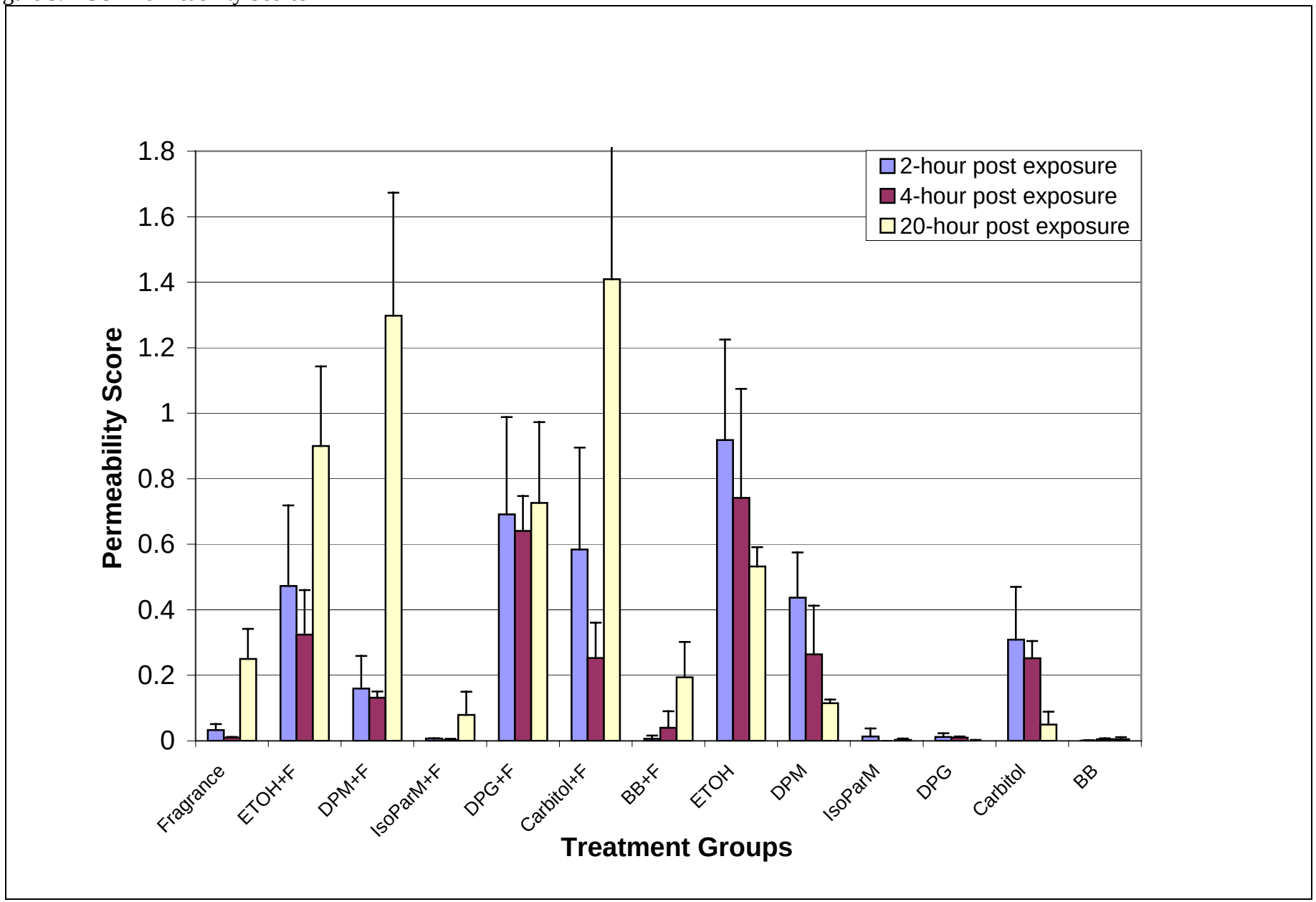


Figure 6. BCOP In Vitro Scores

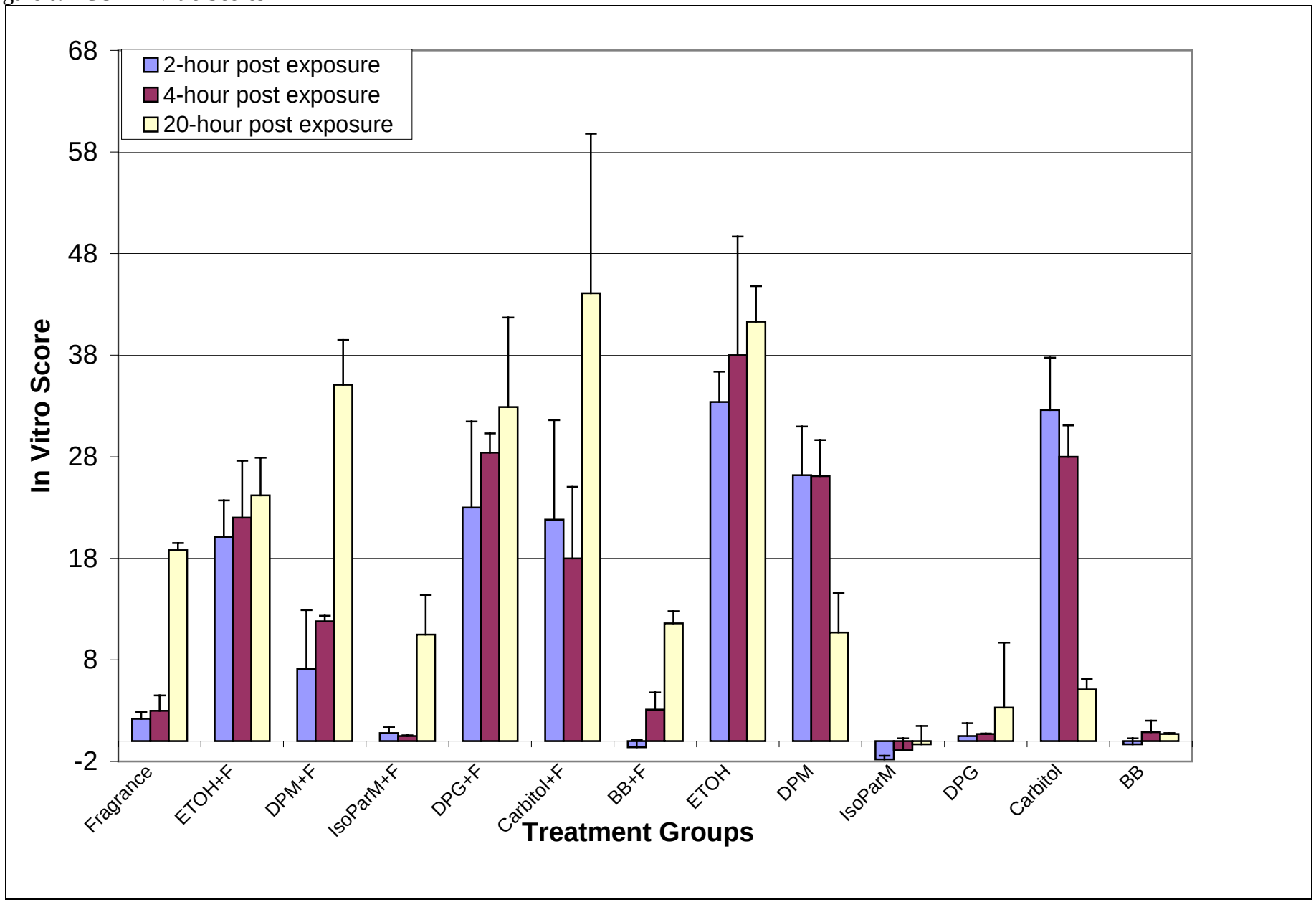
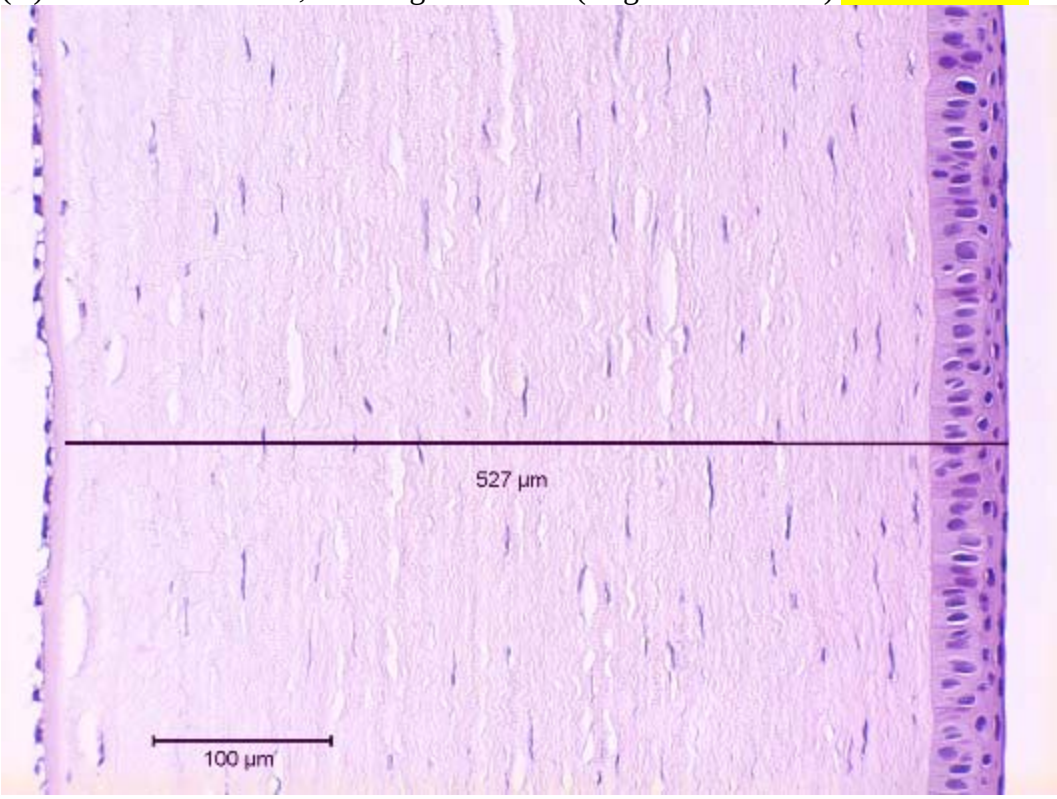


Figure 7. In Vivo Group 1: BB & Isopar M

(A) Center of the cornea, no changes observed (magnification 230x) File 100902-77



(B) Area, away from limbus, showing separation of squamous epithelium (magnification 430x) File 100902-61

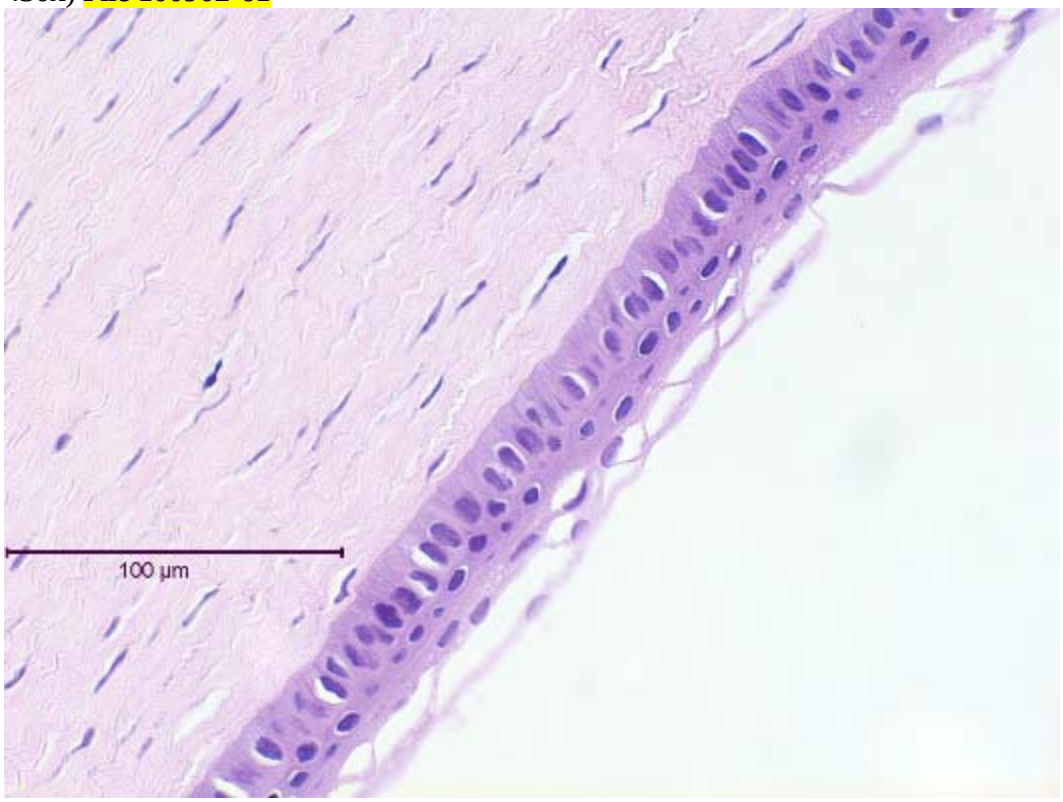
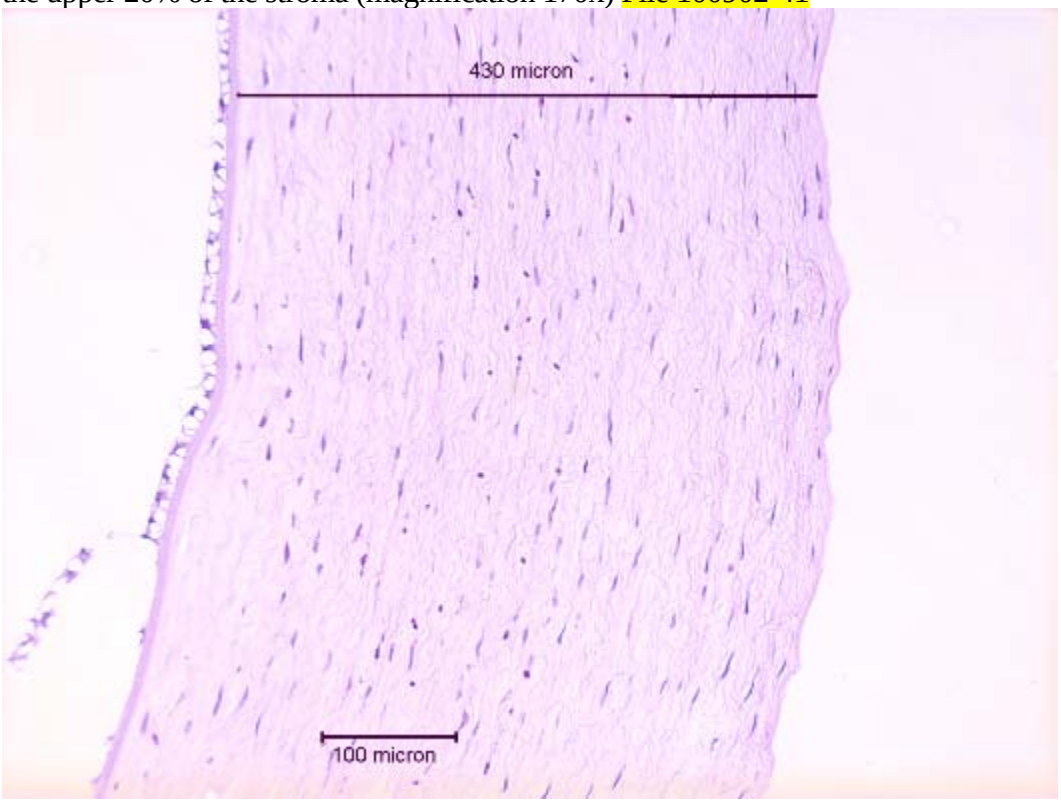


Figure 8. In Vivo Group 4: ETOH.

(A) Central cornea showing loss of epithelium, inflammation, and marked increase in larger dark staining keratocyte nuclei in area of inflammatory infiltrate. Note - the cells were not in the upper 20% of the stroma (magnification 170x) File 100902-41



(B) Area in denuded area showing keratocyte changes and swelling (magnification 430x) File 100902-42

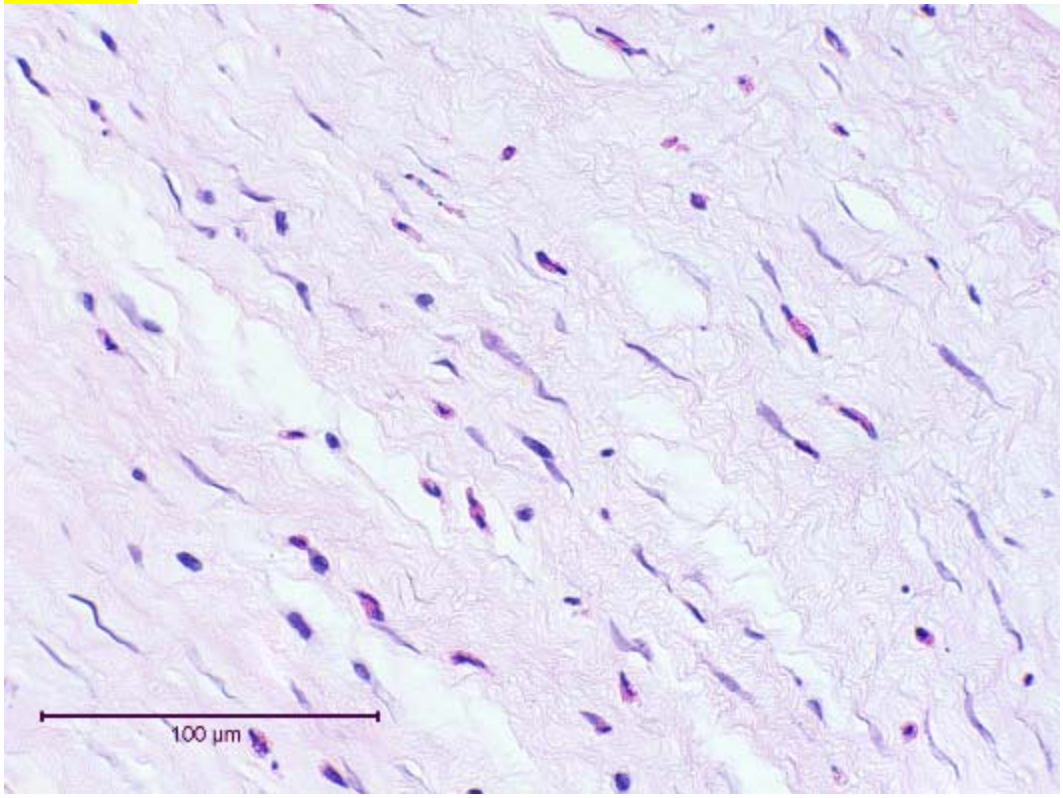
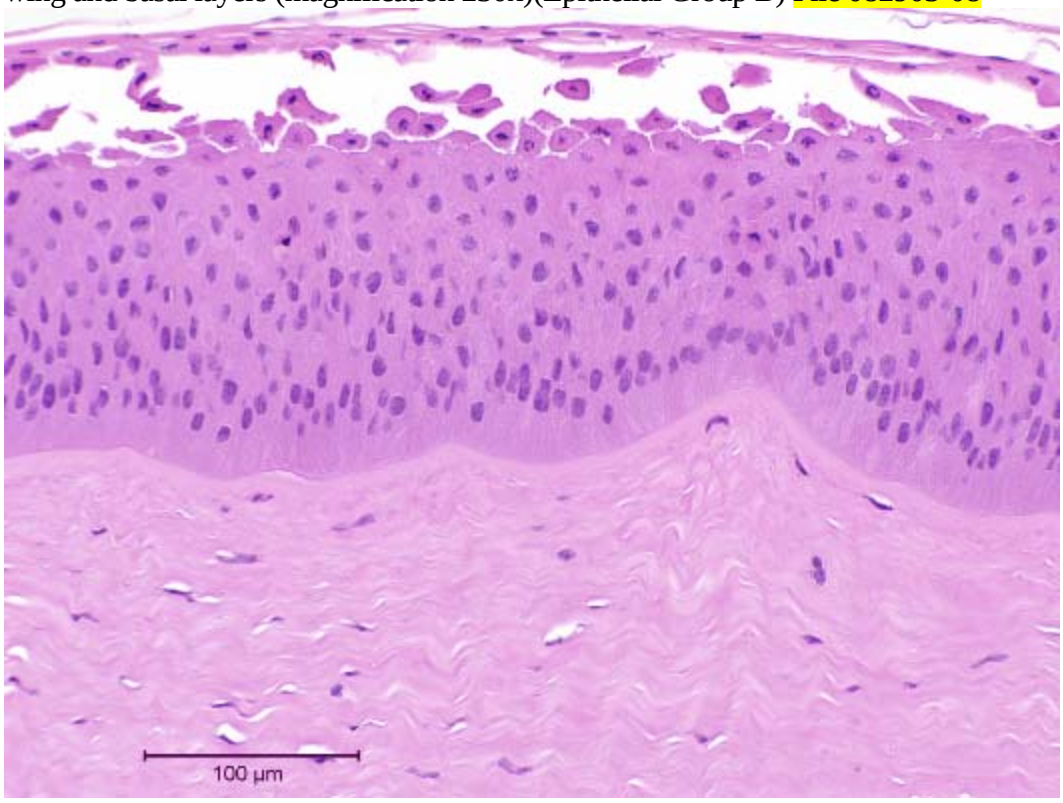


Figure 9. BCOP Fragrance alone: 3-minute exposure, 2-hour post-exposure

(A) Epithelium – Loss of surface squamous epithelium and some necrotic cells within the wing and basal layers (magnification 230x)(Epithelial Group B) File 082903-08



(B) Stroma – Very similar to the time-matched negative control-treated corneas (magnification 430x)(Stromal Group A) File 082903-10

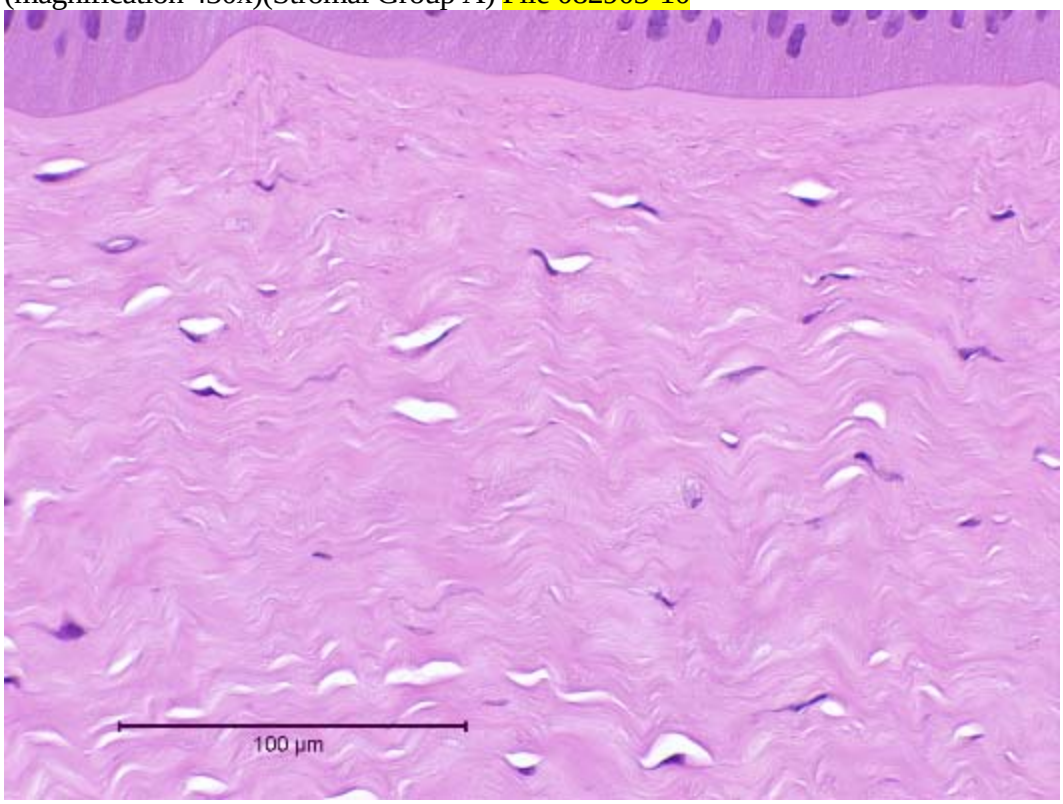
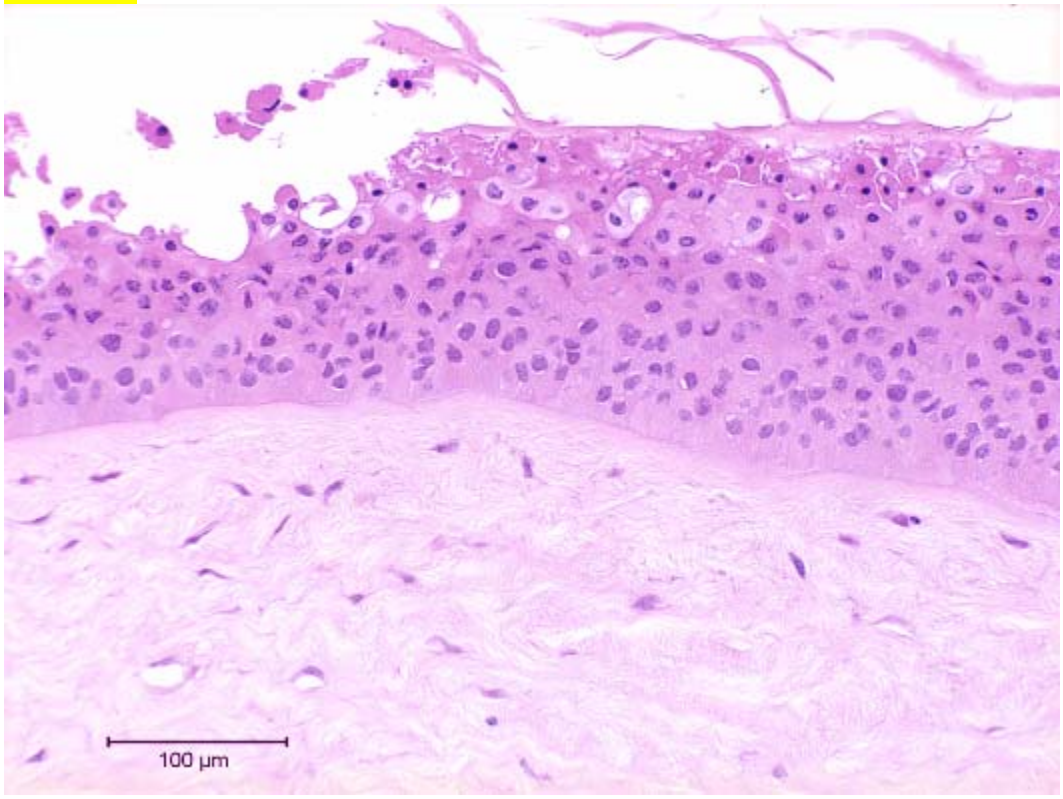


Figure 10. BCOP Fragrance alone: 3-minute exposure, 20-hour post-exposure

(A) Epithelium – Loss of the squamous epithelium and marked nuclear pyknosis and cytoplasmic eosinophilia in the wing cell layer (magnification 230x) (Epithelial Group D) File 111702-12



(B) Stroma – Marked collagen matrix vacuolization to 20% depth and keratocyte nuclear swelling and cytoplasmic eosinophilia (magnification 430x) (Stromal Group D) File 111702-14

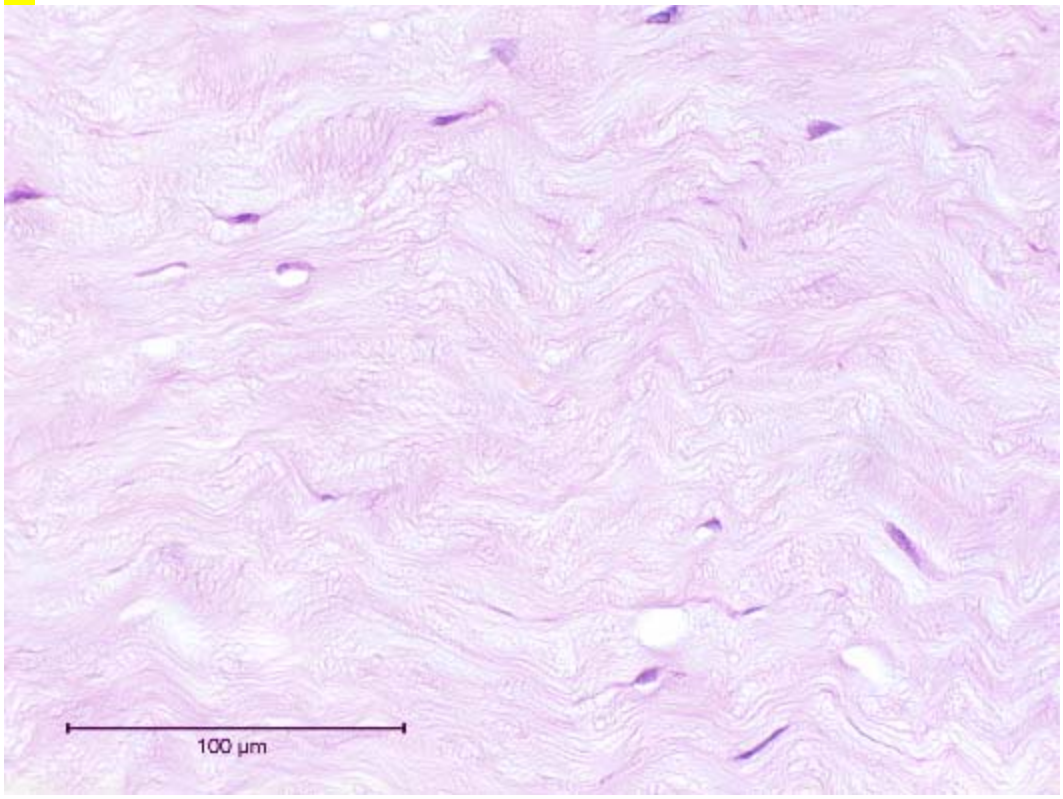
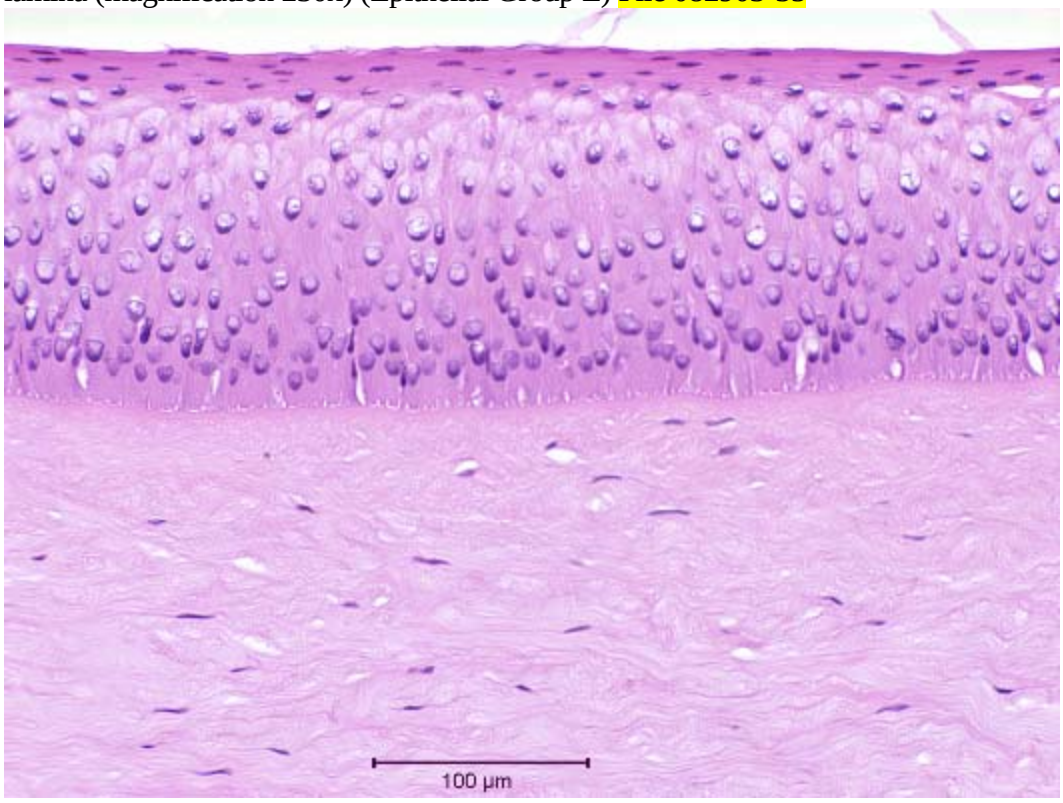


Figure 11. BCOP ETOH alone: 3-minute exposure, 2-hour post-exposure

(A) Epithelium – Marked cellular damage and separation between the basal cells and basal lamina (magnification 230x) (Epithelial Group E) File 082903-35



(B) Stroma – Moderate collagen matrix vacuolization to mid depth and moderate increase in keratinocytes with nuclear pyknosis in the upper 25% of the stroma (magnification 430x) (Stromal Group B) File 082903-37

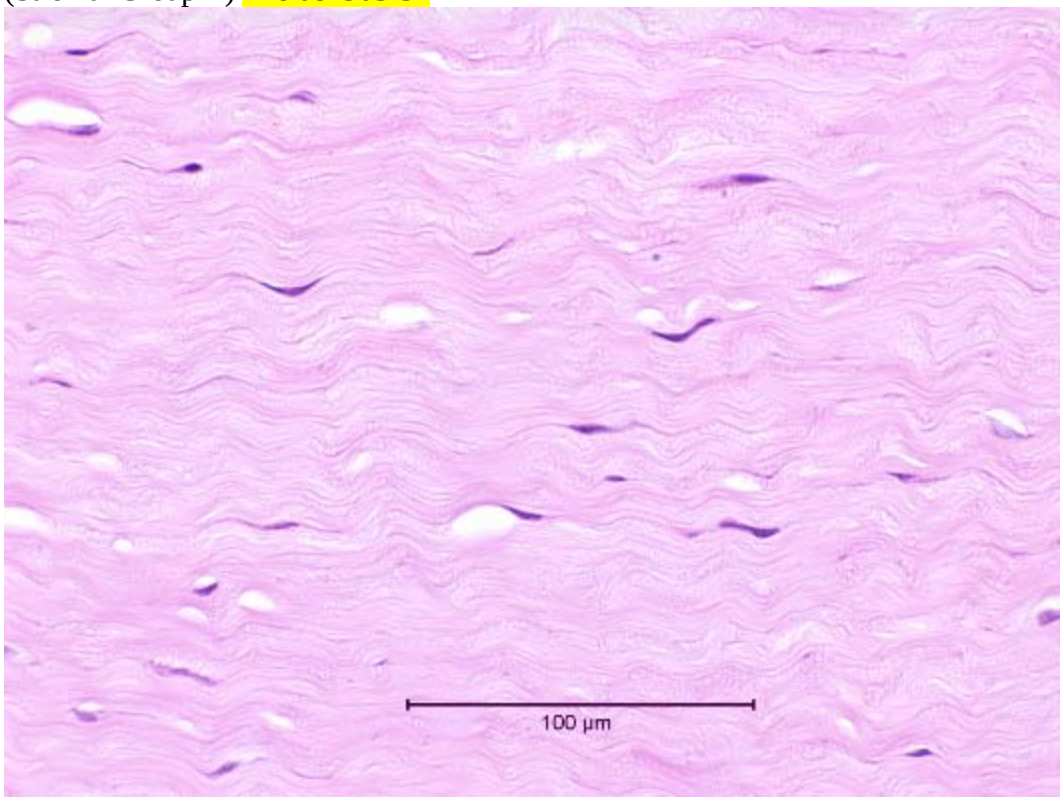
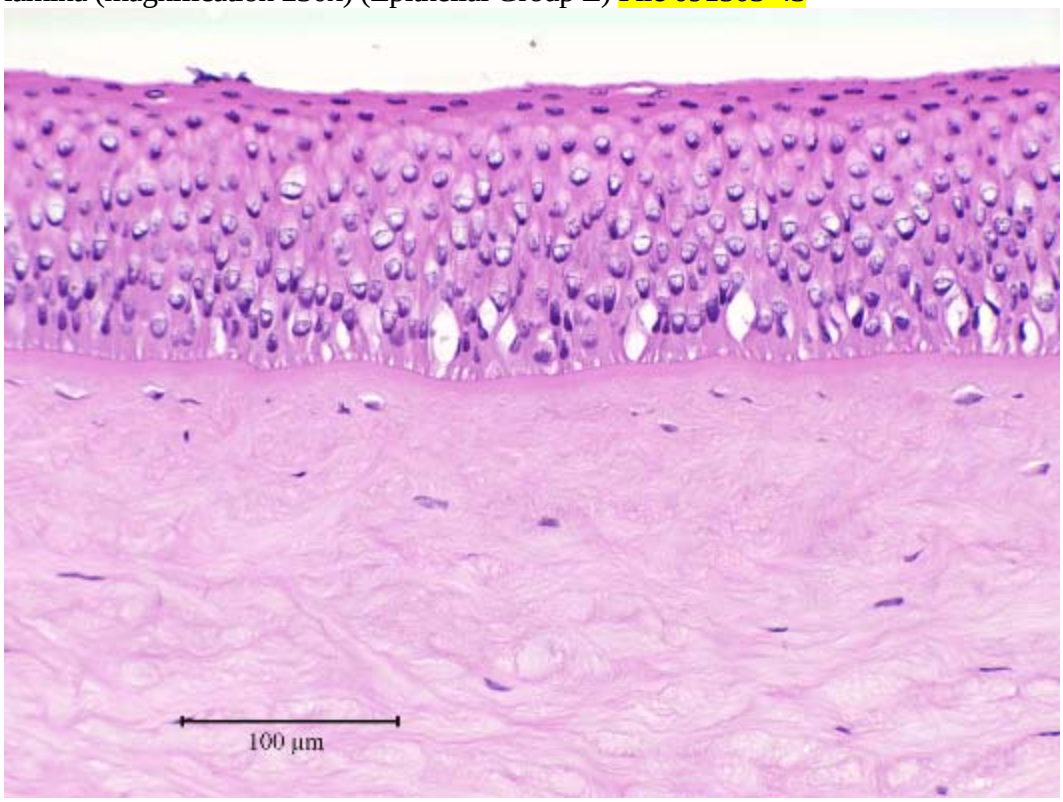
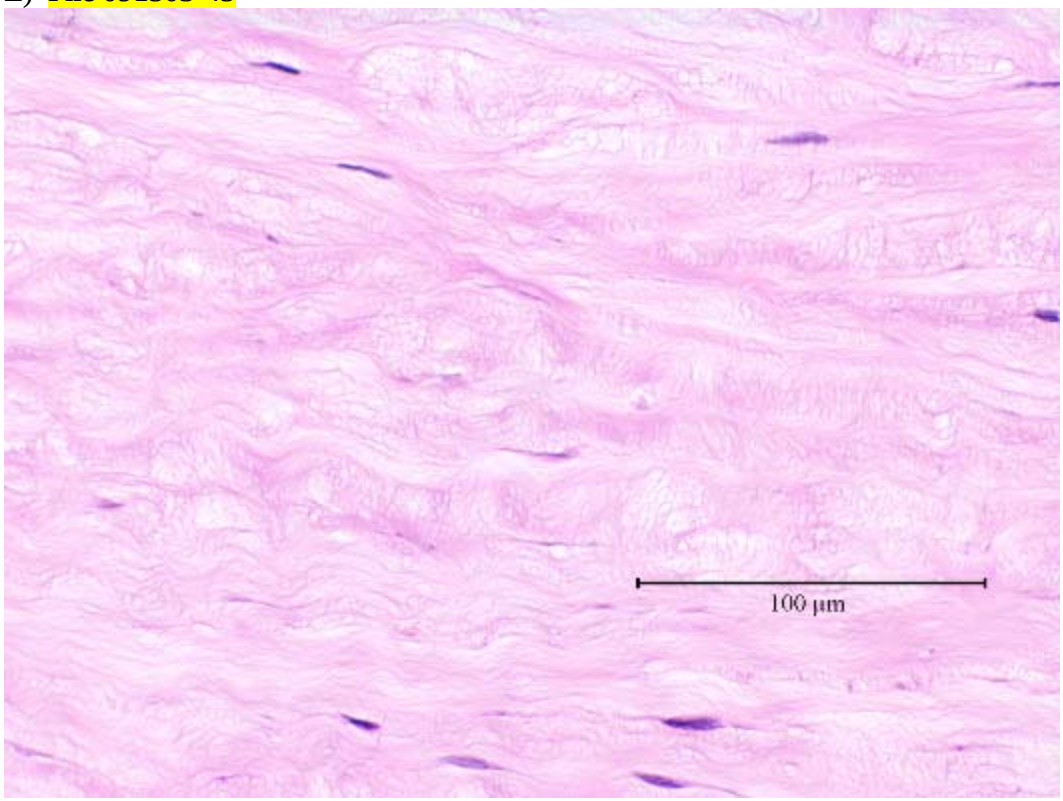


Figure 12. BCOP ETOH alone: 3-minute exposure, 4-hour post-exposure

(A) Epithelium - Marked cellular damage and separation between the basal cells and basal lamina (magnification 230x) (Epithelial Group E) File 091503-43



(B) Stroma – Marked collagen matrix vacuolization and a decrease in viable keratocytes extended to 30% depth. Marked keratocyte nuclear enlargement cytoplasmic eosinophilia was present at mid depth but is not shown in this micrograph (magnification 430x) (Stromal Group E) File 091503-45



FORMULAS

Test Material #	Group	Raw Material	Percentage
1	Fragrance	Benzyl acetate	25
		linalool	25
		Dihydroxymyrcenol	25
		Verdox	25
2	Ethanol	Ethanol	100
3	Dowanol DPM	Dowanol DPM	100
4	Isopar M	Isopar M	100
5	Dipropylene glycol	Dipropylene glycol	100
6	Carbitol	Carbitol	100
7	Benzyl benzoate	Benzyl benzoate	100
8	Ethanol + Fragrance	Ethanol	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20
9	Dowanol DPM + Fragrance	Dowanol DPM	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20
10	Isopar M + Fragrance	Isopar M	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20
11	Dipropylene glycol + Fragrance	Dipropylene glycol	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20
12	Carbitol + Fragrance	Carbitol	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20

FORMULAS

Test Material #	Group	Raw Material	Percentage
13	Benzyl benzoate + Fragrance	Benzyl benzoate	20
		Benzyl acetate	20
		linalool	20
		Dihydroxymyrcenol	20
		Verdox	20

Appendix G2

Dataset Received from S.C. Johnson & Son, Inc. in Support of Cuellar et al. (2002) Poster Presentation

[This Page Intentionally Left Blank]

SC Johnson
A FAMILY COMPANY

S.C. Johnson & Son, Inc.
Worldwide Consumer Products, RD & E
Global Safety Assessment and Regulatory Affairs, Product Toxicology
MS 139 1525 Howe Street, Racine WI 53403

September 3, 2004

Christina Inhof, MSPH
Senior Project Coordinator/Technical Writer ILS, Inc.
NICEATM
P.O. Box 12233
NIEHS MD EC-17
Research Triangle Park, NC 27709

Christina,

Hi! How are you? I am happy to be submitting the data on benchmark and fragrance formulations, which were partially discussed in the poster citation listed below:

Cuellar, N., Merrill, J.C., Clear, M.L.,. Mun G., and Harbell J.W. 2002. The application of benchmarks for the evaluation of the potential ocular irritancy of aerosol fragrances. The Toxicologist 66(1-S): 243-244.

Included with this submission are the following documents:

1. Cover letter
2. Data spreadsheet
3. Fragrance graphs
4. Coded formula spreadsheet

Study Protocols:

Standard Draize protocol was used for 4 of the in-vivo studies. The EPA aerosol protocol was used for test material #3. Standard BCOP protocol was used for the in-vitro work at IIVS. BCOP exposure times were 3 and 10 minutes with post exposure of 2 hours.

Formula Spreadsheet:

The formulas listed in this spreadsheet are coded similarly to past submissions. For benchmarks, test material number is the unique sample number whereas the group description is referenced in the poster as type of benchmark. Test material #3 and #4 (Alcohol-based benchmark) is not listed in the poster. For fragrance formulas, test material denotes the category (formula) whereas the group describes the formula form (i.e aerosol vs membrane (gel). Fragrances formulas are referenced in the poster. For both benchmarks and formulations, raw materials are listed followed by their percentages.

Poster:

Benchmark poster not included. John Harbell previously sent it to you.

Graphs:

Graphs plot the BCOP total score per each unique fragrance formulation. Graphs include reference benchmarks (see formula spreadsheet) and the ethanol control. The bars show one standard deviation from the mean value for each benchmark and the ethanol. Please not that the ethanol values displayed on the 3-minute exposure graphs are for a 3-minute exposure to ethanol (not the 10-minute exposure ethanol that was performed as the positive control). There are 9 tabs in this spreadsheet. Graphs are labeled per form ((2 for aerosol and 2 for membrane (gel)) and exposure time (3 or 10 minutes) in tabs 1-8. The first two graphs refer to the aerosol formulas and the second two graphs refer to the membrane. Each graph includes the corresponding raw data in the next tab. There are 44 aerosol formulas depicted in the aerosol graphs and 36 membrane formulas depicted in the membrane graphs. The final tab describes the basic statistics on the ethanol control and each benchmark by exposure time.

Data Worksheet:

The data worksheet consists of 4 tabs at the bottom of the page. We are including all tabs to clearly demonstrate how we analyzed the data into the GHS and EPA categories in the summary spreadsheet shown in tab #4.

Summary spreadsheet:

The summary spreadsheet is sorted by test material. The test material number refers to the benchmark formula or ethanol listed in the formula spreadsheet. GHS and EPA categories are in the next 8 columns in yellow. The last 2 columns consist of the mean BCOP total scores (3 and 10 minute) in blue. EPA and GHS criteria are summarized in this spreadsheet.

Data was analyzed per formula. Raw data scores from the first three days and days to clear per each of 6 rabbits were randomly put into combinations of three and categorized accordingly based on EPA or GHS criteria. This analysis resulted in a total of 20 combinations per formula. Each combination was listed in the appropriate GHS or EPA category. Scoring assumptions are also listed in this spreadsheet based on protocol differences.

Protocol used for the BCOP assay was the same for all benchmarks and ethanol formulas. In-vivo studies were conducted using the standard Draize protocol with the exception of the ethanol/fragrance benchmark. The ethanol/fragrance benchmark utilized the EPA aerosol dosing Draize protocol.

The ethanol/fragrance benchmark is used to evaluate specific aerosol formulations. This benchmark shows that even though the formulation would be irritating in the standard Draize Assay, the product is provided in a form that restricts exposure to the aerosol route by limiting exposure via a metered dose. The metered dosing approach demonstrates that new formulations would not exceed the irritation potential of the benchmark.

As shown in the graphs and the summary table, the BCOP is able to rank irritation of a variety of formulations in comparison to the benchmarks. Severe irritants can be identified using this methodology. The fragrance level in the formula does impact the irritation potential of the formula.

These data demonstrate the importance of assessing all new formulations relative to an appropriate fixed benchmark.

If you have any questions or comments on this data set, please feel free to contact either Judith Swanson or myself at the following:

Nicole Cuellar
(262) 260-6916
ncuellar@scj.com

Judith Swanson
(262) 260-2688
jeswanso@scj.com

Sincere regards,

Nicole Cuellar
Sr. Research Toxicologist

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F38948	#1	24	1	2	1	2	2	2	27	EPA
			48	1	1	0	2	1	0	11	14
			72	1	1	0	0	1	0	7	GHS
			7 days	0	0	0	2	0	0	4	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	F38948	#1	27	1.0	1.3	0.3	1.3	1.3	0.7	14	14

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	#1	27	1.0	1.3	0.3	1.3	1.3	0.7	14	14
	2	#1	34	0.7	1.3	0.3	2.3	1.3	0.7	14	14
	3	#1	37	0.7	2.0	0.3	2.0	1.3	0.7	7	14
	4	#1	35	1.0	2.3	1.0	2.0	1.3	0.3	7	14
	5	#1	39	1.0	2.7	1.0	2.7	1.3	2.0	14	14
	6	#1	30	1.0	1.7	1.0	2.7	1.3	0.3	14	14
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	R2266	#2	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	R2266	#2	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	#2	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	2	#2	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	3	#2	4	0.0	0.0	0.0	0.7	0.3	0.0	0	3
	4	#2	2	0.0	0.0	0.0	0.7	0.0	0.0	0	3
	5	#2	2	0.0	0.0	0.0	0.7	0.0	0.0	0	3
	6	#2	4	0.0	0.0	0.0	0.7	0.7	0.0	0	3
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F38949	#1	24	1	3	1	3	2	2	34	EPA
			48	1	1	0	2	1	0	11	14
			72	0	0	0	2	1	0	6	GHS
			7 days	1	1	0	1	0	0	7	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F38949	#1	34	0.666667	1.333333	0.333333	2.333333333	1.333333333	0.666666667	14	14

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	1,2,3	0.833333	0.333333	2.166667	1.333333	14	14	Combina-	1,3,4	1	0.666667
tion block	GHS Rating	4	4	2	4	14	14	tion block	GHS Rating	2	4
#1	1,2,4	1	0.666667	2.166667	1.333333	14	14	#2	1,3,5	1	0.666667
	GHS Rating	2	4	2	4	14	14		GHS Rating	2	4
	1,2,5	1	0.666667	2.5	1.333333	14	14		1,3,6	1	0.666667
	GHS Rating	2	4	2	4	14	14		GHS Rating	2	4
	1,2,6	1	0.666667	2.5	1.333333	14	14		1,4,5	1	1
	GHS Rating	2	4	2	4	14	14		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	R2317	#2	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	R2317	#2	2	0	0	0	0.5	0	0	0	2

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	1,2,3	0	0	0.583333	0.166667	0	3	Combina-	1,3,4	0	0
tion block	GHS Rating	4	4	4	4	0	3	tion block	GHS Rating	4	4
#1	1,2,4	0	0	0.583333	0	0	3	#2	1,3,5	0	0
	GHS Rating	4	4	4	4	0	3		GHS Rating	4	4
	1,2,5	0	0	0.583333	0	0	3		1,3,6	0	0
	GHS Rating	4	4	4	4	0	3		GHS Rating	4	4
	1,2,6	0	0	0.583333	0.333333	0	3		1,4,5	0	0
	GHS Rating	4	4	4	4	0	3		GHS Rating	4	4

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	F38950	#1	24	1	4	1	2	2	2	37	EPA
			48	1	2	0	2	1	0	16	7
			72	0	0	0	2	1	0	6	GHS
			7 days	0	0	0	1	0	0	2	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F38950	#1	37	0.666667	2	0.333333	2	1.33333333	0.666666667	7	14

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2	1.333333	14	14	Combina-	1,4,6	1.0	1.0	2.3	1.3	14	14
2	4	14	14	tion block	GHS Ratinç	2	4	2	4	14	14
2.333333	1.333333	14	14	#3	1,5,6	1.0	1.0	2.7	1.3	14	14
2	4	14	14		GHS Ratinç	2	4	2	4	14	14
2.333333	1.333333	14	14		2,3,4	0.8	0.7	2.2	1.3	14	14
2	4	14	14		GHS Ratinç	4	4	2	4	14	14
2.333333	1.333333	14	14		2,3,5	0.8	0.7	2.5	1.3	14	14
2	4	14	14		GHS Ratinç	4	4	2	4	14	14

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	R2314	#2	24	0	0	0	1	1	0	4	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	R2314	#2	4	0	0	0	0.666666667	0.33333333	0	0	3

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0.666667	0.166667	0	3	Combina-	1,4,6	0.0	0.0	0.7	0.3	0	3
4	4	0	3	tion block	GHS Ratinç	4	4	4	4	0	3
0.666667	0.166667	0	3	#3	1,5,6	0.0	0.0	0.7	0.3	0	3
4	4	0	3		GHS Ratinç	4	4	4	4	0	3
0.666667	0.5	0	3		2,3,4	0.0	0.0	0.7	0.2	0	3
4	4	0	3		GHS Ratinç	4	4	4	4	0	3
0.666667	0	0	3		2,3,5	0.0	0.0	0.7	0.2	0	3
4	4	0	3		GHS Ratinç	4	4	4	4	0	3

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F38951	#1	24	1	4	1	2	2	1	35	EPA
			48	1	2	1	2	1	0	21	7
			72	1	1	1	2	1	0	16	GHS
			7 days	0	0	0	1	0	0	2	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F38951	#1	35	1	2.333333	1	2	1.333333333	0.333333333	7	14
	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.8	0.7	2.5	1.3	14	14	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratinç	4	4	2	4	14	14	tion block	GHS Rating	2	4
#4	2,4,5	1.0	1.0	2.5	1.3	14	14	#5	3,4,6	1.0	1.0
	GHS Ratinç	2	4	2	4	14	14		GHS Rating	2	4
	2,4,6	1.0	1.0	2.5	1.3	14	14		3,5,6	1.0	1.0
	GHS Ratinç	2	4	2	4	14	14		GHS Rating	2	4
	2,5,6	1.0	1.0	2.7	1.3	14	14		4,5,6	1.0	1.0
	GHS Ratinç	2	4	2	4	14	14		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	R2299	#2	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	R2299	#2	2	0	0	0	0.66666667	0	0	0	3
	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.7	0.5	0	3	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratinç	4	4	4	4	0	3	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	0.7	0.0	0	3	#5	3,4,6	0.0	0.0
	GHS Ratinç	4	4	4	4	0	3		GHS Rating	4	4
	2,4,6	0.0	0.0	0.7	0.3	0	3		3,5,6	0.0	0.0
	GHS Ratinç	4	4	4	4	0	3		GHS Rating	4	4
	2,5,6	0.0	0.0	0.7	0.3	0	3		4,5,6	0.0	0.0
	GHS Ratinç	4	4	4	4	0	3		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F38952	#1	24	1	4	1	2	2	3	39	EPA
			48	1	3	1	3	1	2	32	14
			72	1	1	1	3	1	1	20	GHS
			7 days	1	1	0	1	1	0	9	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F38952	#1	39	1	2.666667	1	2.66666667	1.333333333	2	14	14

Redness	Chemosis	DtC EPA	DtC GHS	Summary			
2.3	1.3	14	14	#1	1,2,3	2	14
2	4	14	14		1,2,4	2	14
2.3	1.3	14	14		1,2,5	2	14
2	4	14	14		1,2,6	2	14
2.7	1.3	14	14		1,3,4	2	14
2	4	14	14		1,3,5	2	14
2.7	1.3	14	14		1,3,6	2	14
2	4	14	14		1,4,5	2	14
					1,4,6	2	14
					1,5,6	2	14

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	R2275	#2	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	R2275	#2	2	0	0	0	0.66666667	0	0	0	3

Redness	Chemosis	DtC EPA	DtC GHS	Summary			
0.7	0.2	0	3	#2	1,2,3	4	3
4	4	0	3		1,2,4	4	3
0.7	0.5	0	3		1,2,5	4	3
4	4	0	3		1,2,6	4	3
0.7	0.5	0	3		1,3,4	4	3
4	4	0	3		1,3,5	4	3
0.7	0.3	0	3		1,3,6	4	3
4	4	0	3		1,4,5	4	3
					1,4,6	4	3
					1,5,6	4	3

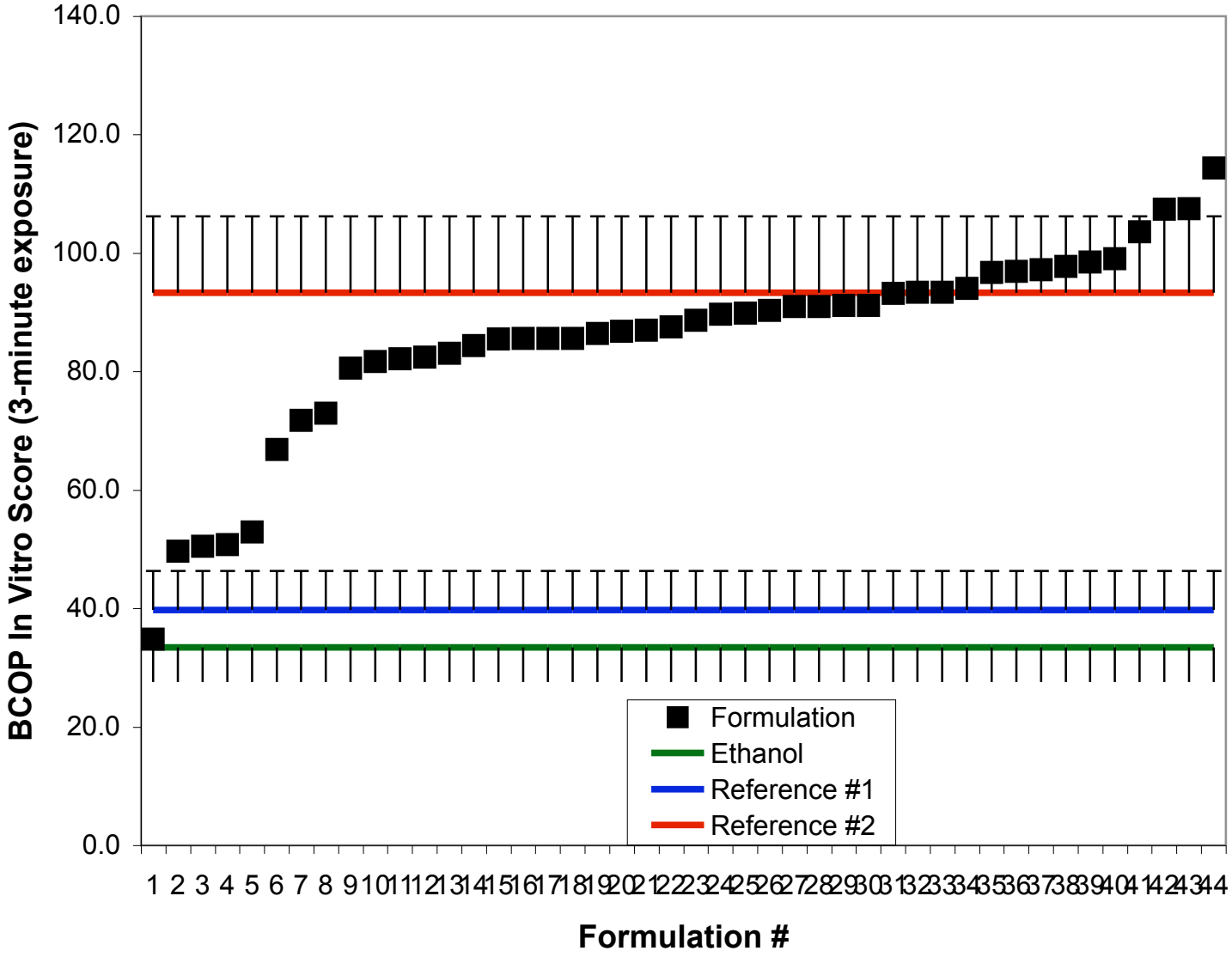
Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F38953	#1	24	1	3	1	2	2	1	30	EPA
			48	1	1	1	3	1	0	18	14
			72	1	1	1	3	1	0	18	GHS
			7 days	0	0	0	2	1	0	6	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F38953	#1	30	1	1.666667	1	2.66666667	1.333333333	0.333333333	14	14
14		2,3,4	2	14		14					
14		2,3,5	2	14		14					
14		2,3,6	2	14		14					
14		2,4,5	2	14		14					
14		2,4,6	2	14		14					
14		2,5,6	2	14		14					
14		3,4,5	2	14		14					
14		3,4,6	2	14		14					
14		3,5,6	2	14		14					
14		4,5,6	2	14		14					

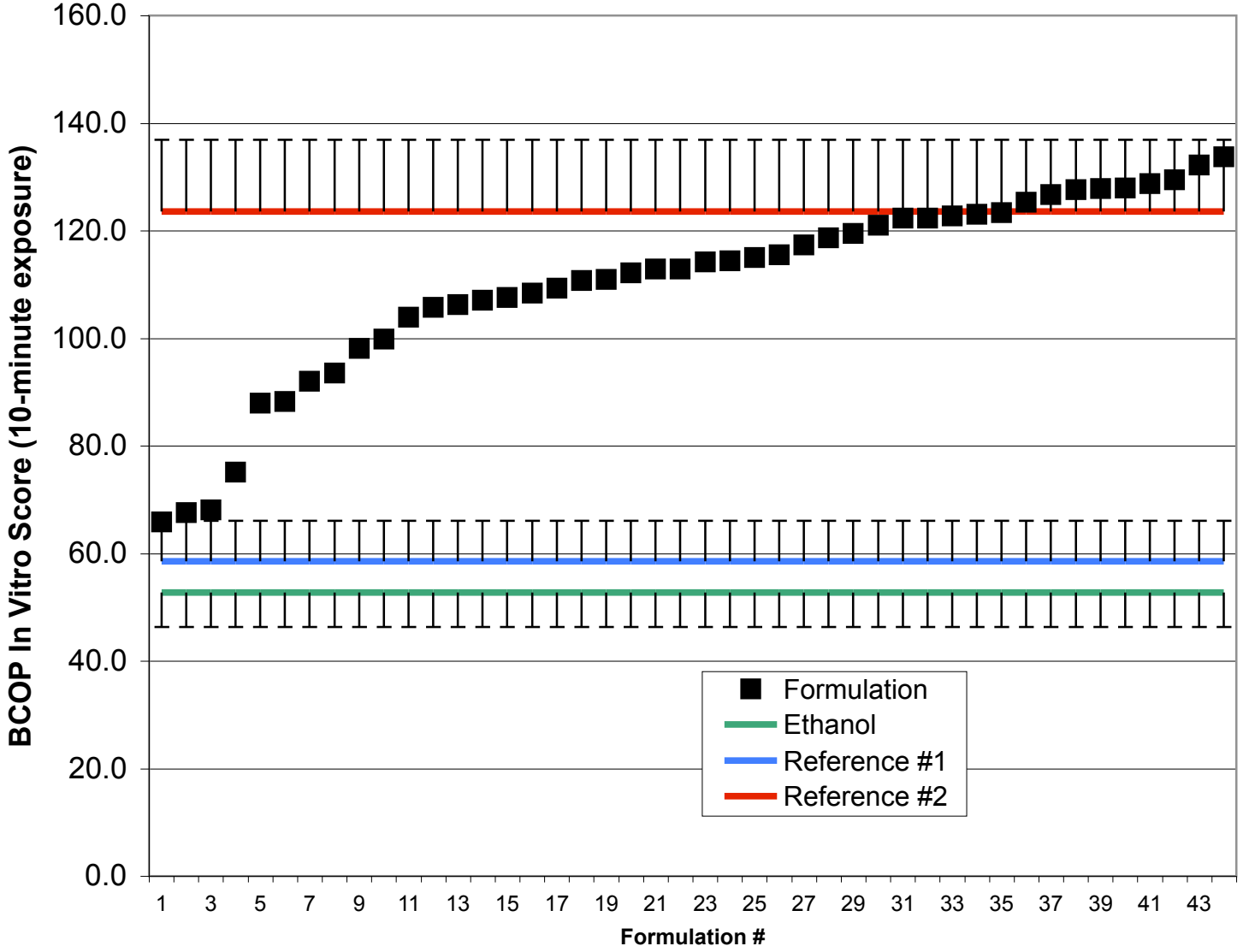
Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	R2267	#2	24	0	0	0	1	1	0	4	EPA
			48	0	0	0	1	1	0	4	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	R2267	#2	4	0	0	0	0.66666667	0.666666667	0	0	3
0		2,3,4	4	3		0					
0		2,3,5	4	3		0					
0		2,3,6	4	3		0					
0		2,4,5	4	3		0					
0		2,4,6	4	3		0					
0		2,5,6	4	3		0					
0		3,4,5	4	3		0					
0		3,4,6	4	3		0					
0		3,5,6	4	3		0					
0		4,5,6	4	3		0					

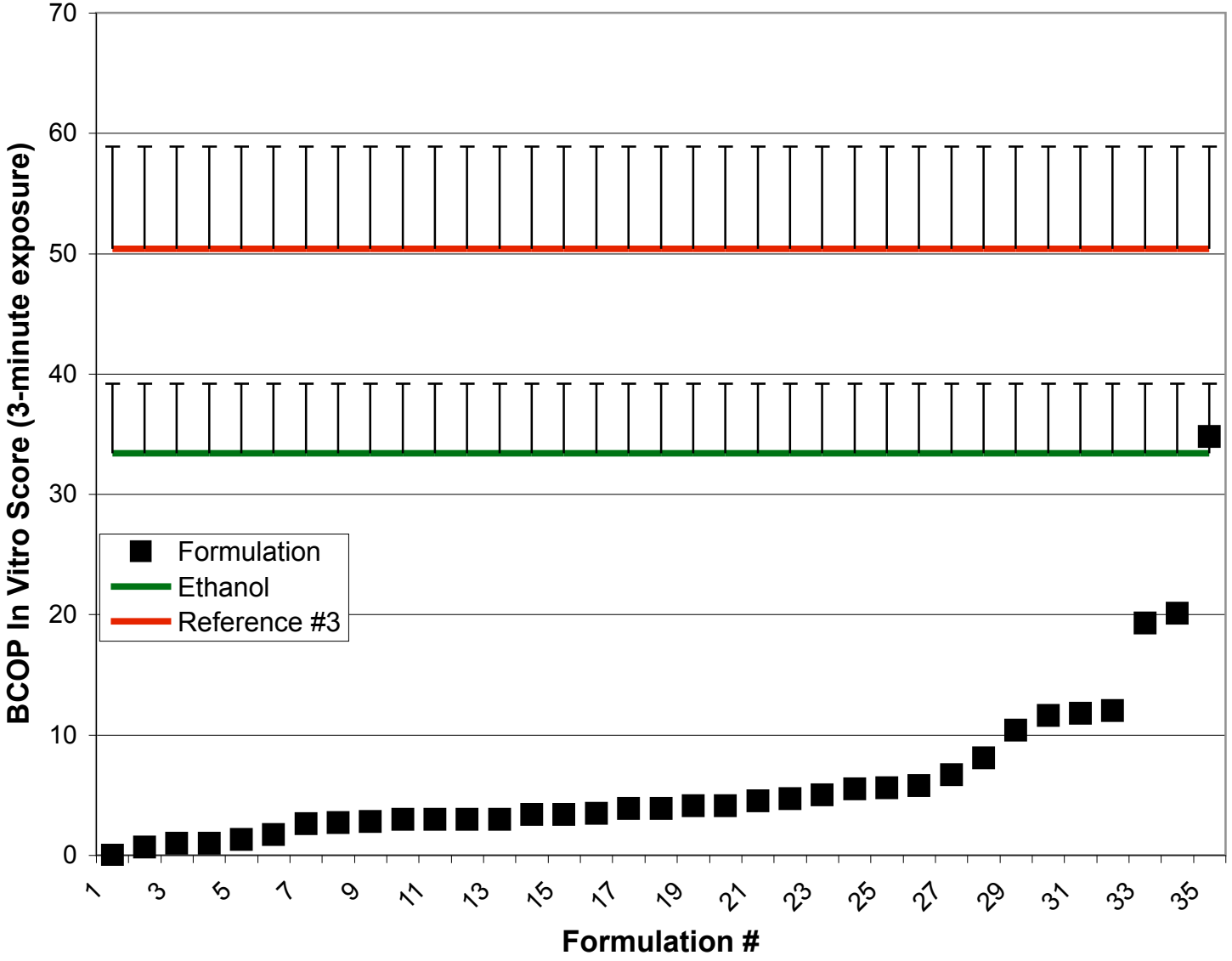
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1															
2					Test				Count per Category					BCOP Mean In Vitro Score	
3					Material #	GHS 1	GHS 2a	GHS 2b	GHS NI	EPA 1	EPA 2	EPA 3	EPA 4	3-minute	10-minute
4	Note #1	Fragrance Benchmark #3			#1	0	20	0	0	0	20	0	0	50.1	90.1
5	Note #2	Ethanol/Fragrance Benchmark #2			#2	0	0	0	20	0	0	0	20	93.3	123.6
6	Note #3	Alcohol-based Benchmark #1			#3	10	10	0	0	10	10	0	0	39.7	58.9
7	Note #4	Alcohol-based Benchmark #1			#4	20	0	0	0	20	0	0	0	39.7	58.9
8	Note #5	Ethanol			#5	10	10	0	0	10	10	0	0	33.4	52.7
9															
10	reference #s refer to the benchmark study														
11	data and figures														
12															
13	Note #1	Full volume rabbit eye irritation test													
14	Note #2	Aerosol protocol where eye is sprayed for one second at a distance of 10 cm							**Not a normal installation Draize test**						
15	Note #3	Full volume eye test. Details of BCOP Reference #1 provided in the earlier submission of ethanol containing formulations													
16	Note #4	second study also reported with the ethanol containing formulations													
17	Note #5	Full volume eye test. Data reported with ethanol formulations													
18															
19	Raw animal data already provided for Reference #1 and ethanol														
20															
21															
22		Opacity	Iris	Conjunct	Days to Clear		Notes:	GHS							
23	GHS 1	3 or >	1.5 or >	NA	>21		Tissue scores for 2 most irritated rabbits								
24	GHS 2a	1 or >	1 or >	2 or >	<21		Days to clear for the last rabbit to clear (all tissues)								
25	GHS 2b	1 or >	1 or >	2 or >	7 or <		(i.e., tissue scores of 0)								
26	GHS NI	<1	<1	<2	<21		Discharge not scored								
27								EPA							
28	EPA 1				>21		Rabbit has cleared if:								
29	EPA 2				<21		Opacity	Iris	Conjunct						
30	EPA 3				7 or <		<1	<1	<2						
31	EPA 4				1 or <		Discharge not scored								
32															
33															


BCOP In Vitro Score (3-minute exposure)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
Formulation #
Formulation
Ethanol
Reference #1
Reference #2

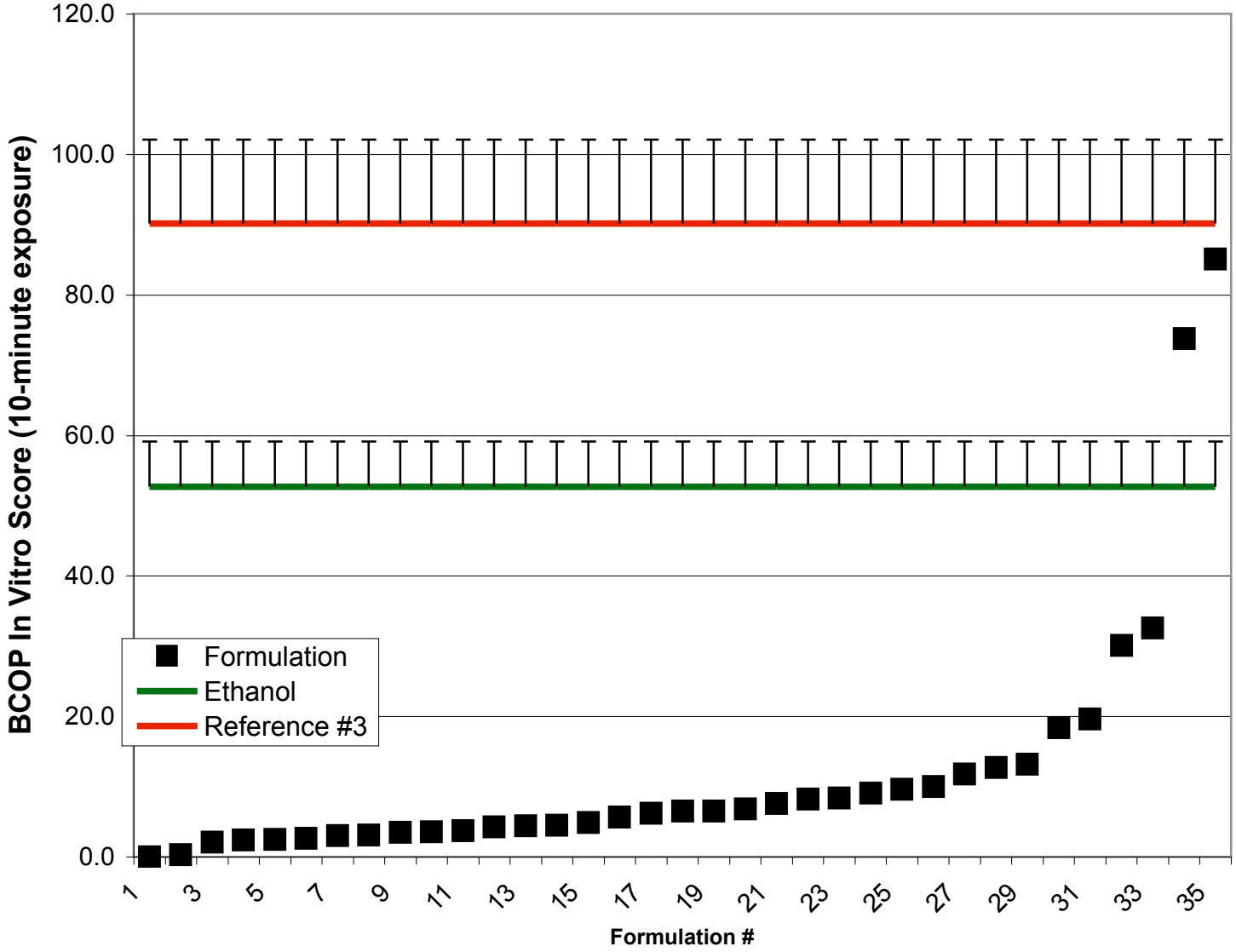
Fragrance Graphs for
SC Johnson Submission
Dated September 3, 2004



Fragrance Graphs for
SC Johnson Submission
Dated September 3, 2004



Fragrance Graphs for
SC Johnson Submission
Dated September 3, 2004



Ethanol 3-minute exposure

	Opacity	OD490	In Vitro Score
Mean	21.1	0.820	33.4
STD	2.9	0.238	5.8
CV	13.7%	29.0%	17.5%

n=7

Ethanol 10-minute exposure (normal positive control)

	Opacity	OD490	In Vitro Score
Mean	31.2	1.422	52.7
STD	4.8	0.345	6.4
CV	15.3%	24.3%	12.1%

n = 632
Oct 1997 to the present

Reference #1 Alcohol-based benchmark
3-minute exposure
Used as the first benchmark formulation for the aerosol formulations

	Opacity	OD490	IV Score
Mean	20.6	1.270	39.7
STD	3.5	0.308	6.6
CV	16.8%	24.2%	16.7%

n=21
10-minute exposure

	Opacity	OD490	IV Score
Mean	28.6	2.001	58.5
STD	4.1	0.415	7.6
CV	14.3%	20.7%	13.0%

n=43

Reference #2 Ethanol Fragrance benchmark
3-minute exposure

	Opacity	OD490	IV Score
Mean	53.7	2.6	93.3
STD	8.5	0.5	12.9
CV	15.8%	20.0%	13.8%

n=32

10-minute exposure

	Opacity	OD490	IV Score
Mean	81.5	2.805	123.6
STD	11.9	0.520	13.3
CV	14.6%	18.5%	10.8%

n=32

Reference #3 Fragrance benchmark (no ethanol)

3-minute exposure

	Opacity	OD490	IV Score
Mean	39.9	0.693	50.4
STD	6.0	0.238	8.5
CV	14.9%	34.3%	16.8%

n=84

10-minute exposure

	Opacity	OD490	IV Score
Mean	61.0	1.941	90.1
STD	7.9	0.459	12.0
CV	12.9%	23.7%	13.3%

n=90

FORMULAS

Test Material #	Group	Raw Material	Percentage
1	Fragrance Benchmark (Reference #3)	Fragrance Thickener	95-100 0-5
2	Ethanol/ Fragrance Benchmark (Reference #2)	Ethanol Fragrance	70-75 25-30
3, 4	Alcohol-based Benchmark (Reference #1)	Alcohol Active Dimethicone	85-90 10-15 1-5
5	Ethanol	Fragrance Ethanol	< 1 100
Fragrance Formulas	Membrane Formula	Fragrance Thickener	95-100 0-5
Fragrance Formulas	Aerosol Formula-1[1]	Alcohol Fragrance	70-75 25-30
Fragrance Formulas	Aerosol Formula-2	Alcohol Fragrance	80-85 15-20
Fragrance Formulas	Aerosol Formula-3	Alcohol Fragrance	90-95 5-10

[1]Most aerosol formulas fall within this category

Appendix G3

Dataset Received from S.C. Johnson & Son, Inc. in Support of Gran et al. (2003) Poster Presentation

[This Page Intentionally Left Blank]

SC Johnson
A FAMILY COMPANY

S.C. Johnson & Son, Inc.
Worldwide Consumer Products, RD & E
Global Safety Assessment and Regulatory Affairs, Product Toxicology
MS 139 1525 Howe Street, Racine WI 53403

October 13, 2004

Christina Inhof, MSPH
Senior Project Coordinator/Technical Writer ILS, Inc.
NICEATM
P.O. Box 12233
NIEHS MD EC-17
Research Triangle Park, NC 27709

Christina,

Hi! How are you? I am happy to be submitting data on sodium percarbonate, which was discussed in the poster citation listed below:

Gran B.P., Swanson J.E., Merrill J.C., and Harbell J.W. 2003. Evaluating the irritancy potential of sodium percarbonate: A case study using the bovine corneal opacity and permeability (BCOP) assay. The Toxicologist, Abstract Number 1066, Volume 72, Number S-1, March 2003.

Included with this submission are the following documents:

1. Cover letter
2. Poster text
3. Histology slides
4. Coded formula spreadsheet

Study Protocols:

The standard Draize protocol was used by the supplier for the *in vivo* studies. We have been granted permission to share this data on sodium percarbonate for the purpose of this review. Due to the powdered form of the raw material, a bulk density determination was made to determine the weight equivalent of a 100 uL dose. Because significant irritation was observed in the acute dermal study, anesthetic was applied to the rabbit eyes five minutes prior to dosing in the primary eye irritation study.

The standard BCOP protocol for solid test articles was not used for the *in vitro* work at IIVS. Test articles were tested as 50% (w/w) slurry suspensions in sterile, deionized water. Treated corneas were incubated for 10 and 30 minutes with post-exposure incubation

periods of 4- and 20-24 hours. The details of the protocol are provided in the poster text. Concurrent positive and negative controls were performed with each assay. Negative control corneas were prepared for each post-exposure incubation time.

Formula Spreadsheet:

The formulas listed in this spreadsheet are coded similarly to formulas listed in the poster. Test material number is the unique sample number and the group name denotes formula description. Raw materials are listed followed by their percentages in each formula.

Poster:

The poster offprint is not included. John Harbell sent it to you previously.

Poster Text:

A word document consisting of poster text and tables is included in this submission for ease of reading. This document highlights where the histology slides should be inserted for ease of understanding.

Histology Slides:

Histology slides should be referenced on page 8.

Data Worksheet:

Since the rabbit study was terminated at 96 hours because of the severe nature of the responses, we must assume that the *in vivo* response fits Category 1 (both GHS and EPA). The 96-hours readings are listed in the table below:

Animal	Opacity	Area	Iris	Redness	Chemosis	Discharge
1	3	1	1	3	2	2
2	3	4	a	3	3	0
3	3	1	a	3	3	2
4	1	1	0	3	2	0
5	3	4	a	3	4	3
6	2	4	1	3	3	1

a – Iris could not be scored because of severe corneal opacity

Summary:

The standard BCOP protocol for solids was not utilized in this investigation of sodium percarbonate. The standard protocol, developed for pharmaceutical intermediates that are relatively insoluble, calls for using a 20% suspension with a 4-hour exposure time. Based on past experience with the BCOP assay, the eye irritancy potential of more aqueous-soluble

solids such as laundry powders using the standard solids protocol is vastly overpredictive of the outcome resulting from accidental human exposure. Furthermore, experience has shown that reactive/oxidizing chemistries (such as bleach, percarbonates and peroxides) have a delayed toxicity response in the assay necessitating increased post-exposure observation time.

The question the investigators faced in this case study of sodium percarbonate was what protocol parameters were needed to model the bolus exposure for an extended period that occurs in the Draize eye irritation protocol as well as what might be expected to be a realistic maximum exposure for humans. The following parameters were chosen: A 50% suspension of the solid with a 30-minute exposure time to model the *in vivo* exposure and 10-minute exposure time to model maximum accidental human exposure. While post-exposure time in the BCOP is typically 2 hours, times of 4 and 20-24 hours were chosen.

Utilizing the protocol considerations discussed above, the BCOP assay was able to adequately predict the irritancy potential of two different concentrations of sodium percarbonate for both a realistic human exposure scenario and an *in vivo* exposure scenario. Reduction of sodium percarbonate concentration predictably reduced the irritancy potential of the end-use formulation. Histology as a third endpoint in the BCOP assay was critical in evaluating the depth and degree of injury.

If you have any questions or comments on this data set, please feel free to contact either Judith Swanson or myself at the following:

Nicole Cuellar
(262) 260-6916
ncuellar@scj.com

Judith Swanson
(262) 260-2688
jeswanso@scj.com

Sincere regards,

Nicole Cuellar

Nicole Cuellar
Sr. Research Toxicologist

POSTER TEXT FOR S.C. JOHNSON SUBMISSION DATED OCTOBER 13, 2004

TITLE

EVALUATING THE IRRITANCY POTENTIAL OF SODIUM PERCARBONATE: A CASE STUDY USING THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

B.P. Gran[1], J.E. Swanson[1], J.C. Merrill[2] and J.W. Harbell[2]
[1]S.C. Johnson & Son, Inc. Racine, WI; [2]Institute for In Vitro Sciences, Inc., Gaithersburg, MD.

ABSTRACT

Sodium percarbonate ($2Na_2CO_3 \bullet 3H_2O_2$) is a component in cleaning products but the neat powder has the potential to be highly irritating to the ocular tissue of rabbits (EPA Category I). This injury results from the chemical's reactivity and dosing method that may trap the powder against the eye. In the BCOP assay, experience has now shown that oxidizing/reactive materials often require a longer post-exposure time to fully manifest cytopathic changes. When testing reactive chemistries, the post-exposure incubation times are increased from 2 hours to 4 and 24 hours. Exposure times of 10, 20, 30 and 60 minutes were used in this study. Sodium percarbonate and percarbonate-based formulations were evaluated as 50% suspensions in water. Abattoir-derived corneas were received, mounted, exposed to test materials, and opacity, permeability and histological endpoints measured as previously reported Curren et al.(2000). Opacity and permeability scores increased with increasing exposure times and concentration of percarbonate. After the 10-minute exposure to percarbonate alone, the 4-hour post-exposure corneas showed focal epithelial layer changes that progressed to a loss of epithelium after 24 hours. Stromal damage included collagen matrix vacuolization and loss of basophilic components in the keratocyte cytoplasm. Exposures of 20 minutes or greater led to rapid destruction of both the epithelial and stromal cells and marked collagen matrix swelling. Tissue lesions declined rapidly with decreasing percarbonate concentration. Thus, the marked ocular damage induced by neat percarbonate in the rabbit, could also be reproduced in the modified BCOP. These data suggest that the modified BCOP assay can be effectively used to evaluate the safety of percarbonate-based formulations and lead to appropriate labeling and packaging decisions.

INTRODUCTION

The sodium percarbonate molecule owes its current popularity in cleaning products to its capability to be a powerful oxygen generator when combined with water. This crystalline solid is a highly reactive molecule that has been shown to be very irritating to rabbit eye tissue. When rabbits were exposed in the standard EPA Guideline eye irritation assay, corneal epithelial peeling, iridial involvement and severe conjunctival irritation occurred. (supplier data)

An initial investigation of the eye irritation potential of sodium percarbonate using the standard BCOP Assay protocol resulted in a relatively benign profile, a strikingly different result from the *in vivo* study.

This case study of sodium percarbonate presents an effort to understand how a non-animal assay, the BCOP Assay, can be utilized to realistically predict human eye irritancy potential of reactive molecules. The BCOP Assay was chosen for this work as it allows exact control over the exposure times and provides several measures of tissue damage. Exposure times were chosen to encompass the range of effective exposures that might occur in the rabbit. This enabled us to identify the exposure time in the BCOP Assay that resulted in a comparable level of injury to that seen in the rabbit study. Corneal injury was evaluated using the standard BCOP endpoints, opacity and permeability, as well as histological examination.

MATERIALS AND METHODS Error! Bookmark not defined.

Bovine Eyes

The BCOP assay was performed following the methods of Sina et al. (1995). Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals. The eyes were grossly examined for damage and those exhibiting defects were discarded. The corneas were excised such that a 2 to 3 mm rim of sclera was present around the cornea. The corneas were mounted in the holders and the two chambers filled with Minimum Essential Medium Eagle (MEM) without phenol red, supplemented with 1% fetal bovine serum (complete MEM). The corneal holders were incubated at 32 ± 1ºC for a minimum of 1 hour.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the medium replaced in both chambers and the opacity was determined for each cornea using a Spectro Designs OP-KIT opacitometer. Three corneas, whose opacity readings were close to the median opacity for all the corneas, were selected as the negative control corneas. The medium was then removed from the anterior chamber and replaced with the test article, positive control, or negative control.

Method for Testing Liquid or Surfactant Materials

The test articles were tested as 50% (w/w) slurry suspension in sterile, deionized water. An aliquot of 750µl of the test article, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. A total of three corneas per treatment group were incubated in the presence of each test article at 32 ± 1ºC for 10, 20, or 30 minutes with a post-exposure incubation period of 4, 20, or 24 hours. The negative control was tested, in groups of 3 corneas each, to match the short and long post-exposure incubation periods. The positive control was tested in three corneas at 32 ± 1ºC for 10 minutes with a post-exposure incubation period of two hours. After the test or control article exposure, the epithelial side of the corneas was washed at least three times with complete MEM to ensure total removal of the test or control articles. The anterior chamber was refilled with fresh complete MEM and an opacity measurement was performed. After the post-exposure incubation period, a second measure of opacity was obtained. The corneas designated for the post-exposure incubation periods of 2 or 4 hours did not require refeeding with fresh medium prior to the second measure of opacity. The corneas designated for the over night post-exposure incubation periods were refed with fresh medium approximately every 6 hours and immediately prior to the second measure of opacity.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was refilled with complete MEM, and 1 ml of a 4 mg/ml fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1ºC. After the incubation, an aliquot of 360 µl from each chamber was placed into the designated well on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader.

Opacity Measurement: The change in opacity for each cornea was calculated by subtracting the pre-treatment opacity readings from the final opacity readings. The corrected opacity value of each cornea was calculated by subtracting the average change in opacity of the time-matched negative control corneas from that of each treated cornea. The mean opacity values of each treatment group were then calculated.

Permeability Measurement: The corrected OD_{490} was calculated by subtracting the mean OD_{490} of the time-matched negative control corneas from the OD_{490} value of each treated cornea. The mean OD_{490} values of each treatment group were then calculated.

Histology

The corneas were placed in individual, prelabelled cassettes and fixed for at least 24 hours in 10% buffered formalin. The fixed corneas were transferred to Pathology Associates - A Charles River Company (Frederick, MD) for embedding, sectioning and staining. Each slide was then stained with hematoxylin and eosin. Slides were returned to the Institute for In Vitro Sciences, Inc. for evaluation. Cornea sections were examined for the presence of changes in the epithelial, stromal, and endothelial areas of the tissue. Treated tissues were compared to concurrent negative and positive control tissues. Photomicrographs and thickness measurements were prepared using a Spot Insight (Spot Diagnostic Instruments) digital camera and associated software.

Primary Eye Irritation Study of FB Sodium Percarbonate in Rabbits (1982 EPA Guidelines 81-4)

The primary eye irritation study of sodium percarbonate in six albino rabbits [Hra: (NZW)SPF] was conducted in 1989 according to the 1982 EPA Guidelines for Acute Eye Irritation (81-4). The study was also in accordance with GLP standards of 1983 since the in-life portion of the study was completed before the effective date of the revised standards (9/18/89). A bulk density determination was made to determine the weight equivalent of a 100 µl dose. Due to irritation observed in the acute dermal study, anesthetic was applied to the eyes five minutes prior to dosing. The weight equivalent of 100 µl was placed in the conjunctival sac of rabbit and the eyelids were gently held together for one second. The contralateral eye served as the untreated control. Observations for ocular irritation were made at 1, 24, 48, 72 and 96 hours after treatment. The study was terminated at 96 hours after consultation with Sponsor due to severity of irritation observed. Acute irritation seemed to peak at 48 hours after instillation. (Study information provided by supplier under confidentiality agreement.)

RESULTS

The BCOP Assay was chosen as a non-whole animal tool for evaluating the potential eye irritancy of sodium percarbonate because it allowed exact control over exposure and observation times, and provided several measures of tissue damage. All assays were performed using a 50% slurry of the percarbonate salt in water to model a concentrated solution of the powder when tearing occurs following accidental exposure.

Exposure times were chosen to encompass the range of what might be a realistic worst possible case in accidental human exposure to an exposure time that would approximate the level of injury found in the *in vivo* study. Corneal injury was evaluated initially using opacity and permeability endpoints, the standard BCOP Assay measures of irritation. Since the full manifestation of oxidative damage to cells may be delayed for some hours after exposure with some materials, several post-exposure periods were selected to compare the manifestation of damage over time. Table 1 shows the impact of exposure times and post-exposure observations on the quantitative BCOP endpoints and the effects of reducing the concentration of sodium percarbonate in formulation at two exposure times..

Table 1. BCOP Opacity and Permeability Scores from Sodium Percarbonate Exposure: Impact of Exposure and Post-Exposure Time

Test Material	Exposure Time	Post-Exposure Incubation Time	Opacity	Permeability	In Vitro Score
1. Sodium Percarbonate (500 mg/ml suspension) pH 10.5	10 minutes	4 hours	8.3	0.123	10.2
	30 minutes	4 hours	14.0	2.598	53.0
	60 minutes	4 hours	19.8	4.344	85.0
	10 minutes	24 hours	16.0	0.636	25.5
	30 minutes	24 hours	27.7	1.392	48.5
	60 minutes	24 hours	27.3	1.333	47.3
2. Sodium Percarbonate (500 mg/ml suspension)	10 minutes	20 hours	11.0	0.025	11.4
	20 minutes	20 hours	14.0	2.810	56.1
3. Sodium Percarbonate* (300 mg/ml suspension)	10 minutes	20 hours	6.0	0.015	6.2
	20 minutes	20 hours	13.3	0.366	18.8

*Formulation with 60% sodium percarbonate

Additionally, the corneas were sectioned, stained and examined microscopically for depth of injury and histological markers of irritancy for this oxidative material. Table 2 summarizes morphological changes seen in the corneas for different exposure times at both short-and long-term post-exposure times.

Table 2. Morphological Changes is Corneas Treated with Sodium Percarbonate: Impact of Exposure and Post-Exposure Time

Test Article Exposure time	Post Exposure time 4 hours			Post Exposure time 20 to 24		
	Epithelium	Stromal Collagen	Keratocytes	Epithelium	Stromal Collagen	Keratocytes
10 min	Surface cells lost but deeper layers remained. Marked focal lesions observed	Increased stromal thickness and moderate CMV* to 40% depth	Moderate increase in cytoplasmic eosinophilia to 40% depth	Surface cells lost and upper wing cells were pyknotic. Deeper cells lost in some fields	Increased stromal thickness and moderate CMV >50% depth	Marked cytoplasmic eosinophilia to 40% depth
20 min**				Epithelium completely lost	Severe CMV throughout the stroma	Few viable cells remained
30 min	Surface cells lost, remaining cells in place but damaged	Marked increase in stromal thickness and CMV past 50% depth	Marked nuclear pyknosis and cytoplasmic eosinophilia – full depth	Epithelium completely lost	Severe CMV throughout the stroma	Few if any viable cells remained
60 min	Epithelium present but not viable	Marked increase in stromal thickness, gas pockets visible	Marked nuclear pyknosis and cytoplasmic eosinophilia – full depth	Epithelium completely lost	Severe CMV throughout the stroma	Few if any viable cells remained

* CMV = Collagen Matrix Vacuolization
** 20-hour post-exposure time

See attached FIGURES for specific histology slides.

The following Figures illustrate the qualitative changes in corneal tissue that are summarized in Table 2.

- Figures 1 and 2, A, B, & C show normal untreated corneal tissue to afford a basis for comparison with the tissues that have been exposed to percarbonate slurries.
- Figures 3-7, A, B & C show injury to corneal structures at different time periods.
- Figures 8, A, B & C shows the effects of a reduced concentration end-use formulation compared to full-strength percarbonate in Figures 4, A, B & C.

DISCUSSION

- With reactive molecules like sodium percarbonate, reliance on the traditional 2-hour post exposure incubation in the BCOP assay can be misleading. The delayed manifestation of toxicity requires an increased post-exposure incubation time (see Table 1).
- The opacity and permeability endpoints may underestimate the toxicity where the epithelium remains physically intact. The focal lesions do not lead to an appreciable increase in permeability scores (see for example Table 1, 10-minute exposures).
- The pattern of lesions in the corneal epithelium suggests that focal lesions develop which breach the epithelial barrier and allow subsequent penetration into the stroma. This pattern of damage is different from what is observed with exposure to surfactants or solvents where the lesions tend to be more uniformly progressive across the epithelial surface of the cornea.
- Loss of the corneal epithelium leads to extensive fluorescein permeability while the corneal stroma is in the process of swelling (Table 1, 30- and 60-minute exposures at 4 hours post-exposure). However, once the corneal stroma has swollen, the relative fluorescein permeability decreases (Table 1, 30- and 60-minute exposures at 24 hours post-exposure). Note the stromal thickness in Figures 5C and 6C.
- The degree and depth of injury to the stromal keratocytes has been shown to be predictive of the degree and duration of ocular injury in vivo (Maurer et al., 2002). Histological evaluation of the bovine corneas, treated in vitro, provides data on keratocyte damage. This damage may not be fully reflected in the opacity and permeability measurements.
- The Draize Test protocol leads to an overestimation of the irritancy of powders. The effects resulting from the Draize methodology greatly exceed what could realistically be expected from accidental human exposure.. The differences in exposure include: the quantity and location of material instilled, the occlusion and pressure of the crystalline material against the cornea, mechanical abrasion and a different tearing response. (see Bruner's discussion of ocular irritation in Frazier's In Vitro Toxicity Testing, pp.160-161, Wilkie and Wyman's chapter in Hobson's Dermal and Ocular Toxicology, p.487. and Maurer et al., 2002)

CONCLUSION

- Testing reactive molecules, such as sodium percarbonate, requires a modification of the BCOP protocol to fully evaluate the potential for delayed effects on corneal tissue.
- Because important changes may come at the cellular rather than tissue level (see Jester et al. [1998] and Maurer et al. [2001]), histology evaluation is critical as a third end-point in the BCOP Assay for this type of molecule. These combined endpoints allow for the determination of depth and degree of injury that is required to predict irritation potential (see Maurer et al [2002]).
- In the BCOP assay, exposures of greater than 10 minutes to a 50% suspension of sodium percarbonate are required to achieve tissue damage consistent with the damage reported for the rabbit eyes in the Draize test. These data suggest that trapping of the powder against the cornea in the conjunctival sac may appreciably impact its toxicity in the rabbit.
- Reduction of sodium percarbonate concentration greatly reduced the irritancy potential of the test formulation, even in the more exaggerated 20-minute exposure.

REFERENCES

Curren, R., Evans, M., Raabe, H., Ruppalt, R., Harbell, J. (2000) Correlation of histopathology, opacity, and permeability of bovine corneas exposed in vitro to known ocular irritants. Veterinary Pathology 37(5):557.

Bruner, Leon H. 1992. Ocular Irritation. In *In Vitro Toxicity Testing*, ed. John M. Frazier, 149-190. New York, Marcel Dekker.

Wilkie, David A. and Wyman, Milton. 1991. Comparative Anatomy and Physiology of the Mammalian Eye. In *Dermal and Ocular Toxicology*, ed. David W. Hobson, 433-492. Florida, CRC Press.

Jester, J.V., Li, H.F., Petroll, W.M., Parker, R.D., Cavanaugh, H.D., Carr, G.J., Smith, B., and Maurer, J.K. (1998) Area and depth of surfactant-induced corneal injury correlates with cell death. Invest Ophthalmol Vis Sci 39:922-936.

Maurer, J.K., Molai, A., Parker, R.D., Li, L., Carr, G., Petroll, W.M., Cavanagh, H.D., and Jester, J.V. (2001) Pathology of ocular irritation with bleaching agents in the rabbit low-valume eye test. Toxicological Pathology 29(3):308-319.

Maurer, J.K., Parker, R.D., and Jester, J.V., (2002) Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. Regulatory Toxicology and Pharmacology 36(1):106-117.

Rees, W.M., Swanson, J.E., Burdick, J.D., Hilgers, D.S., and Harbell, J.W. (2001) Evaluating toxic synergism in hypochlorite-containing solutions using the bovine corneal opacity and permeability (BCOP) assay. ABS# 473, The Toxicologist 60:99.

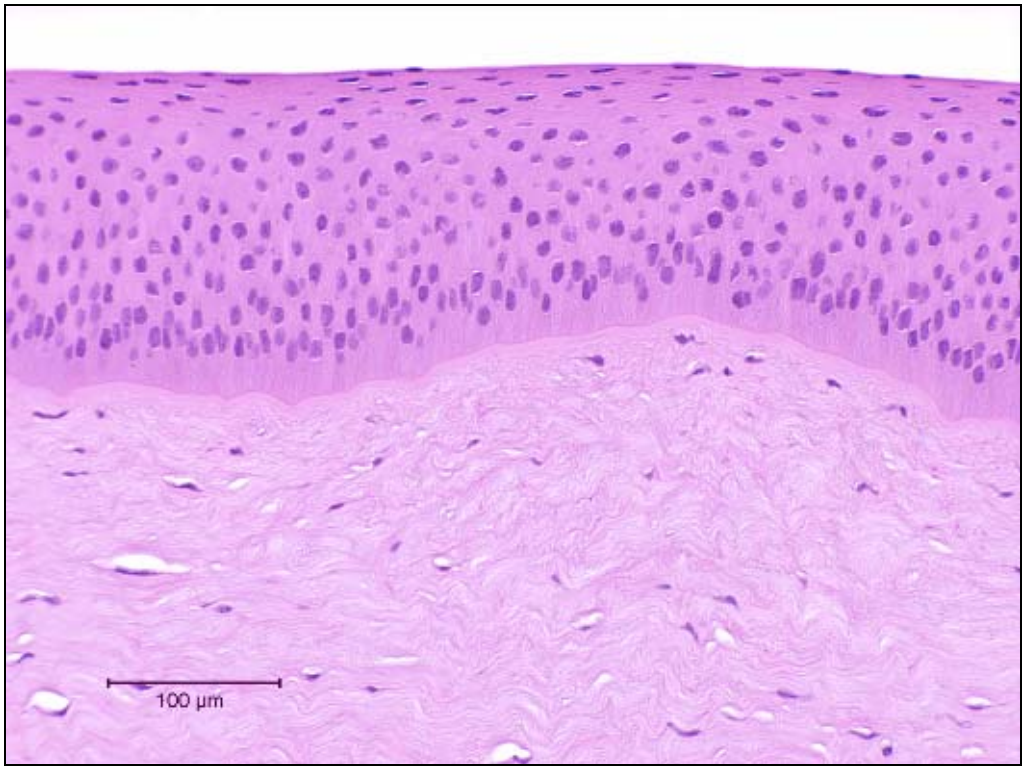
Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. Fundamental and Applied Toxicology 26:20-31.

Swanson, J.E. and Harbell, J.W. (2000) Evaluating the eye irritancy potential of ethanolic test materials with the bovine corneal opacity and permeability assay. The Toxicologist 54(1):188-189.

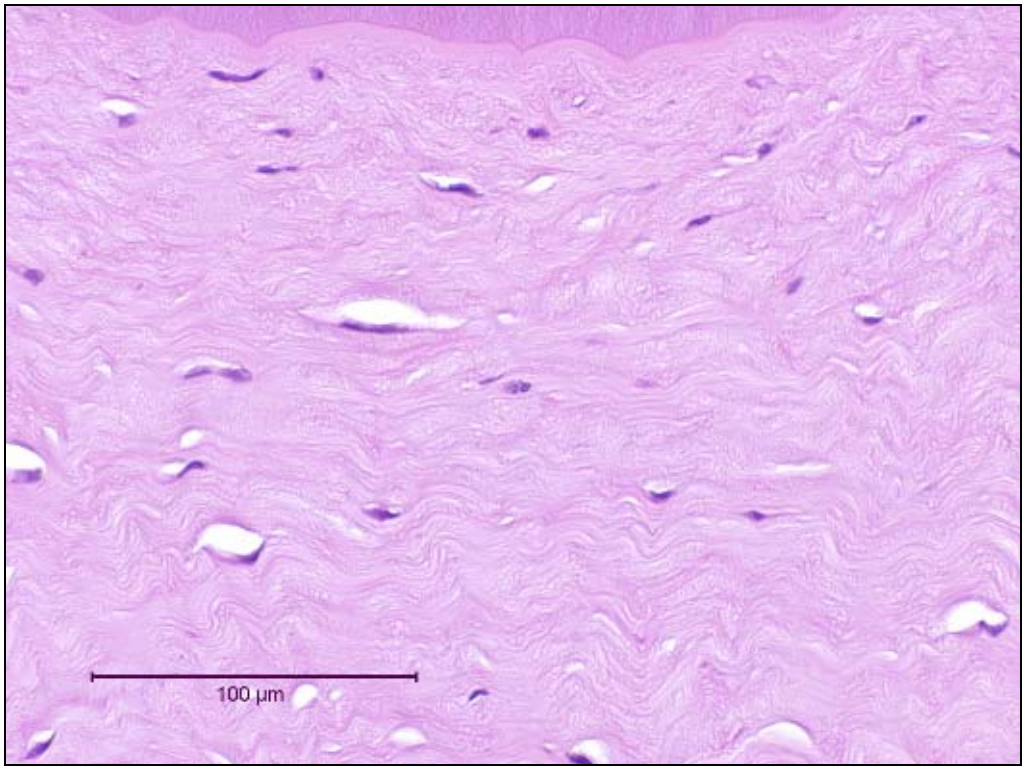
J.E. Swanson, B.T. White, B.P. Gran, J.C. Merrill and J.W. Harbell; 42nd Annual SOT, Poster #1068.

Proposed figure legends (1066)

Figure 1. Negative Control, 4-hour post-exposure
(A) Epithelium (magnification 230x)



(B) Stroma directly below the Bowman's Layer (magnification 430x)



(C) Full thickness (magnification 45x)

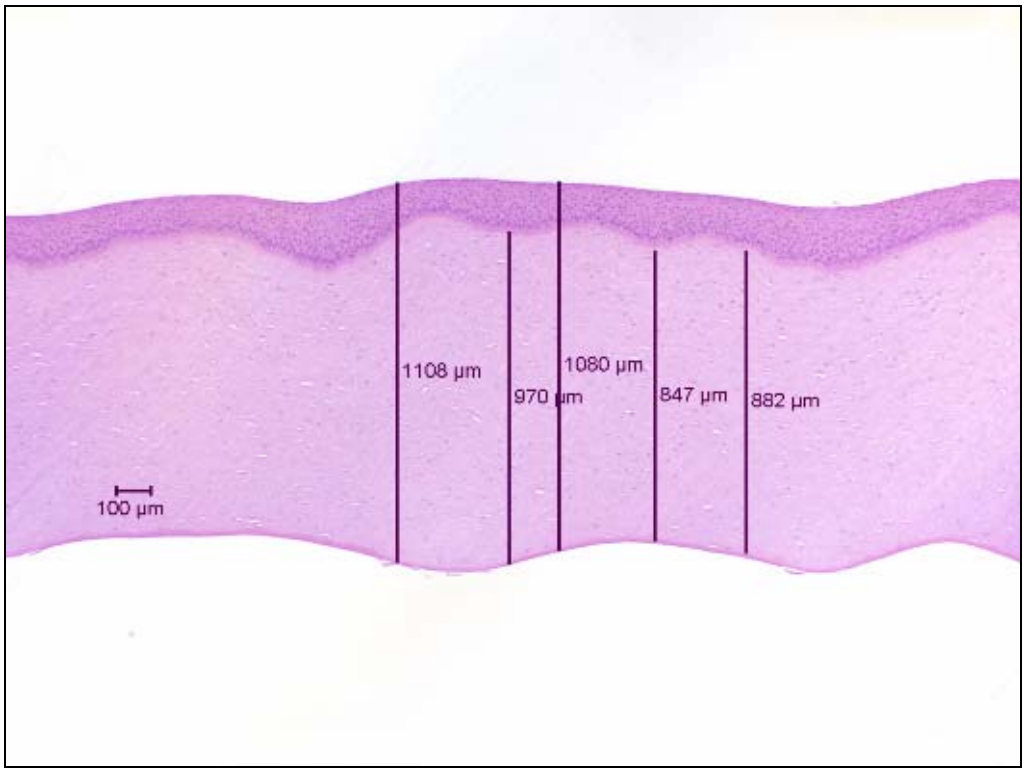
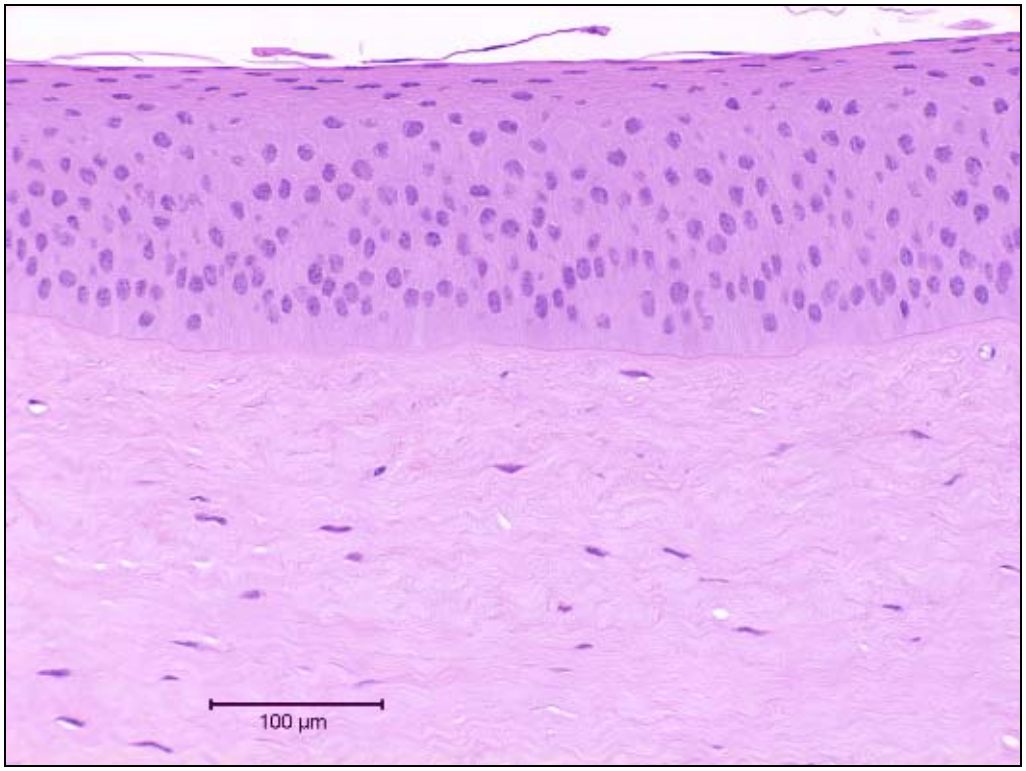
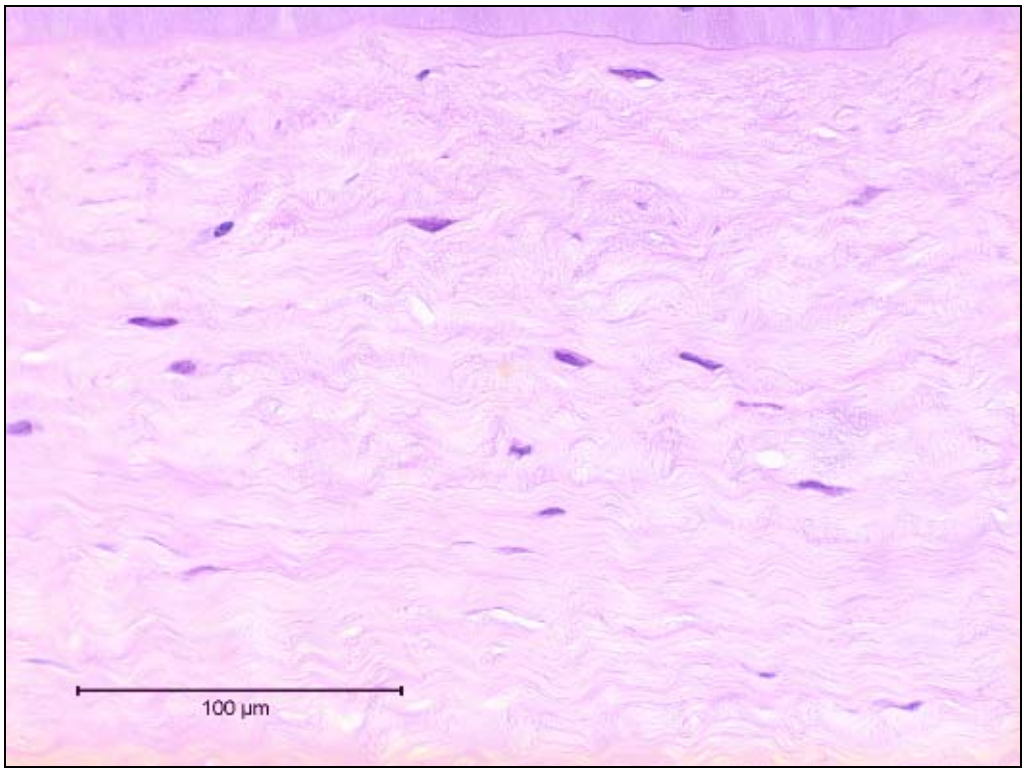


Figure 2. Negative Control, 20-hour post-exposure
(A) Epithelium (magnification 230x)



(B) Stroma directly below the Bowman's Layer (magnification 430x)



(C) Full thickness (magnification 45x)

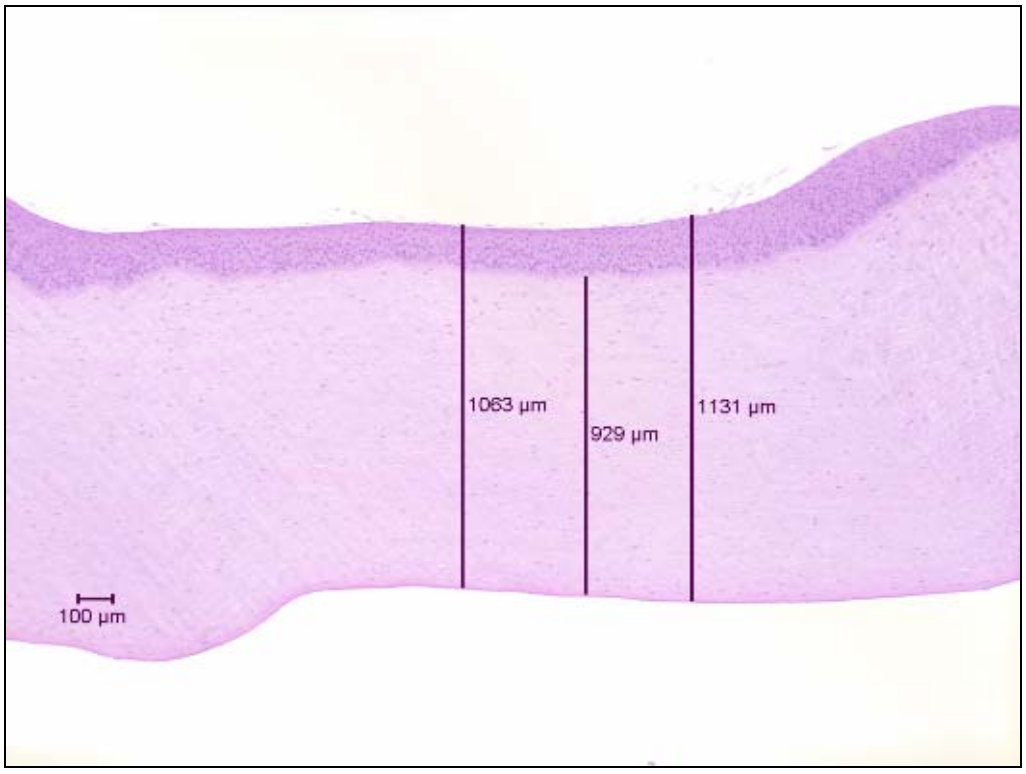
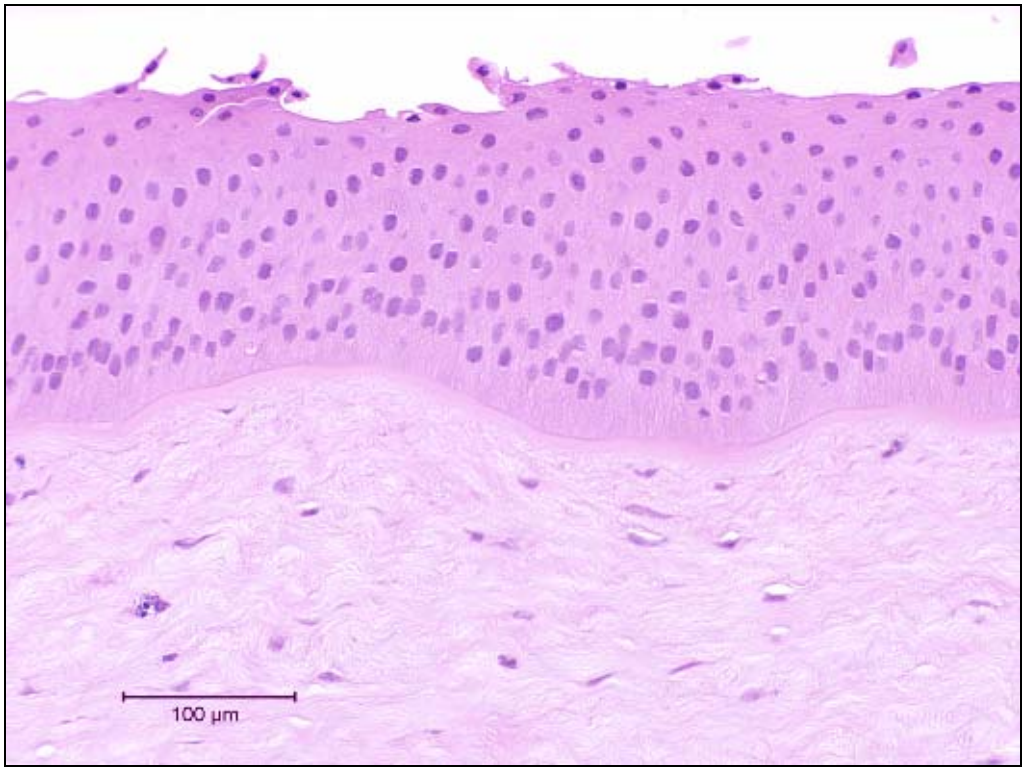
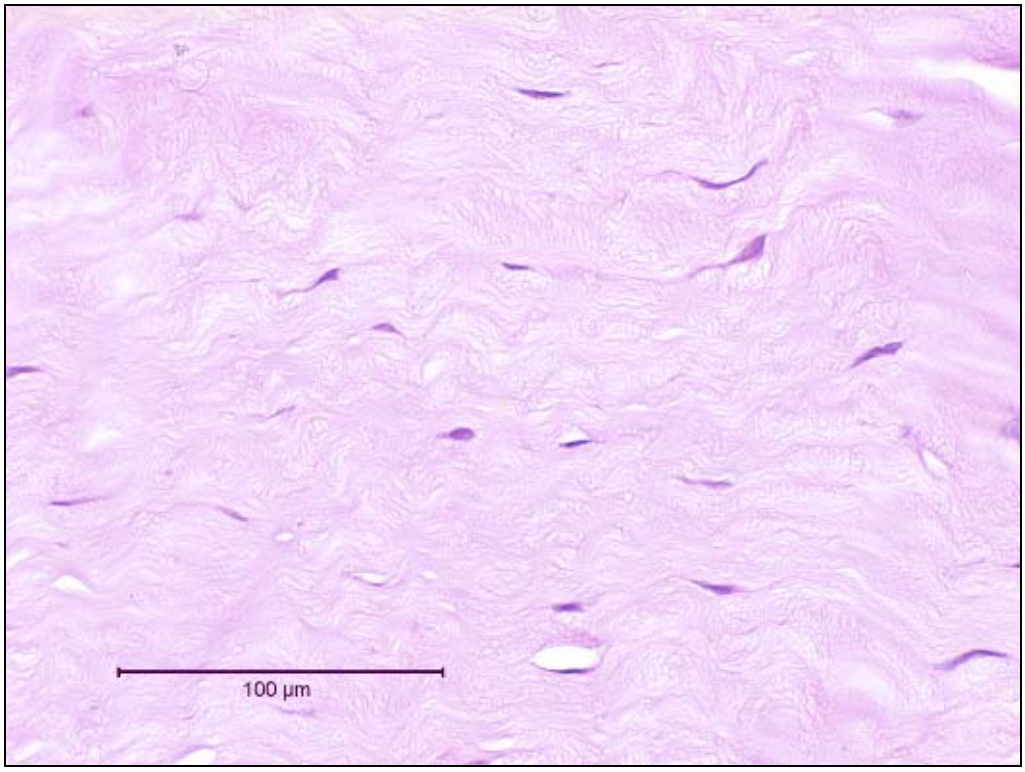


Figure 3. Sodium Percarbonate 50% (w/v) suspension, 10-minute exposure, 4-hour post-exposure
(A) Epithelium (magnification 230x)



(B) Stroma at mid-depth (magnification 230x)



(C) Full thickness (magnification 45x)

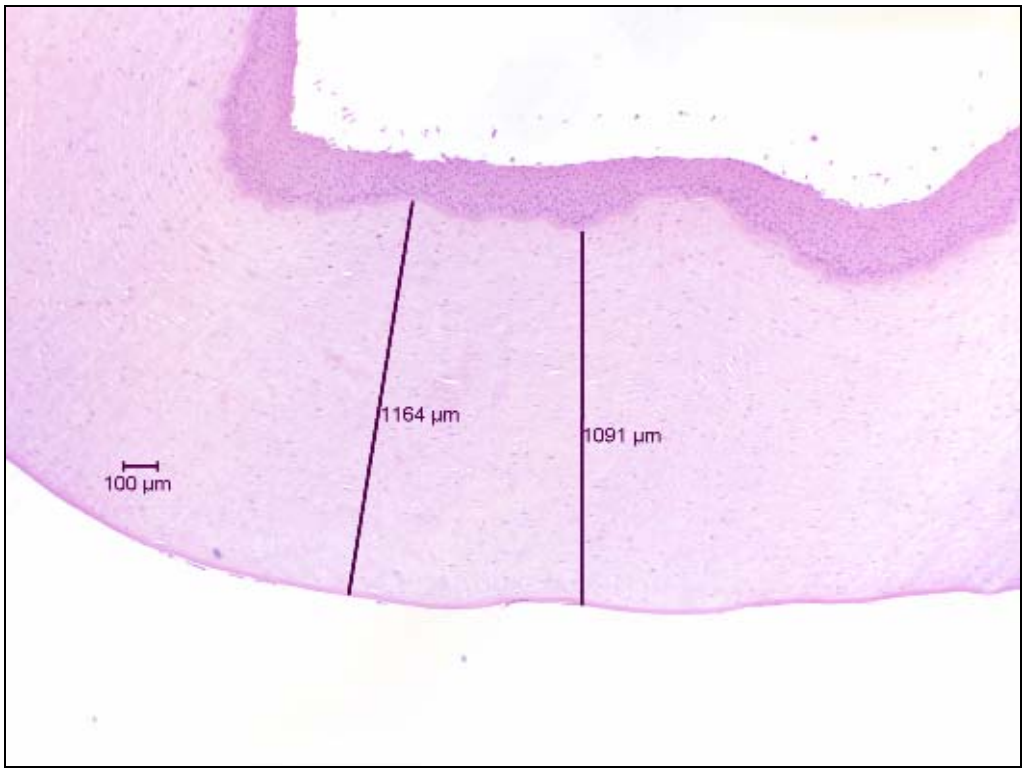
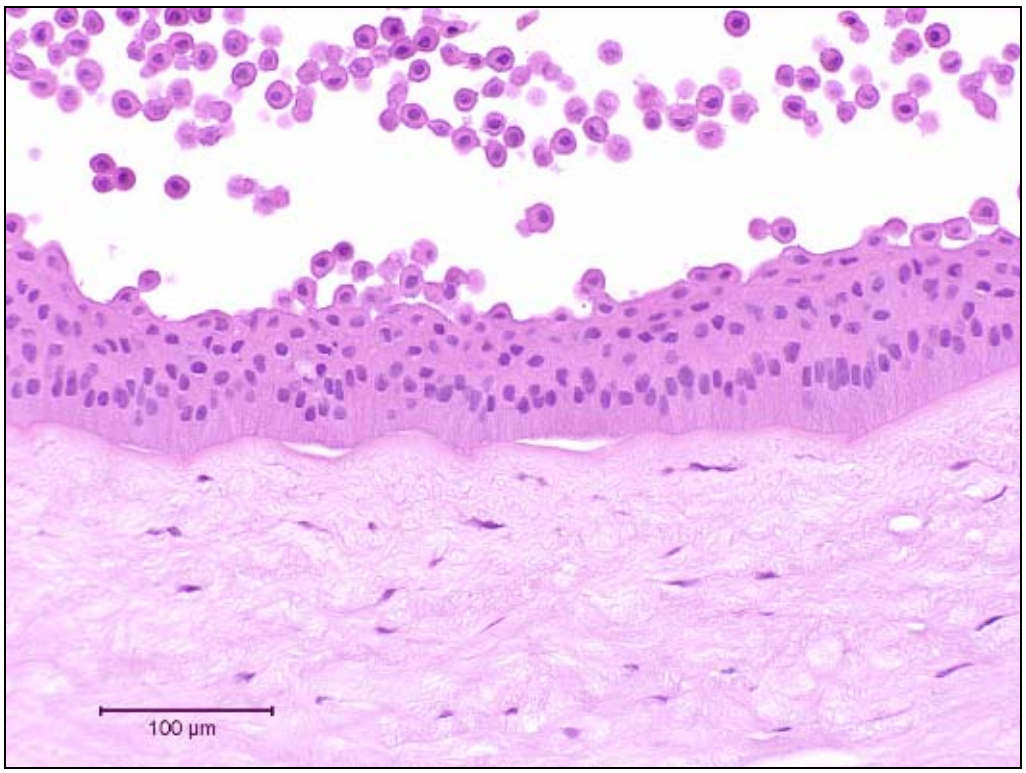
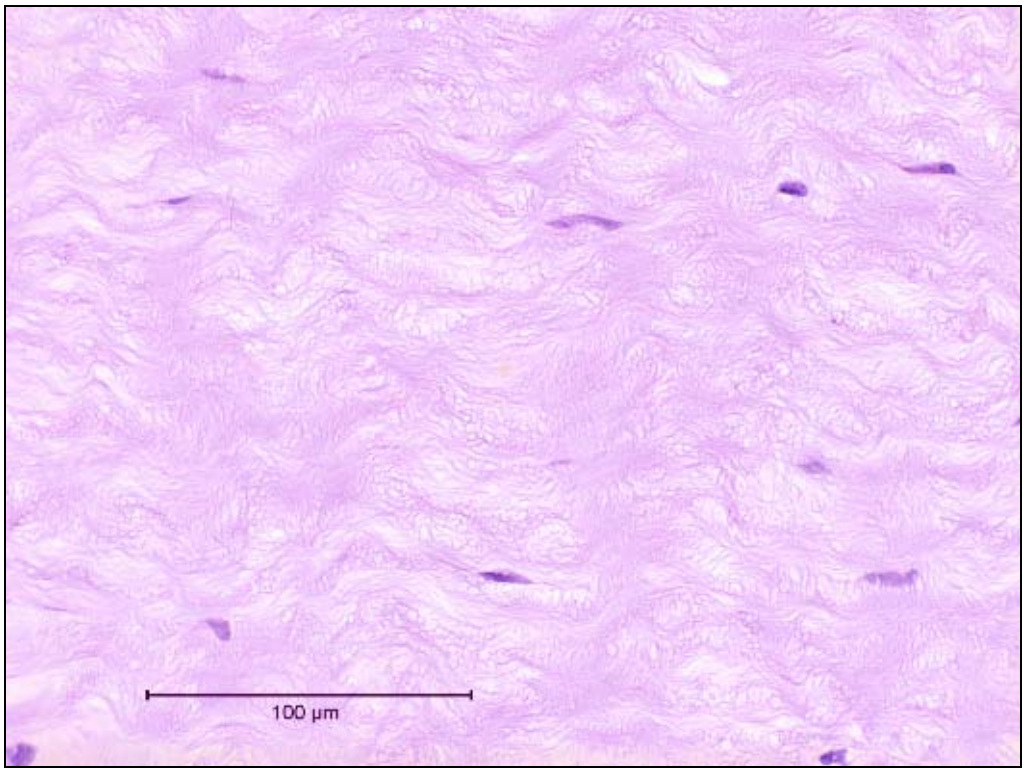


Figure 4. Sodium Percarbonate 50% (w/v) suspension, 10-minute exposure, 24-hour post-exposure
(A) Epithelium showing marked cell loss (magnification 230x)



(B) Stroma at mid depth showing increased collagen matrix vacuolization (magnification 230x)



(C) Full thickness (magnification 45x)

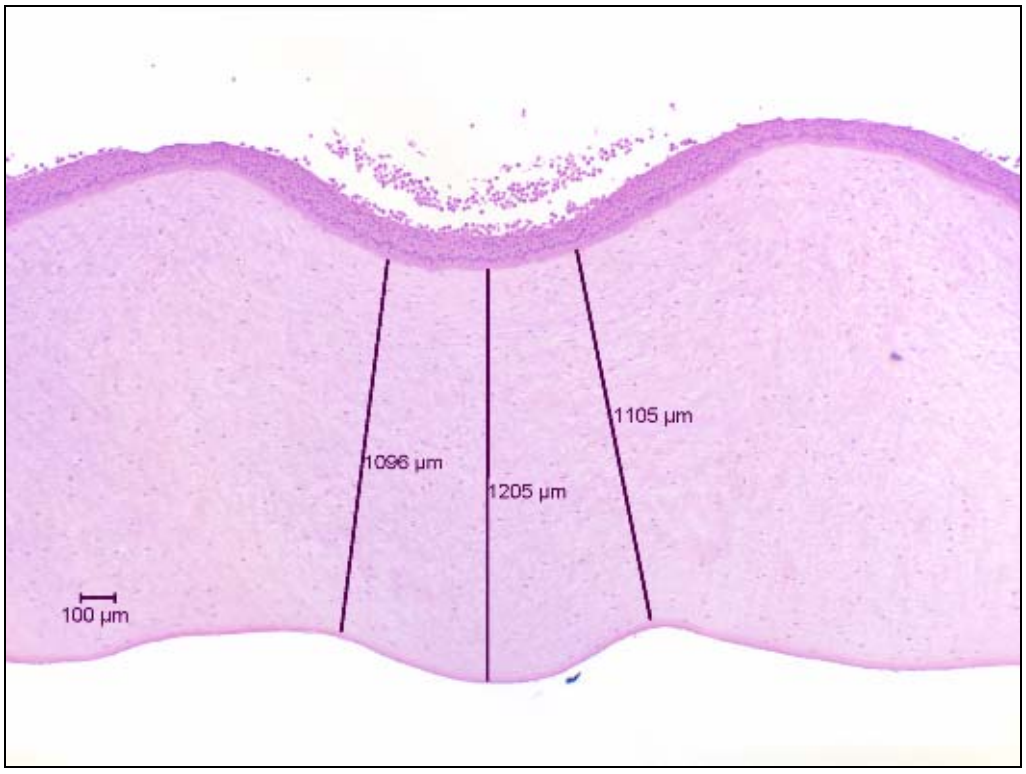
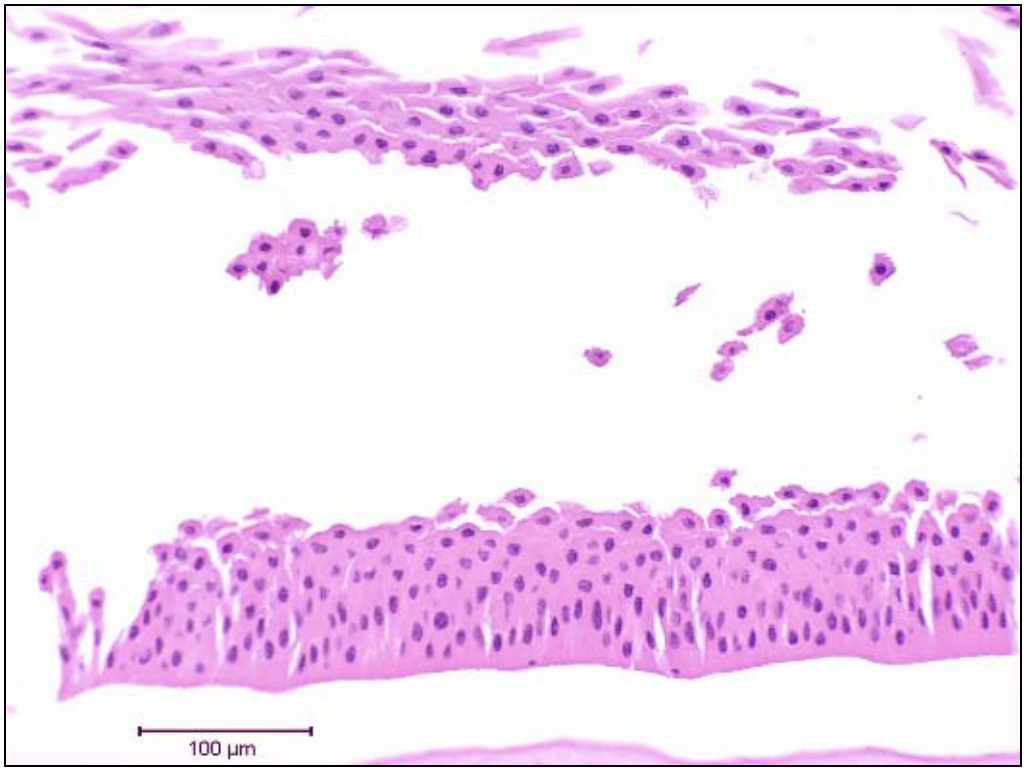
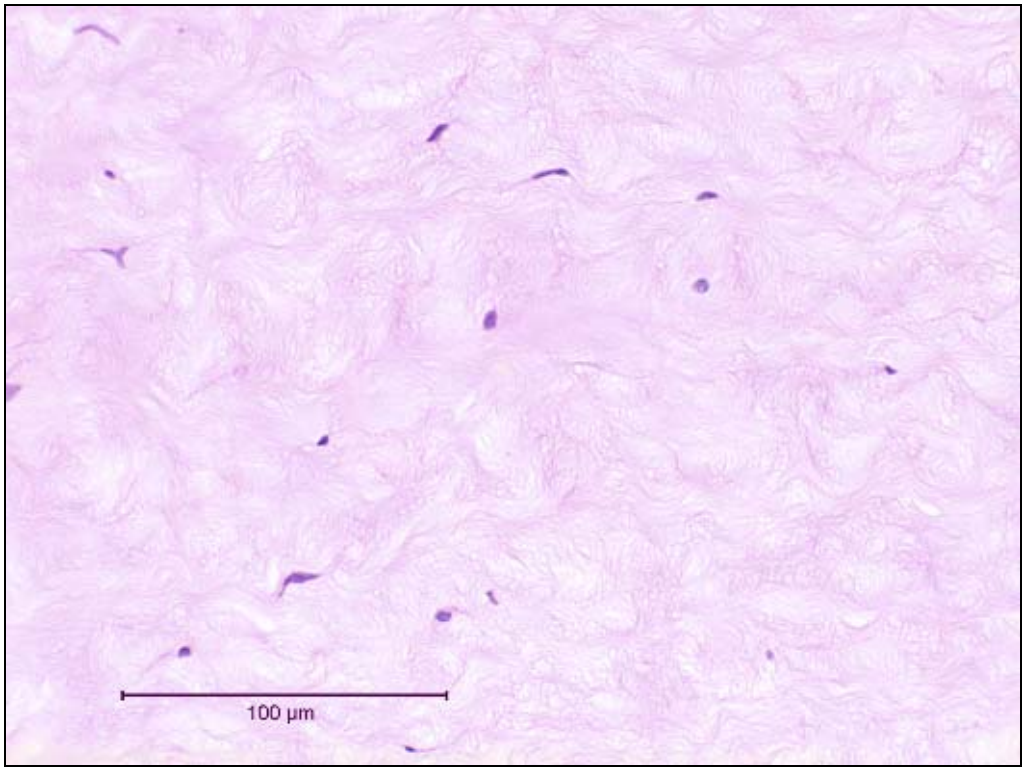


Figure 5. Sodium Percarbonate, 50% (w/v) suspension, 30-minute exposure, 4-hour post-exposure
(A) Epithelium separated from the basal lamina (magnification 230x)



(B) Stroma at mid depth showing marked nuclear pyknosis (magnification 430x)



(C) Full thickness (magnification 45x)

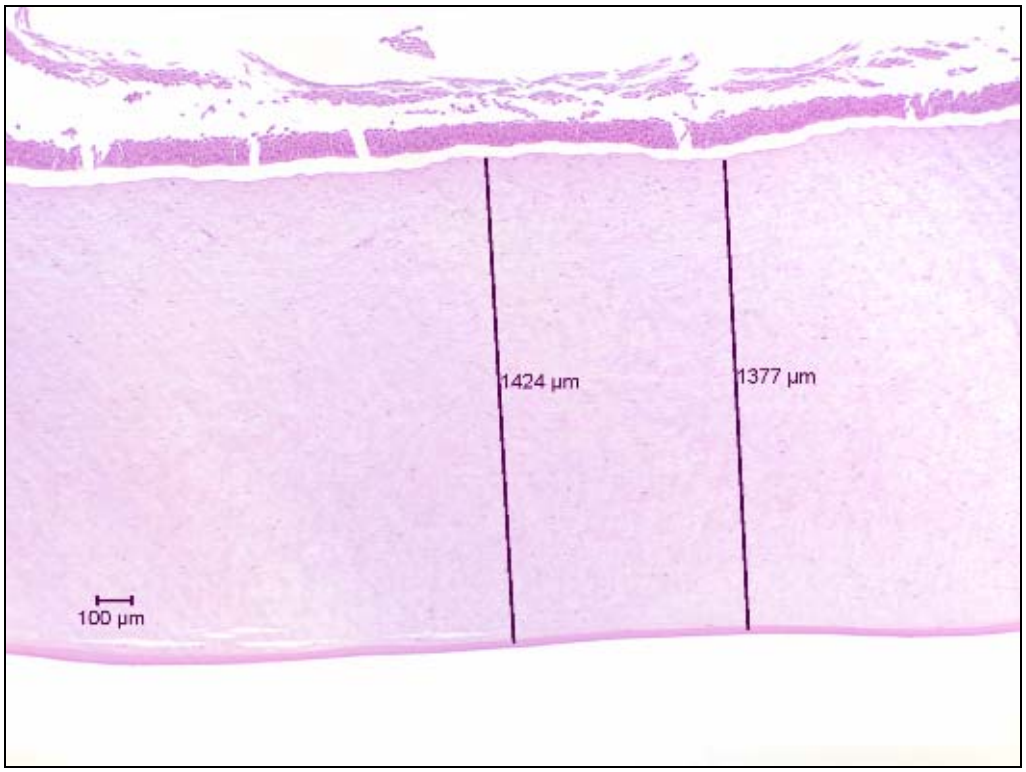
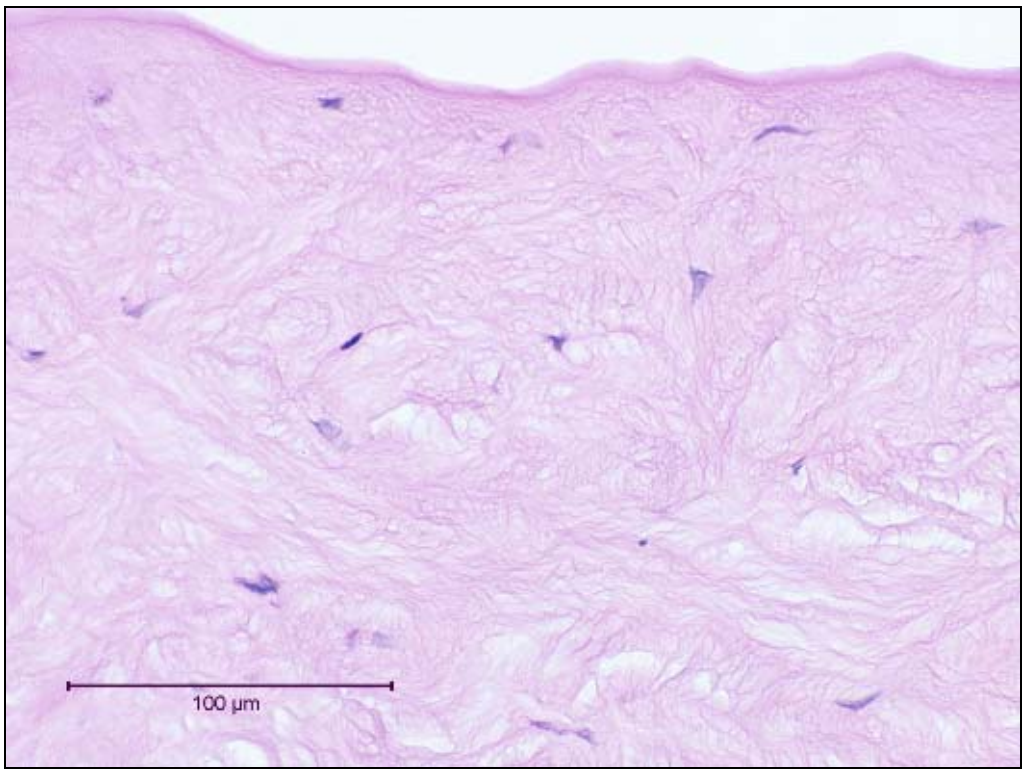
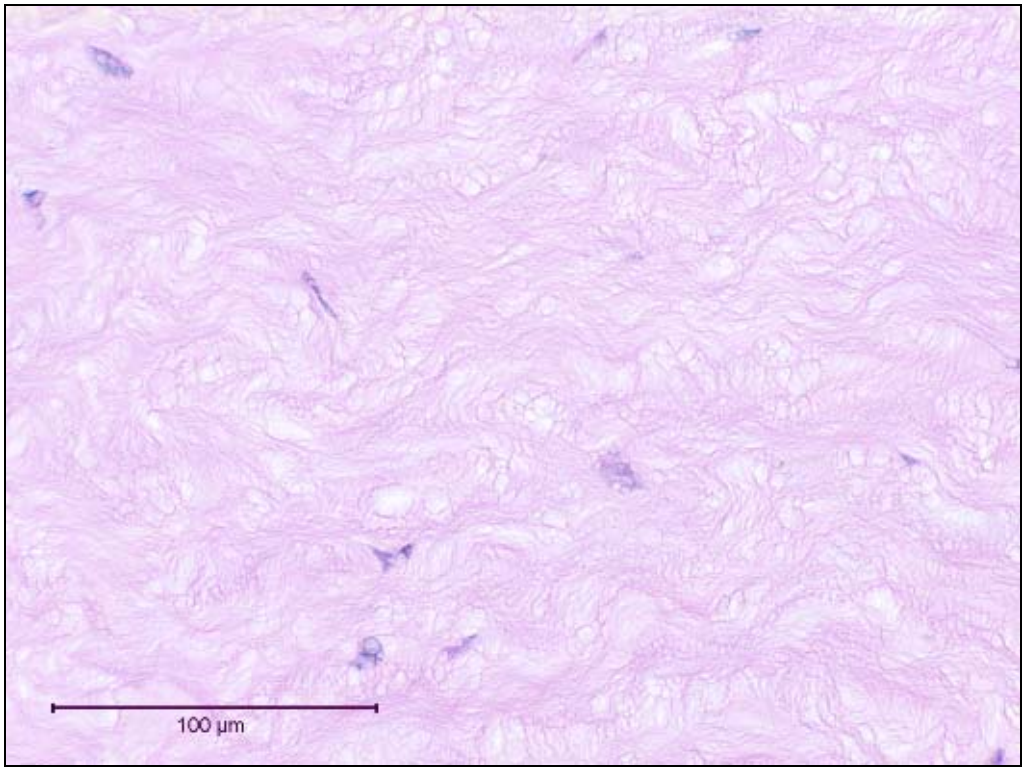


Figure 6. Sodium Percarbonate, 50% (w/v) suspension, 30-minute exposure, 24-hour post-exposure
(A) Epithelium (lost) (magnification 430x)



(B) Stroma at mid depth showing marked collagen matrix vacuolization and dead keratocytes (magnification 430x)



(C) Full thickness (magnification 45x)

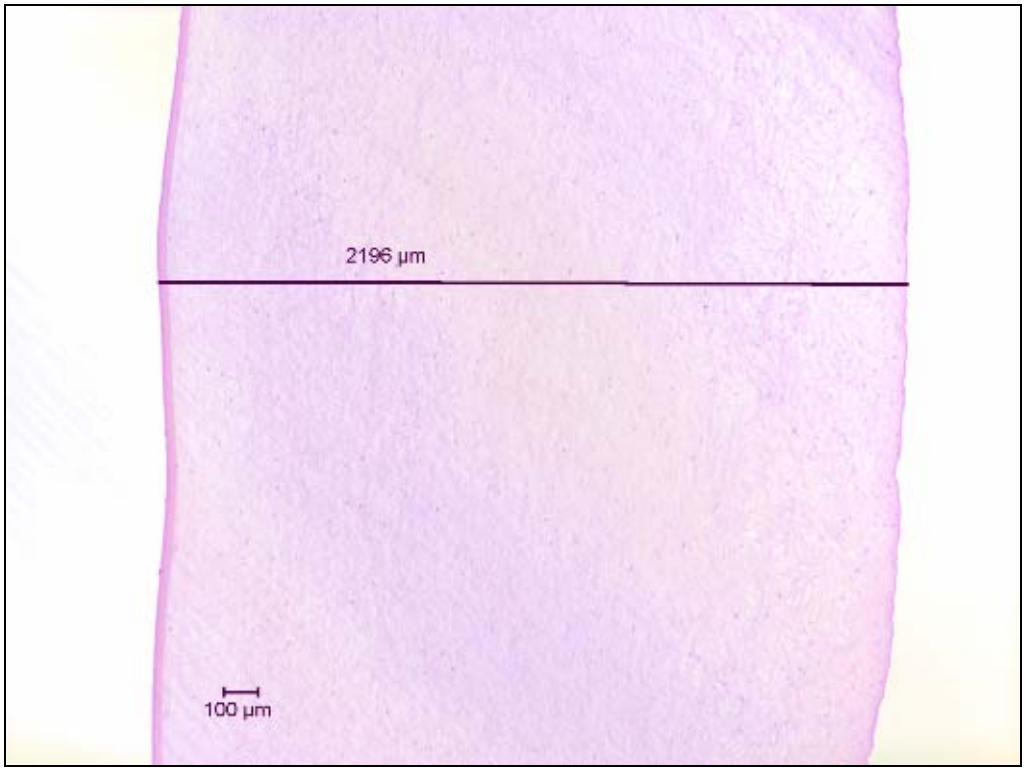
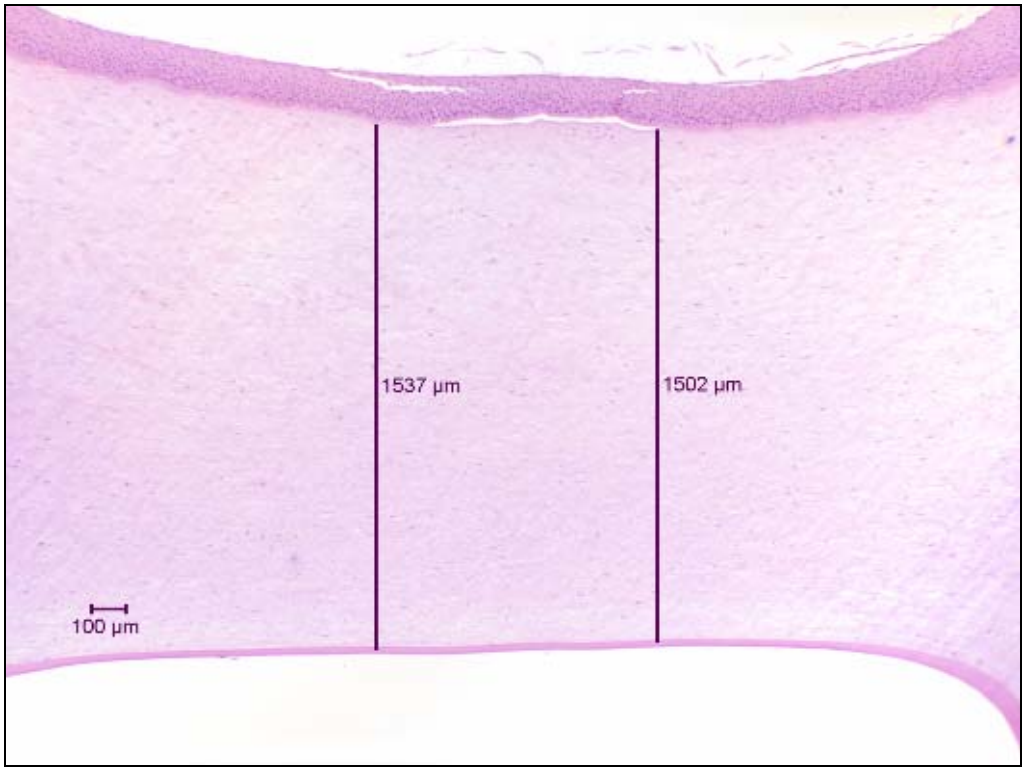


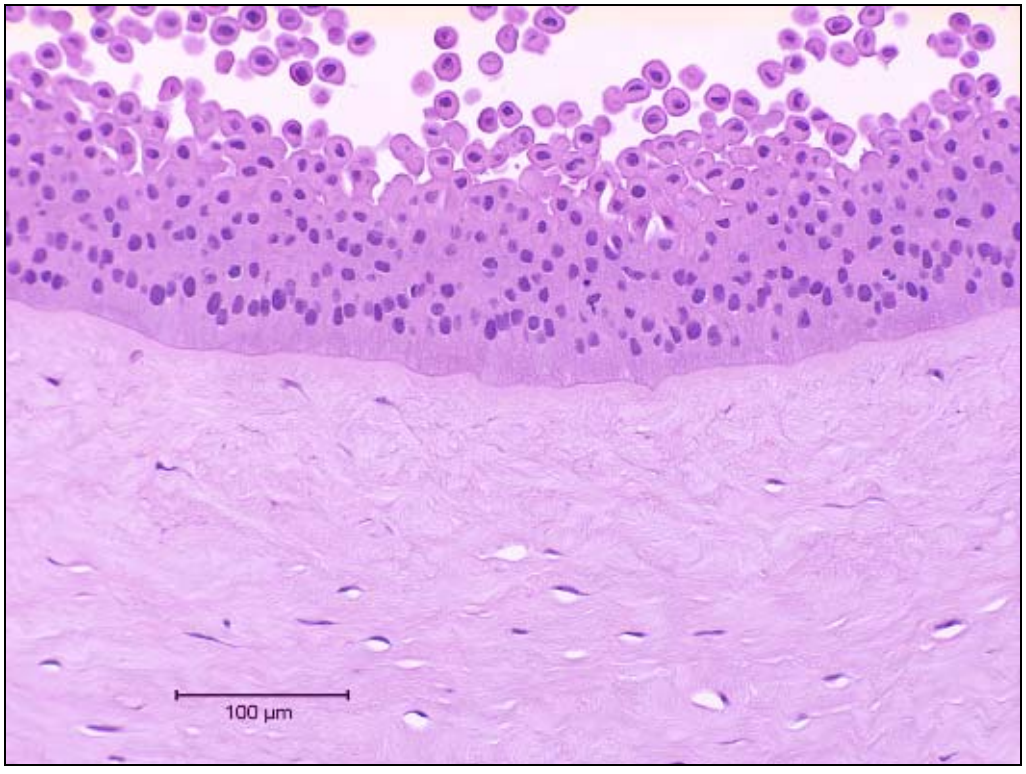
Figure 7. Sodium Percarbonate, 50% (w/v) suspension, 60-minute exposure, 4-hour post-exposure
(A) Epithelium (nonviable) (magnification 430x)

(C) Full thickness (magnification 45x)

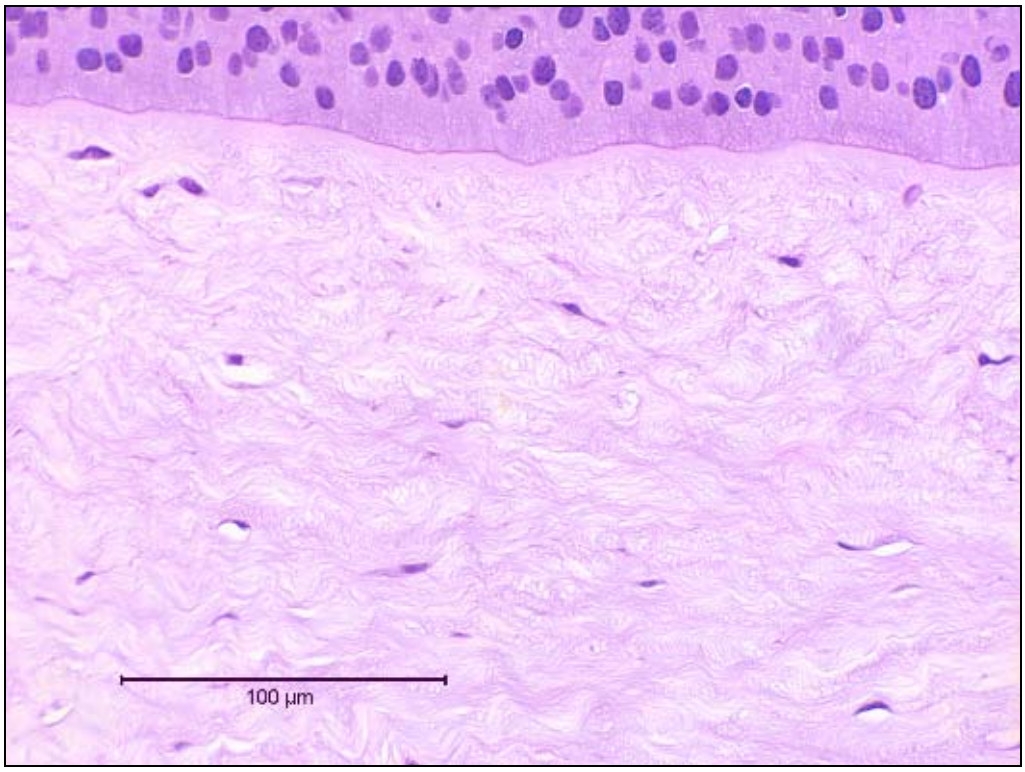


(C) Full thickness showing collagen delamination (magnification 45x)

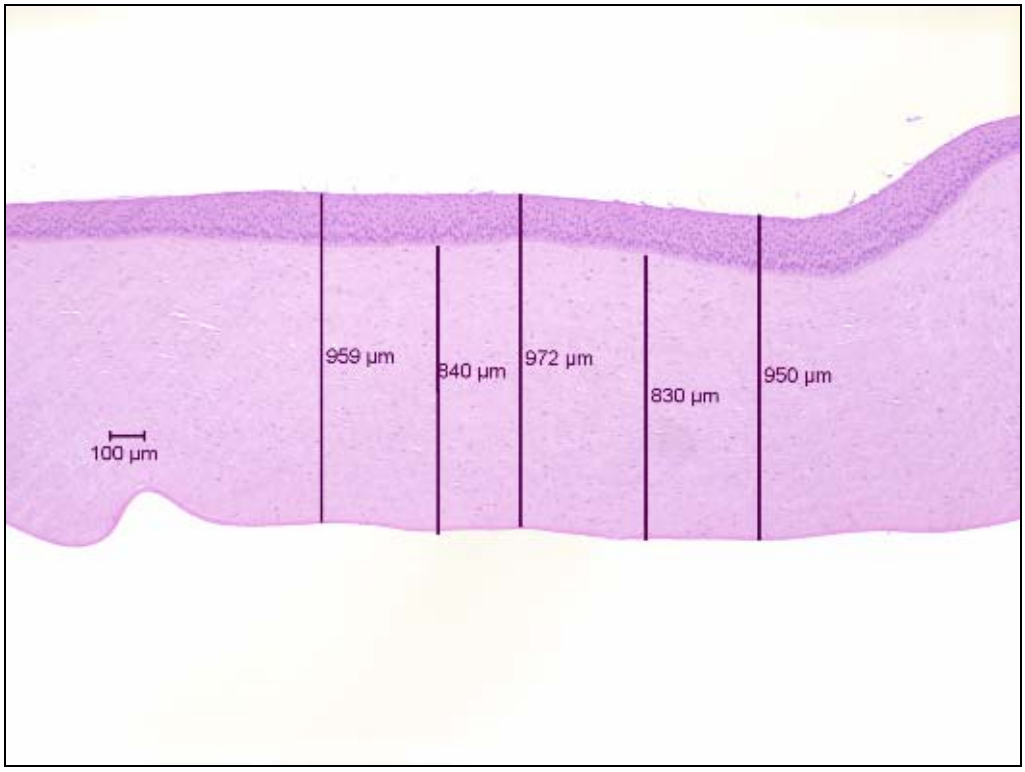
Figure 8. Sodium Percarbonate (60% in formulation), 50% (w/v) suspension, 10-minute exposure, 20-hour post-exposure
(A) Epithelium (magnification 230x)



(B) Stroma directly below Bowman's Layer showing the slight increase in collagen matrix vacuolization (magnification 430x)



(C) Full thickness (magnification 45x)



FORMULAS

Test Material #	Group	Raw Material	Percentage
1	Sodium Percarbonate (CAS #15630-89-4)	Reactive Chemical Mixture	40-45
	Sodium carbonate (CAS #497-19-8	Reactive Chemical Mixture	5-10
		Water	45-50
2	Sodium Percarbonate (CAS #15630-89-4)	Reactive Chemical Mixture	40-45
	Sodium carbonate (CAS #497-19-8	Reactive Chemical Mixture	5-10
		Water	45-50
3	Sodium Percarbonate (CAS #15630-89-4)	Reactive Chemical Mixture	25-30
	Sodium carbonate (CAS #497-19-8	Reactive Chemical Mixture	20-25
		Water	45-50

[This Page Intentionally Left Blank]

Appendix G4

Dataset Received from L'OREAL Advanced Research for an In-House Porcine Corneal Opacity and Permeability Assay

[This Page Intentionally Left Blank]

Preliminary comments :

L'OREAL Advanced Research understood very early the usefulness of isolated cornea assay as a predictive tool for ocular irritancy. Due to constraints of supply and use of bovine eyes, we decided to use porcine corneas.
We have developped an in-house PCOP protocol to provide adequate safety data for cosmetic ingredients. Our PCOP protocol is basically that previously described by P. Gautheron, with some changes linked to species.

For liquid form and water-soluble materials we obtained a prediction model based on only one endpoint (O.D. $_{30}$) allowing to distinguish non irritating compounds (MAS ≤ 15 if O.D. $_{30}$ < 0. 35) from irritating compounds (MAS > 15 if O.D. $_{30}$ ≥ 0. 35).

Using this two classes scheme, 49 of the 50 raw ingredients tested in our PCOP assay were accurately classified. Only one was over-predicted. Agreement between in vivo and *in vitro* classification was high (concordance 98% -Kappa = 0. 96, p < 0.01).

	Observed class (*in vivo*)	
Predicted class (*in vitro*)	Mild irritant MAS ≤15	Moderate/ Irritant / Severe MAS >15
Mild irritant MAS ≤15	25	0
Moderate/ Irritant / Severe MAS >15	1	24

As MAS were available for 43 of the materials, a linear regression was carried out to predict the Draize Maximum Average Score (MAS).
An equation PM was obtained to predict MAS value, but despite satisfactory statistical coefficients - R^2Y= 0.84, concordance = 90 % - this algorithm is not recommended .Data analysis showed that 95% confidence interval was wide and there was too much uncertainty of the MAS prediction for it to be used.

We used the Draize test classification scheme according to French regulations applied to cosmetics (J. O. R. F. June 1992.) as follows :

MAS value	Class
≤ 15	mild irritant
>15 and ≤ 30	moderate irritant
> 30 and ≤ 50	irritant
> 50	severe irritant

Table 1 summarized our PCOP results obtained on 50 liquid and water-soluble compounds, selected on the basis of preexisting *in vivo* data - including 32 surfactants, 7 polymers, 5 solvents, 4 active ingredients and 2 dyes.

All compounds were tested *in vitro* at the same concentration as *in vivo*.

Some of them , including references , were tested in both PCOP and BCOP - See Table II and III for details .

Taking into account those results PCOP seems to be more suitable to predict class irritation than BCOP. Future work need to be done to complete this comparison .

Abbreviations used :
PCOP = Porcine cornea permeability and opacity assay;
BCOP = Bovine cornea permeability and opacity assay;
MAS = maximum average score;
OP_{10} or OP_{30} = opacity induced by a 10-min or 30 min- exposure of corneas, respectively;
$O.D._{10}$ or $O.D._{30}$ = optical density measured after a 10-min or 30 min- exposure of corneas, respectively;
PM = prediction model.

Table 1: PCOP results obtained on 50 liquid and water-soluble compounds

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	PCOP class	MAS (Class deducted)	MAS	class
1	0.5%	4	-0.043	0.3	0.084	Mild	10.2 (Mild)	0.7	Mild
2	10%	-0.3	-0.006	6.3	0.008	Mild	8.3 (Mild)	2.3	Mild
3	20%	-1	-0.003	0.4	0.003	Mild	8.2 (Mild)	3.7	Mild
4	10%	6.7	0.041	53.9	0.092	Mild	10.4 (Mild)	4.0	Mild
5	5%	7.7	0.042	12.7	0.028	Mild	8.8 (Mild)	4.7	Mild
6	10%	-0.7	-0.001	2.7	0.096	Mild	10.5 (Mild)	5.3	Mild
7	10%	-1.3	-0.004	5.7	0.044	Mild	9.2 (Mild)	5.7	Mild
8	100%	1.7	0.014	1.3	0.066	Mild	9.8 (Mild)	8.5	Mild
9	100%	2.6	0.036	-2.7	-0.034	Mild	7.2 (Mild)	9.8	Mild
10	3.1%	0.3	-0.009	-0.3	-0.008	Mild	7.9 (Mild)	10.7	Mild
11	100%	-5.6	-0.002	-4.0	0.003	Mild	8.2 (Mild)	10.7	Mild
12	100%	3.3	-0.002	2.0	0.013	Mild	8.4 (Mild)	10.8	Mild
13	100%	4.3	-0.002	39.0	0.133	Mild	11.5 (Mild)	11.0	Mild
14	100%	5	-0.004	-2.3	-0.003	Mild	8.0 (Mild)	11.3	Mild
15	10%	0.3	-0.017	2.8	0.021	Mild	8.6 Mild)	11.3	Mild
16	5%	5.6	0.421	19.0	1.040	**Irrg.**	29.4 (Mod.)	12.0	Mild
17	100%	1	0.007	4.7	0.028	Mild	8.8 (Mild)	12.0	Mild
18	100%	3	-0.001	12.3	-0.007	Mild	7.9 (Mild)	12.3	Mild
19	6.7%	4.7	0.212	15.0	0.296	Mild	15.3 (Mod.)	12.7	Mild
20	100%	7.3	0.056	21.2	0.014	Mild	8.4 (Mild)	12.8	Mild

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	PCOP class	MAS (Class deducted)	MAS	class
21	2%	3.3	0.042	5.0	0.056	Mild	9.5 (Mild)	13.0	Mild
22	100%	9.3	0.049	17.0	0.089	Mild	Not AppL	NA	Mild
23	100%	2	0.053	7.3	-0.007	Mild	Not AppL	NA	Mild
24	100%	-2.4	0.012	-1.0	0.003	Mild	Not AppL	NA	Mild
25	10%	0.3	0.003	2.7	0.014	Mild	Not AppL	NA	Mild
26	100%	20.7	0.118	67.1	0.367	Irrg.	16.9 (Mod.)	16.0	Mod.
27	6%	16	1.641	14.0	2.060	Irrg.	38.7 (Irr. to Sev.)	30.6	Irr.
28	10%	6.3	0.516	40.5	2.081	Irrg.	38.8 (Irr. to Sev.)	31.0	Irr.
29	0.5%	23	0.739	25.5	0.451	Irrg.	18.8 (mod.)	31.0	Irr.
30	10%	31.3	0.876	38.7	1.268	Irrg.	32.5 (Irr. to Sev.)	31.3	Irr.
31	10%	7.3	0.343	7.7	2.202	Irrg.	39.2 (Irr. to Sev.)	31.7	Irr.
32	10%	24.7	0.979	17.3	0.918	Irrg.	27.8 (Irr. to Sev.)	31.7	Irr.
33	10%	13.7	1.041	3.0	1.764	Irrg.	37.2 (Irr. to Sev.)	32.7	Irr.
34	10%	20	0.868	46.0	1.488	Irrg.	34.9 (Irr. to Sev.)	33.7	Irr.
35	6%	14	0.659	32.5	1.896	Irrg.	37 (Irr. to Sev.)	34.7	Irr.
36	6%	35.3	0.919	30.3	1.548	Irrg.	35.5 (Irr. to Sev.)	35.3	Irr.
37	10%	13	0.613	11.7	1.123	Irrg.	30.6 (Mod.)	35.7	Irr.
38	10%	16.7	1.917	22.3	2.132	Irrg.	38.9 (Irr. to Sev.)	37.4	Irr.
39	10%	14.3	0.750	16.7	2.016	Irrg.	38.6 (Irr. to Sev)	39.3	Irr.
40	10%	9.3	2.433	22.3	1.738	Irrg.	37 (Irr. to Sev.)	40.3	Irr.
41	10%	8.3	0.668	23.0	1.667	Irrg.	36.5 (Irr. to Sev.)	40.7	Irr.
42	10%	1.3	0.308	38.3	1.714	Irrg.	36.8 (Mod.)	43.0	Irr.
43	10%	11.3	0.786	18.0	2.094	Irrg.	38.9 (Irr. to Sev.)	45.0	Irr.
44	10%	29	1.568	33.3	1.978	Irrg.	38.4	46.0	Irr.

		PCOP Data				Predicted data		In vivo data (historical)	
N° product	Concentration tested	OP_{10}	O.D.$_{10}$	OP_{30}	O.D.$_{30}$	PCOP class	MAS (Class deducted)	MAS	class
							(Irr. to Sev.)		
45	10%	23	1.288	19.7	2.174	Irrg.	39.1 (Irr. to Sev.)	47.2	Irr.
46	10%	17.3	0.995	8.0	1.538	Irrg.	Not AppL	63.7	Sev.
47	10%	21.3	1.618	11.3	1.679	Irrg.	Not AppL	63.7	Sev
48	10%	16.7	0.495	27.3	0.878	Irrg.	Not AppL	NA	Irr.
49	10%	6.7	0.232	18.0	1.112	Irrg.	Not AppL	NA	Irr.
50	10%	10.7	0.639	48.3	0.997	Irrg.	Not AppL	NA	Irr.

OP_{10}. OP_{30}. O.D. $_{10}$ O.D. $_{30}$: Each data represents mean corrected value of three corneas.
NA: not available.
Not AppL: Not applicable

Predicted class is determined by the PM: O.D. $_{30} < 0.35 \Rightarrow$ predict nonirritating (MAS ≤15 - mild irritant) - O.D. $_{30} \geq$
$0.35 \Rightarrow$ predict irritating (Irrg.) corresponding to MAS >15, covering three French Draize classes - moderate, irritant, severe.
Predicted MAS is calculated using this algorithm = 8. 08 + 26. 16 x O. D. $_{30} -$ 5. 47 x O.D $_{30}^2$, with deduction of Draize class in brackets. Evaluation of this PM was based on three classes – Mild irritant (MAS ≤ 15), Moderate irritant
(15< MAS ≤ 30) , irritant to severe (MAS >30). Data analysis showed that 95% confidence interval was wide. There is too much uncertainty of the MAS prediction for it to be used.

Table II - References tested in PCOP and BCOP with historical data (N = 8)

		In vivo data (historical)		PCOP data					BCOP data						
N° PRODUCT	CONC	MAS	In vivo Class	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	Predicted class	OP_{10}	$O.D._{10}$	Score 10	OP_{30}	$O.D._{30}$	Score 30	Predicted Class
Propylene glycol (n°20)	100%	12.8	Mild	7.3	0.056	21.2	0.014	Mild	NA	NA	NA	11,7	0.001	1.8	1
Sodium dodecyl sulfate (n°38)	10%	37.4	Irr.	16.7	1.917	22.3	2.132	Irrg	NA	NA	NA	1.5	0.424	18	2
Triton X100 (n°40)	10%	40.3	Irr.	9.3	2.433	22.3	1.738	Irrg	3.2	2.717	43.9	2.1	5.197	79.5	3
Vaseline oil	100%	NI	Mild	-0.3	0.008	-1	-0.03	Mild	NA	NA	NA	NA	0.005	4.0	1
Tween 20 (n° 7)	10%	5.7	Mild	-1.3	0.004	5.7	0.044	Mild	0.3	0.003	0.3	1.8	0.001	1.8	1
Ethanol	100%	37 (at 1h)	Irr.	40	1.260	58.2	0.676	Irrg	26.9	2.912	70.6	NA	NA	NA	3
Ethanol	50%	NA	NA	8.3	0.036	18	0.075	Mild	NA	NA	22.9	NA	1.117	31.3	3
Ethanol	10%	NA	NA	-2.3	0.014	0.3	-0.016	Mild	NA	NA	NA	NA	0.021	0.5	1
Lactic Acid	10%	31.2	Irr.	78	0.928	271	1.552	Irrg	15.2	0.029	15.6	75.2	0.439	81.7	3
Hexadecyl trimethyl ammonium bromide (CTAB)	0.5%	NA	NA	42.3	0.562	53.0	1.270	Irrg	NA	NA	43.3	48.2	5.112	124.9	3

Numbers in brackets refer to table I.

NA: not available.

TABLE III: Results obtained on compounds tested in both PCOP and BCOP (N = 15)

		In vivo data (historical)		PCOP data					BCOP data						
N°	CONC TESTED	MAS	Classe	OP_{10}	$O.D._{10}$	OP_{30}	$O.D._{30}$	Predicted Class	OP_{10}	$O.D._{10}$	Score 10	OP_{30}	$O.D._{30}$	Score 30	Predicted Class
8	100%	8,5	Mild	1,7	0,014	1,3	0,066	Mild	NA	NA	NA	0,8	-0,006	0,7	1
13	100%	11,0	Mild	4,3	-0,002	39,0	0,133	Mild	4,4	0,040	5,0	24,7	0,958	39,1	2 or 3
18	100%	12,3	Mild	3,0	-0,001	12,3	-0,007	Mild	NA	NA	NA	5,8	0,057	6,6	1
20	100%	12,8	Mild	7,3	0,056	21,2	0,014	Mild	NA	NA	NA	11,7	0,424	18,0	2
26	100%	16,0	Mod.	20,7	0,118	67,1	0,367	Irrg	71,5	0,971	86,1	58,6	2,346	93,8	3
A	10%	30,3	Irrg	47,3	1,256	186,0	0,796	Irrg	12,1	1,505	34,7	51,3	4,107	112,9	3
30	10%	31,3	Irrg	31,3	0,876	38,7	1,268	Irrg	56,8	1,786	83,6	98,8	2,327	133,7	3
31	10%	31,7	Irrg	7,3	0,343	7,7	2,202	Irrg	2,5	1,058	18,4	3,0	3,238	51,6	2 or 3
32	10%	31,7	Irrg	24,7	0,979	17,3	0,918	Irrg	3,7	1,977	33,4	3,5	5,231	82,0	3
33	10%	32,7	Irrg	13,7	1,041	3,0	1,764	Irrg	0,3	0,860	13,2	2,7	4,439	69,8	3
39	10%	39,3	Irrg	14,3	0,750	16,7	2,016	Irrg	0,4	0,721	11,2	3,9	1,043	16,9	2
42	10%	43.0	Irrg	1,3	0,308	38,3	1,714	Irrg	5,7	1,082	22,0	6,1	5,392	86,9	3
43	10%	45,0	Irrg	11,3	0,786	18,0	2,094	Irrg	3,6	1,077	19,8	3,6	3,921	62,4	3
45	10%	47,2	Irrg	23,0	1,288	19,7	2,174	Irrg	5,1	3,098	51,6	2,3	5,040	77,9	3
46	10%	63,7	Irrg	17,3	0,995	8,0	1,538	Irrg	4,9	1,812	32,1	2,4	3,854	60,2	3

Product numbers refer to table I .

Our BCOP protocol is a variation on the original protocol developed by P. GAUTHERON .

Corneal score = OP + (15 x O.D.). Classification depends on corneal scores at 30 minutes completed with results at 10 minutes :

Score at 30 min	Class
≤ 10	Class 1 : mild irritant
10 - 25	Class 2 : moderate irritant if score 10 mn ≤ 10 Class 2 or 3 if 10 < score 10 mn ≤ 25 Class 3 : irritant to strong irritant if score 10 mn >25
25 - 55	Class 2 or 3 if score 10 mn ≤ 10 Class 3 : irritant to strong irritant if score 10 mn >10
>55	Class 3 : irritant to strong irritant

Appendix G5

Supporting Analyses Received from IIVS for Gettings et al. (1996) Study

[This Page Intentionally Left Blank]

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZA	24	1	4	1	2	1	1	33	EPA
			48	1	3	0	2	1	0	21	7
			72	1	1	0	2	1	1	13	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZA	33	1.0	2.7	0.3	2.0	1.0	0.7	7	7
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZA	33	1.0	2.7	0.3	2.0	1.0	0.7	7	7
	2	HZA	27	1.0	1.3	0.7	2.3	1.3	1.3	22	22
	3	HZA	34	1.0	1.7	1.0	2.7	1.7	1.3	7	7
	4	HZA	37	1.0	3.0	1.0	2.0	1.7	1.3	14	14
	5	HZA	35	1.0	2.3	0.7	2.3	1.3	0.3	22	22
	6	HZA	39	1.0	3.0	0.7	2.7	1.7	1.7	21	21
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZB*	24	1	1	1	2	1	0	16	EPA
			48	1	1	0	2	1	1	13	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZB*	16	0.7	0.7	0.3	1.3	0.7	0.3	3	3
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZB*	16	0.7	0.7	0.3	1.3	0.7	0.3	3	3
	2	HZB*	4	0.0	0.0	0.0	1.0	0.0	0.0	2	2
	3	HZB*	11	1.0	1.0	0.0	1.3	1.0	0.0	7	7
	4	HZB*	27	0.7	1.0	0.3	1.7	1.0	0.7	3	7
	5	HZB*	35	1.0	2.3	1.0	2.0	1.3	1.0	7	7
	6	HZB*	0	0.3	0.3	0.3	1.7	1.0	0.0	3	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZA	24	1	2	1	2	2	2	27	EPA
			48	1	1	1	3	1	2	22	22
			72	1	1	0	2	1	0	11	GHS
			7 days	1	1	0	2	1	1	13	22
			14 days	1	1	0	2	2	1	15	
			21 days	1	1	0	2	1	0	11	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZA	27	1	1.333333	0.666667	2.333333333	1.333333333	1.333333333	22	22

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	1	0.833333	2.5	1.5	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,4	1	0.833333	2.166667	1.5	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,5	1	0.666667	2.333333	1.333333	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,6	1	0.666667	2.5	1.5	22	22
	GHS Rating	2	4	2	4	22	22

	Combinations	Opacity	Iris
Combination block #2	1,3,4	1	1
	GHS Rating	2	4
	1,3,5	1	0.833333
	GHS Rating	2	4
	1,3,6	1	0.833333
	GHS Rating	2	4
	1,4,5	1	0.833333
	GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZB*	24	0	0	0	2	0	0	4	EPA
			48	0	0	0	0	0	0	0	2
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZB*	4	0	0	0	1	0	0	2	2

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	0.833333	0.166667	1.333333	0.833333	7	7
	GHS Rating	4	4	4	4	7	7
	1,2,4	0.666667	0.333333	1.5	0.833333	3	7
	GHS Rating	4	4	4	4	3	7
	1,2,5	0.833333	0.666667	1.666667	1	7	7
	GHS Rating	4	4	4	4	7	7
	1,2,6	0.5	0.333333	1.5	0.833333	3	7
	GHS Rating	4	4	4	4	3	7

	Combinations	Opacity	Iris
Combination block #2	1,3,4	0.833333	0.333333
	GHS Rating	4	4
	1,3,5	1	0.666667
	GHS Rating	2	4
	1,3,6	0.833333	0.333333
	GHS Rating	4	4
	1,4,5	0.833333	0.666667
	GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZA	24	1	3	1	3	2	2	34	EPA
			48	1	1	1	3	1	2	22	7
			72	1	1	1	2	2	0	18	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZA	34	1	1.666667	1	2.666666667	1.66666667	1.333333333	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.333333	1.666667	14	14	Combina-	1,4,6	1.0	0.8	2.3	1.7	21	21
2	4	14	14	tion block	GHS Ratinç	2	4	2	4	21	21
2.5	1.5	22	22	#3	1,5,6	1.0	0.7	2.5	1.5	22	22
2	4	22	22		GHS Ratinç	2	4	2	4	22	22
2.666667	1.666667	21	21		2,3,4	1.0	1.0	2.5	1.7	22	22
2	4	21	21		GHS Ratinç	2	2	2	4	22	22
2.166667	1.5	22	22		2,3,5	1.0	0.8	2.5	1.5	22	22
2	4	22	22		GHS Ratinç	2	4	2	4	22	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZB*	24	1	1	0	2	1	0	11	EPA
			48	1	1	0	1	1	0	9	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZB*	11	1	1	0	1.333333333	1	0	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.5	1	7	7	Combina-	1,4,6	0.7	0.3	1.7	1.0	3	7
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	3	7
1.666667	1.166667	7	7	#3	1,5,6	0.8	0.7	1.8	1.2	7	7
4	4	7	7		GHS Ratinç	4	4	4	4	7	7
1.5	1	7	7		2,3,4	0.8	0.2	1.5	1.0	7	7
4	4	7	7		GHS Ratinç	4	4	4	4	7	7
1.833333	1.166667	7	7		2,3,5	1.0	0.5	1.7	1.2	7	7
4	4	7	7		GHS Ratinç	2	4	4	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZA	24	1	4	1	2	2	2	37	EPA
			48	1	3	1	2	1	0	26	14
			72	1	2	1	2	2	2	27	GHS
			7 days	1	1	0	1	1	0	9	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZA	37	1	3	1	2	1.666666667	1.333333333	14	14

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.8	2.7	1.7	22	22	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	4	2	4	22	22	tion block	GHS Rating	2	2
#4	2,4,5	1.0	0.8	2.3	1.5	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	4	2	4	22	22		GHS Rating	2	2
	2,4,6	1.0	0.8	2.5	1.7	22	22		3,5,6	1.0	0.8
	GHS Ratin	2	4	2	4	22	22		GHS Rating	2	4
	2,5,6	1.0	0.7	2.5	1.5	22	22		4,5,6	1.0	0.8
	GHS Ratin	2	4	2	4	22	22		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZB*	24	1	2	1	2	2	2	27	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZB*	27	0.666667	1	0.333333	1.66666667	1	0.666666667	3	7

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.7	0.2	1.5	1.0	7	7	Combina-	3,4,5	1.0	0.7
tion block	GHS Ratin	4	4	4	4	7	7	tion block	GHS Rating	2	4
#4	2,4,5	0.8	0.7	1.8	1.2	7	7	#5	3,4,6	0.8	0.3
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,4,6	0.5	0.3	1.7	1.0	3	7		3,5,6	1.0	0.7
	GHS Ratin	4	4	4	4	3	7		GHS Rating	2	4
	2,5,6	0.7	0.7	1.8	1.2	7	7		4,5,6	0.8	0.7
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZA	24	1	4	1	3	2	0	35	EPA
			48	1	2	1	2	1	1	23	22
			72	1	1	0	2	1	0	11	GHS
			7 days	1	1	0	0	0	0	5	22
			14 days	1	1	0	0	0	0	5	
			21 days	1	1	0	0	0	0	5	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZA	35	1	2.333333	0.666667	2.33333333	1.333333333	0.333333333	22	22

Redness	Chemosis	DtC EPA	DtC GHS
2.5	1.7	22	22
2	4	22	22
2.7	1.7	21	21
2	4	21	21
2.7	1.7	22	22
2	4	22	22
2.5	1.7	22	22
2	4	22	22

Summary			
HZA	1,2,3	2	22
	1,2,4	2	22
	1,2,5	2	22
	1,2,6	2	22
	1,3,4	2	14
	1,3,5	2	22
	1,3,6	2	21
	1,4,5	2	22
	1,4,6	2	21
	1,5,6	2	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZB*	24	1	4	1	2	2	1	35	EPA
			48	1	1	1	2	1	1	18	7
			72	1	2	1	2	1	1	23	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZB*	35	1	2.333333	1	2	1.333333333	1	7	7

Redness	Chemosis	DtC EPA	DtC GHS
1.8	1.2	7	7
4	4	7	7
1.7	1.0	7	7
4	4	7	7
1.8	1.2	7	7
4	4	7	7
1.8	1.2	7	7
4	4	7	7

Summary			
HZB*	1,2,3	4	7
	1,2,4	4	7
	1,2,5	4	7
	1,2,6	4	7
	1,3,4	4	7
	1,3,5	2	7
	1,3,6	4	7
	1,4,5	4	7
	1,4,6	4	7
	1,5,6	4	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZA	24	1	4	1	3	2	2	39	EPA
			48	1	3	1	3	2	2	34	21
			72	1	2	0	2	1	1	18	GHS
			7 days	1	1	0	1	1	1	11	21
			14 days	1	1	0	1	0	0	7	
			21 days	0	0	0	0	0	0	0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZA	39	1	3	0.666667	2.66666667	1.666666667	1.666666667	21	21
22		2,3,4	2	22		22					
22		2,3,5	2	22		22					
22		2,3,6	2	22		22					
22		2,4,5	2	22		22					
14		2,4,6	2	22		22					
22		2,5,6	2	22		22					
21		3,4,5	2	22		22					
22		3,4,6	2	21		21					
21		3,5,6	2	22		22					
22		4,5,6	2	22		22					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZB*	24	1	1	1	2	2	0	0	EPA
			48	0	0	0	2	1	0	0	3
			72	0	0	0	1	0	0	0	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZB*	0	0.333333	0.333333	0.333333	1.66666667	1	0	3	7
7		2,3,4	4	7		7					
3		2,3,5	2	7		7					
7		2,3,6	4	7		7					
3		2,4,5	4	7		7					
7		2,4,6	4	7		3					
7		2,5,6	4	7		7					
7		3,4,5	2	7		7					
7		3,4,6	4	7		7					
3		3,5,6	2	7		7					
7		4,5,6	4	7		7					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZC*	24	0	0	0	2	1	2	10	EPA
			48	0	0	0	1	0	0	2	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZC*	10	0.0	0.0	0.0	1.0	0.3	0.7	3	3
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZC*	10	0.0	0.0	0.0	1.0	0.3	0.7	3	3
	2	HZC*	32	1.0	1.7	0.3	2.0	1.3	0.7	7	7
	3	HZC*	35	1.0	2.3	1.0	2.0	1.3	1.3	7	7
	4	HZC*	6	0.0	0.0	0.0	1.7	0.3	0.0	3	7
	5	HZC*	35	1.0	2.7	0.7	2.0	1.3	0.7	7	7
	6	HZC*	11	0.7	0.7	0.0	1.0	0.3	0.0	3	3
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F41401	HZD*	24	1	1	0	3	2	3	21	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	F41401	HZD*	21	1.0	1.0	0.0	2.0	1.3	1.0	7	7
	ANIMAL ID										
Summary block used analysis of the twenty combinations	F41401	HZD*	21	1.0	1.0	0.0	2.0	1.3	1.0	7	7
	2	HZD*	15	0.7	0.7	0.0	1.7	0.7	1.0	3	7
	3	HZD*	19	0.3	0.3	0.0	1.7	1.0	1.0	3	7
	4	HZD*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	F41356	HZD*	11	0.7	0.7	0.0	1.0	0.3	0.0	3	3
	F41386	HZD*	13	0.5	0.5	0.0	1.0	0.5	0.5	2	2
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZC*	24	1	3	1	2	2	2	32	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZC*	32	1	1.666667	0.333333	2	1.333333333	0.666666667	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	0.666667	2	1.333333	7	7	Combination block #2	1,3,4	0.5	0.5
	GHS Rating	2	4	2	4	7	7		GHS Rating	4	4
	1,2,4	0.5	0.166667	1.833333	0.833333	7	7		1,3,5	1	0.833333
	GHS Rating	4	4	4	4	7	7		GHS Rating	2	4
	1,2,5	1	0.5	2	1.333333	7	7		1,3,6	0.833333	0.5
	GHS Rating	2	4	2	4	7	7		GHS Rating	4	4
	1,2,6	0.833333	0.166667	1.5	0.833333	7	7		1,4,5	0.5	0.333333
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZD*	24	1	1	0	2	1	2	15	EPA
			48	1	1	0	2	1	1	13	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZD*	15	0.666667	0.666667	0	1.666666667	0.666666667	1	3	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.833333	0	1.833333	1.166667	7	7	Combination block #2	1,3,4	0.666667	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,4	0.833333	0	1.833333	1	7	7		1,3,5	0.833333	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,5	0.833333	0	1.833333	1	7	7		1,3,6	0.75	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,6	0.833333	0	1.833333	1	7	7		1,4,5	0.833333	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZC*	24	1	4	1	2	1	2	35	EPA
			48	1	2	1	2	1	0	21	7
			72	1	1	1	2	2	2	22	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZC*	35	1	2.333333	1	2	1.33333333	1.333333333	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.833333	0.833333	7	7	Combina-	1,4,6	0.3	0.0	1.3	0.3	3	7
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	3	7
2	1.333333	7	7	#3	1,5,6	0.8	0.3	1.5	0.8	7	7
2	4	7	7		GHS Ratinç	4	4	4	4	7	7
1.5	0.833333	7	7		2,3,4	1.0	0.7	2.0	1.3	7	7
4	4	7	7		GHS Ratinç	2	4	2	4	7	7
1.833333	0.833333	7	7		2,3,5	1.0	0.8	2.0	1.3	7	7
4	4	7	7		GHS Ratinç	2	4	2	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZD*	24	1	1	0	2	2	3	19	EPA
			48	0	0	0	2	1	0	6	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZD*	19	0.333333	0.333333	0	1.666666667	1	1	3	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.833333	1.166667	7	7	Combina-	1,4,6	0.8	0.0	1.5	0.9	7	7
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	7	7
1.833333	1.166667	7	7	#3	1,5,6	0.8	0.0	1.5	0.9	7	7
4	4	7	7		GHS Ratinç	4	4	4	4	7	7
1.833333	1.166667	7	7		2,3,4	0.5	0.0	1.7	0.8	3	7
4	4	7	7		GHS Ratinç	4	4	4	4	3	7
1.5	0.833333	7	7		2,3,5	0.7	0.0	1.7	0.8	3	7
4	4	7	7		GHS Ratinç	4	4	4	4	3	7

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZC*	24	0	0	0	2	1	0	6	EPA
			48	0	0	0	2	0	0	4	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZC*	6	0	0	0	1.66666667	0.333333333	0	3	7

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.7	2.0	1.3	7	7	Combina-	3,4,5	1.0	0.8
tion block	GHS Ratin	2	4	2	4	7	7	tion block	GHS Rating	2	4
#4	2,4,5	1.0	0.5	2.0	1.3	7	7	#5	3,4,6	0.8	0.5
	GHS Ratin	2	4	2	4	7	7		GHS Rating	4	4
	2,4,6	0.8	0.2	1.8	0.8	7	7		3,5,6	1.0	0.8
	GHS Ratin	4	4	4	4	7	7		GHS Rating	2	4
	2,5,6	1.0	0.5	2.0	1.3	7	7		4,5,6	0.8	0.3
	GHS Ratin	2	4	2	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZD*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZD*	2	0	0	0	0.5	0	0	0	2

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.6	0.0	1.7	0.8	3	7	Combina-	3,4,5	0.5	0.0
tion block	GHS Ratin	4	4	4	4	3	7	tion block	GHS Rating	4	4
#4	2,4,5	0.7	0.0	1.3	0.5	3	7	#5	3,4,6	0.4	0.0
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4
	2,4,6	0.6	0.0	1.3	0.6	3	7		3,5,6	0.6	0.0
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4
	2,5,6	0.7	0.0	1.3	0.6	3	7		4,5,6	0.6	0.0
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZC*	24	1	4	1	2	2	1	35	EPA
			48	1	3	1	2	1	1	28	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZC*	35	1	2.666667	0.666667	2	1.333333333	0.666666667	7	7

Redness	Chemosis	DtC EPA	DtC GHS
2.0	1.3	7	7
2	4	7	7
1.8	0.8	7	7
4	4	7	7
2.0	1.3	7	7
2	4	7	7
1.8	0.8	7	7
4	4	7	7

Summary		
HZC*	1,2,3	2
	1,2,4	4
	1,2,5	2
	1,2,6	4
	1,3,4	4
	1,3,5	2
	1,3,6	4
	1,4,5	4
	1,4,6	4
	1,5,6	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F41356	HZD*	24	1	1	0	2	1	0	11	EPA
			48	1	1	0	1	0	0	7	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F41356	HZD*	11	0.666667	0.666667	0	1	0.333333333	0	3	3

Redness	Chemosis	DtC EPA	DtC GHS
1.3	0.7	3	7
4	4	3	7
1.3	0.8	3	7
4	4	3	7
1.3	0.8	3	7
4	4	3	7
1.0	0.4	3	3
4	4	3	3

Summary		
HZD*	1,2,3	4
	1,2,4	4
	1,2,5	4
	1,2,6	4
	1,3,4	4
	1,3,5	4
	1,3,6	4
	1,4,5	4
	1,4,6	4
	1,5,6	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZC*	24	1	1	0	2	1	0	11	EPA
			48	1	1	0	1	0	0	7	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZC*	11	0.666667	0.666667	0	1	0.333333333	0	3	3
7		2,3,4	2	7		7					
7		2,3,5	2	7		7					
7		2,3,6	2	7		7					
7		2,4,5	2	7		7					
7		2,4,6	4	7		7					
7		2,5,6	2	7		7					
7		3,4,5	2	7		7					
7		3,4,6	4	7		7					
3		3,5,6	2	7		7					
7		4,5,6	4	7		7					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F41386	HZD*	24	1	1	0	2	1	1	13	EPA
			48	0	0	0	0	0	0	0	2
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F41386	HZD*	13	0.5	0.5	0	1	0.5	0.5	2	2
7		2,3,4	4	7		3					
7		2,3,5	4	7		3					
7		2,3,6	4	7		3					
7		2,4,5	4	7		3					
7		2,4,6	4	7		3					
7		2,5,6	4	7		3					
7		3,4,5	4	7		3					
7		3,4,6	4	7		3					
7		3,5,6	4	7		3					
7		4,5,6	4	3		3					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZE	24	0	0	0	1	1	0	4	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZE	4	0.0	0.0	0.0	0.7	0.3	0.0	2	3
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZE	4	0.0	0.0	0.0	0.7	0.3	0.0	2	3
	2	HZE	20	0.3	0.3	0.3	1.3	0.7	0.3	3	3
	3	HZE	29	1.0	1.3	0.3	2.0	1.7	1.7	7	7
	4	HZE	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	5	HZE	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	6	HZE	32	1.0	2.7	0.3	2.0	1.7	1.0	22	22
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZF	24	1	3	1	2	2	2	32	EPA
			48	1	2	1	3	2	2	29	14
			72	1	1	1	3	2	1	22	GHS
			7 days	1	1	0	1	1	1	11	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZF	32	1.0	2.0	1.0	2.7	2.0	1.7	14	14
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZF	32	1.0	2.0	1.0	2.7	2.0	1.7	14	14
	2	HZF	29	1.0	1.3	0.7	2.7	1.3	1.0	7	7
	3	HZF	34	1.0	2.3	0.7	2.0	2.0	2.3	22	22
	4	HZF	41	1.0	3.3	1.0	2.0	2.3	2.7	22	22
	5	HZF	39	1.0	2.3	1.0	2.3	2.0	0.7	14	14
	6	HZF	32	1.0	2.0	0.7	1.7	1.7	0.7	7	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZE	24	1	1	1	2	2	1	20	EPA
			48	0	0	0	2	0	0	4	3
			72	0	0	0	0	0	0	0	GHS
			7 days	0	0	0	0	0	0	0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZE	20	0.333333	0.333333	0.333333	1.333333333	0.666666667	0.333333333	3	3

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	0.666667	0.333333	1.666667	1.166667	7	7
	GHS Rating	4	4	4	4	7	7
	1,2,4	0.166667	0.166667	1	0.5	3	3
	GHS Rating	4	4	4	4	3	3
	1,2,5	0.166667	0.166667	1	0.5	3	3
	GHS Rating	4	4	4	4	3	3
	1,2,6	0.666667	0.333333	1.666667	1.166667	22	22
	GHS Rating	4	4	4	4	22	22

	Combinations	Opacity	Iris
Combination block #2	1,3,4	0.5	0.166667
	GHS Rating	4	4
	1,3,5	0.5	0.166667
	GHS Rating	4	4
	1,3,6	1	0.333333
	GHS Rating	2	4
	1,4,5	0	0
	GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZF	24	1	2	1	3	2	2	29	EPA
			48	1	1	1	3	1	1	20	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZF	29	1	1.333333	0.666667	2.666666667	1.333333333	1	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	1	0.833333	2.666667	2	22	22
	GHS Rating	2	4	2	2	22	22
	1,2,4	1	1	2.666667	2.166667	22	22
	GHS Rating	2	2	2	2	22	22
	1,2,5	1	1	2.666667	2	14	14
	GHS Rating	2	2	2	2	14	14
	1,2,6	1	0.833333	2.666667	1.833333	14	14
	GHS Rating	2	4	2	4	14	14

	Combinations	Opacity	Iris
Combination block #2	1,3,4	1	1
	GHS Rating	2	2
	1,3,5	1	1
	GHS Rating	2	2
	1,3,6	1	0.833333
	GHS Rating	2	4
	1,4,5	1	1
	GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZE	24	1	2	1	2	2	3	29	EPA
			48	1	1	0	2	2	2	17	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZE	29	1	1.333333	0.333333	2	1.66666667	1.666666667	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.333333	1	7	7	Combina-	1,4,6	0.5	0.2	1.3	1.0	22	22
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	22	22
1.333333	1	7	7	#3	1,5,6	0.5	0.2	1.3	1.0	22	22
4	4	7	7		GHS Ratinç	4	4	4	4	22	22
2	1.666667	22	22		2,3,4	0.7	0.3	1.7	1.2	7	7
4	4	22	22		GHS Ratinç	4	4	4	4	7	7
0.583333	0.166667	2	3		2,3,5	0.7	0.3	1.7	1.2	7	7
4	4	2	3		GHS Ratinç	4	4	4	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZF	24	1	3	1	2	2	3	34	EPA
			48	1	3	1	2	2	2	32	22
			72	1	1	0	2	2	2	17	GHS
			7 days	1	1	0	3	2	3	21	22
			14 days	1	1	0	2	1	1	13	
			21 days	1	1	0	2	1	1	13	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZF	34	1	2.333333	0.666667	2	2	2.333333333	22	22

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.333333	2.166667	22	22	Combina-	1,4,6	1.0	1.0	2.3	2.2	22	22
2	2	22	22	tion block	GHS Ratinç	2	2	2	2	22	22
2.5	2	22	22	#3	1,5,6	1.0	1.0	2.5	2.0	14	14
2	2	22	22		GHS Ratinç	2	2	2	2	14	14
2.333333	2	22	22		2,3,4	1.0	0.8	2.3	2.2	22	22
2	2	22	22		GHS Ratinç	2	4	2	2	22	22
2.5	2.166667	22	22		2,3,5	1.0	0.8	2.5	2.0	22	22
2	2	22	22		GHS Ratinç	2	4	2	2	22	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZE	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZE	2	0	0	0	0.5	0	0	0	2

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.3	2.0	1.7	22	22	Combina-	3,4,5	0.5	0.2
tion block	GHS Ratin	4	4	2	4	22	22	tion block	GHS Rating	4	4
#4	2,4,5	0.2	0.2	0.9	0.3	3	3	#5	3,4,6	1.0	0.3
	GHS Ratin	4	4	4	4	3	3		GHS Rating	2	4
	2,4,6	0.7	0.3	1.7	1.2	22	22		3,5,6	1.0	0.3
	GHS Ratin	4	4	4	4	22	22		GHS Rating	2	4
	2,5,6	0.7	0.3	1.7	1.2	22	22		4,5,6	0.5	0.2
	GHS Ratin	4	4	4	4	22	22		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZF	24	1	4	1	2	3	3	41	EPA
			48	1	3	1	2	2	3	34	22
			72	1	3	1	2	2	2	32	GHS
			7 days	1	2	0	2	2	1	20	22
			14 days	1	2	0	2	2	2	22	
			21 days	2	1	0	2	2	1	20	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZF	41	1	3.333333	1	2	2.333333333	2.666666667	22	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.7	2.3	1.8	22	22	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	4	2	4	22	22	tion block	GHS Rating	2	2
#4	2,4,5	1.0	1.0	2.5	2.2	22	22	#5	3,4,6	1.0	0.8
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	4
	2,4,6	1.0	0.8	2.3	2.0	22	22		3,5,6	1.0	0.8
	GHS Ratin	2	4	2	2	22	22		GHS Rating	2	4
	2,5,6	1.0	0.8	2.5	1.8	14	14		4,5,6	1.0	1.0
	GHS Ratin	2	4	2	4	14	14		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZE	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZE	2	0	0	0	0.5	0	0	0	2

Redness	Chemosis	DtC EPA	DtC GHS
1.3	0.8	7	7
4	4	7	7
2.0	1.7	22	22
2	4	22	22
2.0	1.7	22	22
2	4	22	22
1.3	0.8	22	22
4	4	22	22

Summary			
HZE	1,2,3	4	7
	1,2,4	4	3
	1,2,5	4	3
	1,2,6	4	22
	1,3,4	4	7
	1,3,5	4	7
	1,3,6	2	22
	1,4,5	4	3
	1,4,6	4	22
	1,5,6	4	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZF	24	1	4	1	3	2	2	39	EPA
			48	1	2	1	2	2	0	23	14
			72	1	1	1	2	2	0	18	GHS
			7 days	1	1	0	1	1	0	9	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZF	39	1	2.333333	1	2.33333333	2	0.666666667	14	14

Redness	Chemosis	DtC EPA	DtC GHS
2.2	2.2	22	22
2	2	22	22
2.0	2.2	22	22
2	2	22	22
2.2	2.0	22	22
2	2	22	22
2.2	2.2	22	22
2	2	22	22

Summary			
HZF	1,2,3	2	22
	1,2,4	2	22
	1,2,5	2	14
	1,2,6	2	14
	1,3,4	2	22
	1,3,5	2	22
	1,3,6	2	22
	1,4,5	2	22
	1,4,6	2	22
	1,5,6	2	14

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZE	24	1	4	0	2	2	2	32	EPA
			48	1	2	0	2	2	1	20	22
			72	1	2	1	2	1	0	21	GHS
			7 days	1	1	0	2	1	0	11	22
			14 days	1	1	0	2	2	1	15	
			21 days	2	1	0	2	2	1	20	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZE	32	1	2.666667	0.333333	2	1.666666667	1	22	22
7		2,3,4	4	7		7					
3		2,3,5	4	7		7					
3		2,3,6	2	22		22					
22		2,4,5	4	3		3					
7		2,4,6	4	22		22					
7		2,5,6	4	22		22					
22		3,4,5	4	7		7					
2		3,4,6	2	22		22					
22		3,5,6	2	22		22					
22		4,5,6	4	22		22					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZF	24	1	3	1	2	2	2	32	EPA
			48	1	2	1	2	2	0	23	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZF	32	1	2	0.666667	1.66666667	1.666666667	0.666666667	7	7
22		2,3,4	2	22		22					
22		2,3,5	2	22		22					
14		2,3,6	2	22		22					
14		2,4,5	2	22		22					
22		2,4,6	2	22		22					
22		2,5,6	2	14		14					
22		3,4,5	2	22		22					
22		3,4,6	2	22		22					
22		3,5,6	2	22		22					
14		4,5,6	2	22		22					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZG*	24	1	1	0	2	0	0	9	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZG*	9	0.3	0.3	0.0	1.0	0.0	0.0	2	3
	ANIMAL ID										
Summary	1	HZG*	9	0.3	0.3	0.0	1.0	0.0	0.0	2	3
block used	2	HZG*	18	0.3	0.3	0.3	1.0	0.3	0.3	3	3
analysis of	3	HZG*	20	0.7	1.0	0.0	1.7	1.0	0.3	3	7
the twenty	4	HZG*	18	0.7	0.7	0.3	1.7	0.3	0.3	3	7
combinations	5	HZG*	15	0.7	0.7	0.0	1.0	1.0	0.3	3	3
	6	HZG*	11	0.3	0.3	0.0	1.7	0.7	0.0	7	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	ANIMAL ID										
Summary	1	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
block used	2	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
analysis of	3	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
the twenty	4	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
combinations	5	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	6	HZH	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZG*	24	1	1	1	2	1	1	18	EPA
			48	0	0	0	1	0	0	2	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZG*	18	0.333333	0.333333	0.333333	1	0.333333333	0.333333333	3	3

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.5	0.166667	1.333333	0.666667	3	7	Combination block #2	1,3,4	0.666667	0.166667
	GHS Rating	4	4	4	4	3	7		GHS Rating	4	4
	1,2,4	0.5	0.333333	1.333333	0.333333	3	7		1,3,5	0.666667	0
	GHS Rating	4	4	4	4	3	7		GHS Rating	4	4
	1,2,5	0.5	0.166667	1	0.666667	3	3		1,3,6	0.5	0
	GHS Rating	4	4	4	4	3	3		GHS Rating	4	4
	1,2,6	0.333333	0.166667	1.333333	0.5	7	7		1,4,5	0.666667	0.166667
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	
			72							0	GHS
			7 days							0	
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZH	0	0	0	0	0	0	0	0	0

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0	0	0	0	0	0	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,4	0	0	0	0	0	0		1,3,5	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,5	0	0	0	0	0	0		1,3,6	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,6	0	0	0	0	0	0		1,4,5	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZG*	24	1	2	0	2	2	1	20	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZG*	20	0.666667	1	0	1.666666667	1	0.333333333	3	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.666667	0.666667	3	7	Combina-	1,4,6	0.5	0.2	1.7	0.5	7	7
4	4	3	7	tion block	GHS Ratinç	4	4	4	4	7	7
1.333333	1	3	7	#3	1,5,6	0.5	0.0	1.3	0.8	7	7
4	4	3	7		GHS Ratinç	4	4	4	4	7	7
1.666667	0.833333	7	7		2,3,4	0.7	0.3	1.7	0.7	3	7
4	4	7	7		GHS Ratinç	4	4	4	4	3	7
1.333333	0.666667	3	7		2,3,5	0.7	0.2	1.3	1.0	3	7
4	4	3	7		GHS Ratinç	4	4	4	4	3	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZH	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0	0	0	0	Combina-	1,4,6	0.0	0.0	0.0	0.0	0	0
4	4	0	0	tion block	GHS Ratinç	4	4	4	4	0	0
0	0	0	0	#3	1,5,6	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0
0	0	0	0		2,3,4	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0
0	0	0	0		2,3,5	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZG*	24	1	1	1	2	1	1	18	EPA
			48	1	1	0	2	0	0	9	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZG*	18	0.666667	0.666667	0.333333	1.66666667	0.333333333	0.333333333	3	7

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.5	0.2	1.7	0.8	7	7	Combina-	3,4,5	0.7	0.2
tion block	GHS Ratin	4	4	4	4	7	7	tion block	GHS Rating	4	4
#4	2,4,5	0.7	0.3	1.3	0.7	3	7	#5	3,4,6	0.7	0.2
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4
	2,4,6	0.5	0.3	1.7	0.5	7	7		3,5,6	0.7	0.0
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,5,6	0.5	0.2	1.3	0.8	7	7		4,5,6	0.7	0.2
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZH	0	0	0	0	0	0	0	0	0

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.0	0.0	0	0	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	0	0	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	0.0	0.0	0	0	#5	3,4,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4
	2,4,6	0.0	0.0	0.0	0.0	0	0		3,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4
	2,5,6	0.0	0.0	0.0	0.0	0	0		4,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZG*	24	1	1	0	2	2	1	15	EPA
			48	1	1	0	1	1	0	9	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZG*	15	0.666667	0.666667	0	1	1	0.333333333	3	3

Redness	Chemosis	DtC EPA	DtC GHS
1.7	1.0	3	7
4	4	3	7
1.7	0.8	7	7
4	4	7	7
1.7	1.0	7	7
4	4	7	7
1.7	0.8	7	7
4	4	7	7

Summary			
HZG*	1,2,3	4	7
	1,2,4	4	7
	1,2,5	4	3
	1,2,6	4	7
	1,3,4	4	7
	1,3,5	4	7
	1,3,6	4	7
	1,4,5	4	7
	1,4,6	4	7
	1,5,6	4	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZH	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0

Summary			
HZH	1,2,3	4	0
	1,2,4	4	0
	1,2,5	4	0
	1,2,6	4	0
	1,3,4	4	0
	1,3,5	4	0
	1,3,6	4	0
	1,4,5	4	0
	1,4,6	4	0

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZG*	24	1	1	0	2	1	0	11	EPA
			48	0	0	0	2	1	0	6	7
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZG*	11	0.333333	0.333333	0	1.66666667	0.666666667	0	7	7
3		2,3,4	4	7		3					
3		2,3,5	4	7		3					
3		2,3,6	4	7		7					
7		2,4,5	4	7		3					
3		2,4,6	4	7		7					
3		2,5,6	4	7		7					
7		3,4,5	4	7		3					
3		3,4,6	4	7		7					
7		3,5,6	4	7		7					
7		4,5,6	4	7		7					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZH	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZH	0	0	0	0	0	0	0	0	0
0		2,3,4	4	0		0					
0		2,3,5	4	0		0					
0		2,3,6	4	0		0					
0		2,4,5	4	0		0					
0		2,4,6	4	0		0					
0		2,5,6	4	0		0					
0		3,4,5	4	0		0					
0		3,4,6	4	0		0					
0		3,5,6	4	0		0					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40840	HZI	24	1	4	1	3	2	2	39	EPA
			48	1	3	1	3	2	2	34	7
			72	1	1	1	3	2	2	24	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	F40840	HZI	39	1.0	2.7	1.0	3.0	2.0	2.0	7	7
	ANIMAL ID										
Summary block used analysis of the twenty combinations	F40840	HZI	39	1.0	2.7	1.0	3.0	2.0	2.0	7	7
	F40855	HZI	36	1.0	2.3	1.0	3.0	2.0	2.3	14	14
	F40881	HZI	39	1.0	3.0	1.0	2.0	1.7	1.7	22	22
	F41365	HZI	43	1.0	3.0	0.7	2.3	2.3	3.0	22	22
	F41379	HZI	29	1.0	1.3	0.3	2.0	1.7	1.3	7	7
	F41405	HZI	39	1.0	3.7	1.0	2.3	2.0	1.7	0	0
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZJ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZJ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZJ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	2	HZJ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	3	HZJ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	4	HZJ	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	5	HZJ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	6	HZJ	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40855	HZI	24	1	3	1	3	2	3	36	EPA
			48	1	2	1	3	2	2	29	14
			72	1	2	1	3	2	2	29	GHS
			7 days	1	1	0	2	1	0	11	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F40855	HZI	36	1	2.333333	1	3	2	2.333333333	14	14

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	1	3	2	22	22	Combination block #2	1,3,4	1	1
	GHS Rating	2	2	2	2	22	22		GHS Rating	2	2
	1,2,4	1	1	3	2.166667	22	22		1,3,5	1	1
	GHS Rating	2	2	2	2	22	22		GHS Rating	2	2
	1,2,5	1	1	3	2	14	14		1,3,6	1	1
	GHS Rating	2	2	2	2	14	14		GHS Rating	2	2
	1,2,6	1	1	3	2	14	14		1,4,5	1	0.833333
	GHS Rating	2	2	2	2	14	14		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZJ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZJ	0	0	0	0	0	0	0	0	0

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0	0	0	0	0	0	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,4	0	0	0.25	0	0	2		1,3,5	0	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4
	1,2,5	0	0	0	0	0	0		1,3,6	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,6	0	0	0.25	0	0	2		1,4,5	0	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40881	HZI	24	1	4	1	2	2	3	39	EPA
			48	1	3	1	2	1	1	28	22
			72	1	2	1	2	2	1	25	GHS
			7 days	1	1	0	2	1	1	13	22
			14 days	1	1	0	1	1	0	9	
			21 days	1	1	0	1	1	0	9	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F40881	HZI	39	1	3	1	2	1.66666667	1.666666667	22	22

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.666667	2.166667	22	22	Combina-	1,4,6	1.0	1.0	2.7	2.2	22	22
2	2	22	22	tion block	GHS Ratinç	2	2	2	2	22	22
2.5	1.833333	22	22	#3	1,5,6	1.0	1.0	2.7	2.0	7	7
2	4	22	22		GHS Ratinç	2	4	2	2	7	7
2.666667	2	22	22		2,3,4	1.0	1.0	2.7	2.2	22	22
2	2	22	22		GHS Ratinç	2	2	2	2	22	22
2.666667	2.166667	22	22		2,3,5	1.0	1.0	2.5	1.8	22	22
2	2	22	22		GHS Ratinç	2	2	2	4	22	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZJ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZJ	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0.25	0	0	2	Combina-	1,4,6	0.0	0.0	0.5	0.0	0	2
4	4	0	2	tion block	GHS Ratinç	4	4	4	4	0	2
0	0	0	0	#3	1,5,6	0.0	0.0	0.3	0.0	0	2
4	4	0	0		GHS Ratinç	4	4	4	4	0	2
0.25	0	0	2		2,3,4	0.0	0.0	0.3	0.0	0	2
4	4	0	2		GHS Ratinç	4	4	4	4	0	2
0.25	0	0	2		2,3,5	0.0	0.0	0.0	0.0	0	0
4	4	0	2		GHS Ratinç	4	4	4	4	0	0

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F41365	HZI	24	1	4	1	3	3	3	43	EPA
			48	1	3	1	2	2	3	34	22
			72	1	2	0	2	2	3	24	GHS
			7 days	1	1	0	2	2	2	17	22
			14 days	2	1	0	2	2	2	22	
			21 days	1	1	0	1	1	0	9	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F41365	HZI	43	1	3	0.666667	2.33333333	2.333333333	3	22	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	1.0	2.7	2.0	22	22	Combina-	3,4,5	1.0	0.8
tion block	GHS Ratin	2	2	2	2	22	22	tion block	GHS Rating	2	4
#4	2,4,5	1.0	0.8	2.7	2.2	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	4	2	2	22	22		GHS Rating	2	2
	2,4,6	1.0	1.0	2.7	2.2	22	22		3,5,6	1.0	1.0
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	4
	2,5,6	1.0	1.0	2.7	2.0	14	14		4,5,6	1.0	0.8
	GHS Ratin	2	4	2	2	14	14		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZJ	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZJ	2	0	0	0	0.5	0	0	0	2

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.3	0.0	0	2	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	0	2	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	0.3	0.0	0	2	#5	3,4,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4
	2,4,6	0.0	0.0	0.5	0.0	0	2		3,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4
	2,5,6	0.0	0.0	0.3	0.0	0	2		4,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4

								1,5,6		4	0	
		TEST		CORNEAL				CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE		DRAIZE	CLEAR
0.1	F41379	HZI	24	1	2	1	2	2	3		29	EPA
			48	1	1	0	2	2	1		15	7
			72	1	1	0	2	1	0		11	GHS
			7 days	0	0	0	0	0	0		0	7
			14 days								0	
			21 days								0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F41379	HZI	29	1	1.333333	0.333333	2	1.666666667	1.333333333	7	7

Redness	Chemosis	DtC EPA	DtC GHS			
2.2	2.0	22	22	Summary	1,2,3	2	22
2	2	22	22	HZI	1,2,4	2	22
2.3	2.2	22	22		1,2,5	2	14
2	2	22	22		1,2,6	2	14
2.2	1.8	22	22		1,3,4	2	22
2	4	22	22		1,3,5	2	22
2.3	2.2	22	22		1,3,6	2	22
2	2	22	22		1,4,5	2	22
					1,4,6	2	22
					1,5,6	2	7

		TEST		CORNEAL				CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE		DRAIZE	CLEAR
0.1	5	HZJ	24	0	0	0	0	0	0		0	EPA
			48	0	0	0	0	0	0		0	0
			72								0	GHS
			7 days								0	0
			14 days								0	
			21 days								0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZJ	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS			
0.3	0.0	0	2	Summary	1,2,3	4	0
4	4	0	2	HZJ	1,2,4	4	2
0.5	0.0	0	2		1,2,5	4	0
4	4	0	2		1,2,6	4	2
0.3	0.0	0	2		1,3,4	4	2
4	4	0	2		1,3,5	4	0
0.5	0.0	0	2		1,3,6	4	2
4	4	0	2		1,4,5	4	2
					1,4,6	4	2
					1,5,6	4	2

0		4,5,6		4	0		0				
		TEST		CORNEAL				CONJUNCTIVAL			DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	F41405	HZI	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	3	2	2	39	
			72	1	3	1	2	2	1	30	GHS
			7 days	1	1	0	1	1	0	9	
			14 days	1	1	0	1	0	0	7	
			21 days	1	1	0	0	0	0	5	
	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	F41405	HZI	39	1	3.666667	1	2.33333333	2	1.666666667	0	0
22		2,3,4		2	22		22				
22		2,3,5		2	22		22				
14		2,3,6		2	22		22				
14		2,4,5		2	22		22				
22		2,4,6		2	22		22				
22		2,5,6		2	14		14				
22		3,4,5		2	22		22				
22		3,4,6		2	22		22				
22		3,5,6		2	22		22				
7		4,5,6		2	22		22				
		TEST		CORNEAL				CONJUNCTIVAL			DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	6	HZJ	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	
	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZJ	2	0	0	0	0.5	0	0	0	2
0		2,3,4		4	2		0				
0		2,3,5		4	0		0				
0		2,3,6		4	2		0				
0		2,4,5		4	2		0				
0		2,4,6		4	2		0				
0		2,5,6		4	2		0				
0		3,4,5		4	2		0				
0		3,4,6		4	2		0				
0		3,5,6		4	2		0				
0		4,5,6		4	2		0				

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZK	24	1	4	1	3	2	3	41	EPA
			48	1	3	1	3	1	2	32	7
			72	1	2	1	2	1	3	27	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZK	41	1.0	3.0	1.0	2.7	1.3	2.7	7	7
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZK	41	1.0	3.0	1.0	2.7	1.3	2.7	7	7
	2	HZK	39	1.0	3.7	1.0	2.7	1.7	2.3	22	22
	3	HZK	41	1.0	2.7	1.0	2.3	2.3	2.7	22	22
	4	HZK	35	1.0	4.0	1.0	2.0	1.7	1.0	22	22
	5	HZK	41	1.0	4.0	1.0	2.3	2.0	2.3	22	22
	6	HZK	41	1.0	3.0	1.0	2.7	2.0	2.3	22	22
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZL	24	1	3	1	2	1	3	32	EPA
			48	1	2	1	3	1	1	25	22
			72	1	1	1	2	1	0	16	GHS
			7 days	1	1	0	2	1	0	11	22
			14 days	1	1	0	1	1	0	9	
			21 days	1	1	0	1	0	0	7	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZL	32	1.0	2.0	1.0	2.3	1.0	1.3	22	22
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZL	32	1.0	2.0	1.0	2.3	1.0	1.3	22	22
	2	HZL	27	1.0	1.3	0.3	2.0	1.3	1.3	7	7
	3	HZL	35	1.0	2.0	0.3	1.7	1.0	1.0	7	7
	4	HZL	41	1.0	2.7	0.7	2.7	1.7	1.0	7	7
	5	HZL	41	1.0	2.0	1.0	3.0	2.0	2.3	22	22
	6	HZL	37	1.0	3.0	1.0	2.3	1.7	1.7	0	22
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZK	24	1	4	1	2	2	3	39	EPA
			48	1	4	1	3	1	2	37	22
			72	1	3	1	3	2	2	34	GHS
			7 days	1	2	0	2	1	0	16	22
			14 days	1	1	0	2	1	1	13	
			21 days	1	1	0	2	1	1	13	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZK	39	1	3.666667	1	2.666666667	1.666666667	2.333333333	22	22

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	1	1	2.666667	2	22	22
	GHS Rating	2	2	2	2	22	22
	1,2,4	1	1	2.666667	1.666667	22	22
	GHS Rating	2	2	2	4	22	22
	1,2,5	1	1	2.666667	1.833333	22	22
	GHS Rating	2	2	2	4	22	22
	1,2,6	1	1	2.666667	1.833333	22	22
	GHS Rating	2	2	2	4	22	22

	Combinations	Opacity	Iris
Combination block #2	1,3,4	1	1
	GHS Rating	2	2
	1,3,5	1	1
	GHS Rating	2	2
	1,3,6	1	1
	GHS Rating	2	2
	1,4,5	1	1
	GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZL	24	1	2	1	2	2	2	27	EPA
			48	1	1	0	2	1	2	15	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZL	27	1	1.333333	0.333333	2	1.333333333	1.333333333	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
Combination block #1	1,2,3	1	0.666667	2.166667	1.166667	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,4	1	0.833333	2.5	1.5	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,5	1	1	2.666667	1.666667	22	22
	GHS Rating	2	4	2	4	22	22
	1,2,6	1	1	2.333333	1.5	22	22
	GHS Rating	2	4	2	4	22	22

	Combinations	Opacity	Iris
Combination block #2	1,3,4	1	0.833333
	GHS Rating	2	4
	1,3,5	1	1
	GHS Rating	2	4
	1,3,6	1	1
	GHS Rating	2	4
	1,4,5	1	1
	GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZK	24	1	4	1	2	3	3	41	EPA
			48	1	2	1	3	2	2	29	22
			72	1	2	1	2	2	3	29	GHS
			7 days	1	2	0	3	2	1	22	22
			14 days	1	1	0	1	1	0	9	
			21 days	1	1	0	2	1	1	13	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZK	41	1	2.666667	1	2.333333333	2.33333333	2.666666667	22	22

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.5	2	22	22	Combina-	1,4,6	1.0	1.0	2.7	1.8	22	22
2	2	22	22	tion block	GHS Ratin	2	2	2	4	22	22
2.5	2.166667	22	22	#3	1,5,6	1.0	1.0	2.7	2.0	22	22
2	2	22	22		GHS Ratin	2	2	2	2	22	22
2.666667	2.166667	22	22		2,3,4	1.0	1.0	2.5	2.0	22	22
2	2	22	22		GHS Ratin	2	2	2	2	22	22
2.5	1.833333	22	22		2,3,5	1.0	1.0	2.5	2.2	22	22
2	4	22	22		GHS Ratin	2	2	2	2	22	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZL	24	1	4	1	2	1	2	35	EPA
			48	1	1	0	2	1	1	13	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZL	35	1	2	0.333333	1.666666667	1	1	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.5	1.333333	22	22	Combina-	1,4,6	1.0	1.0	2.5	1.7	22	22
2	4	22	22	tion block	GHS Ratin	2	2	2	4	22	22
2.666667	1.5	22	22	#3	1,5,6	1.0	1.0	2.7	1.8	22	22
2	4	22	22		GHS Ratin	2	2	2	4	22	22
2.333333	1.333333	22	22		2,3,4	1.0	0.5	2.3	1.5	7	7
2	4	22	22		GHS Ratin	2	4	2	4	7	7
2.833333	1.833333	22	22		2,3,5	1.0	0.7	2.5	1.7	22	22
2	4	22	22		GHS Ratin	2	4	2	4	22	22

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZK	24	1	4	1	2	2	1	35	EPA
			48	1	4	1	2	2	1	35	22
			72	1	4	1	2	1	1	33	GHS
			7 days	1	2	1	2	1	0	21	22
			14 days	1	2	0	1	1	0	14	
			21 days	1	1	0	1	0	0	7	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZK	35	1	4	1	2	1.666666667	1	22	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	1.0	2.7	2.2	22	22	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	2	2	2	22	22	tion block	GHS Rating	2	2
#4	2,4,5	1.0	1.0	2.5	1.8	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2
	2,4,6	1.0	1.0	2.7	1.8	22	22		3,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2
	2,5,6	1.0	1.0	2.7	2.0	22	22		4,5,6	1.0	1.0
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZL	24	1	4	1	3	2	3	41	EPA
			48	1	3	1	3	2	0	30	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZL	41	1	2.666667	0.666667	2.66666667	1.666666667	1	7	7

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.7	2.2	1.5	7	22	Combina-	3,4,5	1.0	0.8
tion block	GHS Ratin	2	4	2	4	7	22	tion block	GHS Rating	2	4
#4	2,4,5	1.0	0.8	2.8	1.8	22	22	#5	3,4,6	1.0	0.8
	GHS Ratin	2	4	2	4	22	22		GHS Rating	2	4
	2,4,6	1.0	0.8	2.5	1.7	7	22		3,5,6	1.0	1.0
	GHS Ratin	2	4	2	4	7	22		GHS Rating	2	4
	2,5,6	1.0	1.0	2.7	1.8	22	22		4,5,6	1.0	1.0
	GHS Ratin	2	4	2	4	22	22		GHS Rating	2	2

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZK	24	1	4	1	3	2	3	41	EPA
			48	1	4	1	2	2	2	37	22
			72	1	4	1	2	2	2	37	GHS
			7 days	1	2	1	2	2	2	27	22
			14 days	2	1	1	2	2	2	27	
			21 days	3	1	0	2	2	1	25	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZK	41	1	4	1	2.33333333	2	2.333333333	22	22

Redness	Chemosis	DtC EPA	DtC GHS
2.3	2.2	22	22
2	2	22	22
2.5	2.2	22	22
2	2	22	22
2.5	2.2	22	22
2	2	22	22
2.5	2.0	22	22
2	2	22	22

Summary			
HZK	1,2,3	2	22
	1,2,4	2	22
	1,2,5	2	22
	1,2,6	2	22
	1,3,4	2	22
	1,3,5	2	22
	1,3,6	2	22
	1,4,5	2	22
	1,4,6	2	22
	1,5,6	2	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZL	24	1	4	1	3	2	3	41	EPA
			48	1	1	1	3	2	2	24	22
			72	1	1	1	3	2	2	24	GHS
			7 days	1	1	0	2	1	1	13	22
			14 days	2	1	0	2	1	1	18	
			21 days	2	1	0	1	1	0	14	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZL	41	1	2	1	3	2	2.333333333	22	22

Redness	Chemosis	DtC EPA	DtC GHS
2.8	1.8	22	22
2	4	22	22
2.5	1.7	7	22
2	4	7	22
2.7	1.8	22	22
2	4	22	22
2.8	1.8	22	22
2	4	22	22

Summary			
HZL	1,2,3	2	22
	1,2,4	2	22
	1,2,5	2	22
	1,2,6	2	22
	1,3,4	2	22
	1,3,5	2	22
	1,3,6	2	22
	1,4,5	2	22
	1,4,6	2	22
	1,5,6	2	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZK	24	1	4	1	3	2	3	41	EPA
			48	1	3	1	3	2	3	36	22
			72	1	2	1	2	2	1	25	GHS
			7 days	1	2	1	2	2	2	27	22
			14 days	1	1	0	1	1	0	9	
			21 days	1	1	0	1	1	0	9	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZK	41	1	3	1	2.66666667	2	2.333333333	22	22
22		2,3,4	2	22		22					
22		2,3,5	2	22		22					
22		2,3,6	2	22		22					
22		2,4,5	2	22		22					
22		2,4,6	2	22		22					
22		2,5,6	2	22		22					
22		3,4,5	2	22		22					
22		3,4,6	2	22		22					
22		3,5,6	2	22		22					
22		4,5,6	2	22		22					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZL	24	1	4	1	2	2	2	37	22
			48	1	3	1	3	2	2	34	
			72	1	2	1	2	1	1	23	GHS
			7 days	1	1	0	2	1	0	11	22
			14 days	1	1	0	0	0	0	5	
			21 days	1	1	0	0	0	0	5	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZL	37	1	3	1	2.33333333	1.666666667	1.666666667	0	22
22		2,3,4	2	7		7					
22		2,3,5	2	22		22					
22		2,3,6	2	22		7					
22		2,4,5	2	22		22					
22		2,4,6	2	22		7					
22		2,5,6	2	22		22					
22		3,4,5	2	22		22					
22		3,4,6	2	22		7					
22		3,5,6	2	22		22					
22		4,5,6	2	22		22					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZM*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZM*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZM*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	2	HZM*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	3	HZM*	11	0.3	0.3	0.0	1.7	0.7	0.0	3	7
	4	HZM*	7	0.3	0.3	0.0	0.7	0.0	0.0	0	3
	5	HZM*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	6	HZM*	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZN*	24	1	1	0	2	1	0	11	EPA
			48	0	0	0	1	1	0	4	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZN*	11	0.3	0.3	0.0	1.0	0.7	0.0	3	3
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZN*	11	0.3	0.3	0.0	1.0	0.7	0.0	3	3
	2	HZN*	7	0.3	0.3	0.0	0.7	0.0	0.0	2	3
	3	HZN*	20	0.7	1.0	0.0	1.3	1.0	0.3	3	3
	4	HZN*	25	0.7	1.0	0.3	1.0	0.3	0.7	3	3
	5	HZN*	20	0.7	0.7	0.3	1.3	0.7	0.7	3	3
	6	HZN*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZM*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZM*	2	0	0	0	0.5	0	0	0	2

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.166667	0	1.083333	0.333333	3	7	Combination block #2	1,3,4	0.333333	0
	GHS Rating	4	4	4	4	3	7		GHS Rating	4	4
	1,2,4	0.166667	0	0.583333	0	0	3		1,3,5	0.166667	0
	GHS Rating	4	4	4	4	0	3		GHS Rating	4	4
	1,2,5	0	0	0.5	0	0	2		1,3,6	0.166667	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4
	1,2,6	0	0	0.5	0	0	2		1,4,5	0.166667	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZN*	24	1	1	0	1	0	0	7	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZN*	7	0.333333	0.333333	0	0.666666667	0	0	2	3

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.5	0	1.166667	0.833333	3	3	Combination block #2	1,3,4	0.666667	0.166667
	GHS Rating	4	4	4	4	3	3		GHS Rating	4	4
	1,2,4	0.5	0.166667	1	0.5	3	3		1,3,5	0.666667	0.166667
	GHS Rating	4	4	4	4	3	3		GHS Rating	4	4
	1,2,5	0.5	0.166667	1.166667	0.666667	3	3		1,3,6	0.5	0
	GHS Rating	4	4	4	4	3	3		GHS Rating	4	4
	1,2,6	0.333333	0	0.833333	0.333333	3	3		1,4,5	0.666667	0.333333
	GHS Rating	4	4	4	4	3	3		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZM*	24	1	1	0	2	1	0	11	EPA
			48	0	0	0	2	1	0	6	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZM*	11	0.333333	0.333333	0	1.666666667	0.66666667	0	3	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.166667	0.333333	3	7	Combina-	1,4,6	0.2	0.0	0.6	0.0	0	3
4	4	3	7	tion block	GHS Ratinç	4	4	4	4	0	3
1.083333	0.333333	3	7	#3	1,5,6	0.0	0.0	0.5	0.0	0	2
4	4	3	7		GHS Ratinç	4	4	4	4	0	2
1.083333	0.333333	3	7		2,3,4	0.3	0.0	1.2	0.3	3	7
4	4	3	7		GHS Ratinç	4	4	4	4	3	7
0.583333	0	0	3		2,3,5	0.2	0.0	1.1	0.3	3	7
4	4	0	3		GHS Ratinç	4	4	4	4	3	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZN*	24	1	2	0	2	2	1	20	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZN*	20	0.666667	1	0	1.333333333	1	0.333333333	3	3

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.166667	0.833333	3	3	Combina-	1,4,6	0.5	0.2	1.0	0.5	3	3
4	4	3	3	tion block	GHS Ratinç	4	4	4	4	3	3
1.333333	0.833333	3	3	#3	1,5,6	0.5	0.2	1.2	0.7	3	3
4	4	3	3		GHS Ratinç	4	4	4	4	3	3
1.166667	0.833333	3	3		2,3,4	0.7	0.2	1.2	0.7	3	3
4	4	3	3		GHS Ratinç	4	4	4	4	3	3
1.166667	0.666667	3	3		2,3,5	0.7	0.2	1.3	0.8	3	3
4	4	3	3		GHS Ratinç	4	4	4	4	3	3

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZM*	24	1	1	0	1	0	0	7	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZM*	7	0.333333	0.333333	0	0.66666667	0	0	0	3

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.2	0.0	1.1	0.3	3	7	Combina-	3,4,5	0.3	0.0
tion block	GHS Ratin	4	4	4	4	3	7	tion block	GHS Rating	4	4
#4	2,4,5	0.2	0.0	0.6	0.0	0	3	#5	3,4,6	0.3	0.0
	GHS Ratin	4	4	4	4	0	3		GHS Rating	4	4
	2,4,6	0.2	0.0	0.6	0.0	0	3		3,5,6	0.2	0.0
	GHS Ratin	4	4	4	4	0	3		GHS Rating	4	4
	2,5,6	0.0	0.0	0.5	0.0	0	2		4,5,6	0.2	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZN*	24	1	2	1	2	1	2	25	EPA
			48	1	1	0	1	0	0	7	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZN*	25	0.666667	1	0.333333	1	0.333333333	0.666666667	3	3

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.5	0.0	1.0	0.5	3	3	Combina-	3,4,5	0.7	0.3
tion block	GHS Ratin	4	4	4	4	3	3	tion block	GHS Rating	4	4
#4	2,4,5	0.7	0.3	1.2	0.5	3	3	#5	3,4,6	0.7	0.2
	GHS Ratin	4	4	4	4	3	3		GHS Rating	4	4
	2,4,6	0.5	0.2	0.8	0.2	3	3		3,5,6	0.7	0.2
	GHS Ratin	4	4	4	4	3	3		GHS Rating	4	4
	2,5,6	0.5	0.2	1.0	0.3	3	3		4,5,6	0.7	0.3
	GHS Ratin	4	4	4	4	3	3		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZM*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZM*	2	0	0	0	0.5	0	0	0	2

Redness	Chemosis	DtC EPA	DtC GHS
1.2	0.3	3	7
4	4	3	7
1.2	0.3	3	7
4	4	3	7
1.1	0.3	3	7
4	4	3	7
0.6	0.0	0	3
4	4	0	3

Summary			
HZM*	1,2,3	4	7
	1,2,4	4	3
	1,2,5	4	2
	1,2,6	4	2
	1,3,4	4	7
	1,3,5	4	7
	1,3,6	4	7
	1,4,5	4	3
	1,4,6	4	3
	1,5,6	4	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZN*	24	1	1	1	2	1	2	20	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZN*	20	0.666667	0.666667	0.333333	1.33333333	0.666666667	0.666666667	3	3

Redness	Chemosis	DtC EPA	DtC GHS
1.3	0.8	3	3
4	4	3	3
1.2	0.7	3	3
4	4	3	3
1.3	0.8	3	3
4	4	3	3
1.2	0.5	3	3
4	4	3	3

Summary			
HZN*	1,2,3	4	3
	1,2,4	4	3
	1,2,5	4	3
	1,2,6	4	3
	1,3,4	4	3
	1,3,5	4	3
	1,3,6	4	3
	1,4,5	4	3
	1,4,6	4	3
	1,5,6	4	3

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZM*	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZM*	0	0	0	0	0	0	0	0	0
3		2,3,4	4	7		3					
0		2,3,5	4	7		3					
0		2,3,6	4	7		3					
0		2,4,5	4	3		0					
3		2,4,6	4	3		0					
3		2,5,6	4	2		0					
3		3,4,5	4	7		3					
0		3,4,6	4	7		3					
0		3,5,6	4	7		3					
0		4,5,6	4	3		0					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZN*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZN*	2	0	0	0	0.5	0	0	0	2
3		2,3,4	4	3		3					
3		2,3,5	4	3		3					
3		2,3,6	4	3		3					
3		2,4,5	4	3		3					
3		2,4,6	4	3		3					
3		2,5,6	4	3		3					
3		3,4,5	4	3		3					
3		3,4,6	4	3		3					
3		3,5,6	4	3		3					
3		4,5,6	4	3		3					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZP	24	0	0	0	2	0	0	4	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZP	4	0.0	0.0	0.0	1.0	0.0	0.0	2	3
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZP	4	0.0	0.0	0.0	1.0	0.0	0.0	2	3
	2	HZP	4	0.0	0.0	0.0	1.0	0.0	0.0	2	2
	3	HZP	2	0.0	0.0	0.0	0.7	0.0	0.0	0	3
	4	HZP	2	0.0	0.0	0.0	0.7	0.0	0.0	0	3
	5	HZP	6	0.0	0.0	0.0	1.0	0.3	0.0	2	3
	6	HZP	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZQ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZQ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZQ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	2	HZQ	34	1.0	1.7	0.7	2.0	1.3	1.0	7	7
	3	HZQ	8	0.0	0.0	0.0	1.0	0.3	0.3	2	3
	4	HZQ	6	0.0	0.0	0.0	1.3	0.0	0.3	3	3
	5	HZQ	6	0.0	0.0	0.0	1.0	0.3	0.0	2	3
	6	HZQ	11	0.3	0.3	0.0	1.7	0.7	0.0	3	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZP	24	0	0	0	2	0	0	4	EPA
			48	0	0	0	0	0	0	0	2
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZP	4	0	0	0	1	0	0	2	2

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0	0	1	0	2	3	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	2	3		GHS Rating	4	4
	1,2,4	0	0	1	0	2	3		1,3,5	0	0
	GHS Rating	4	4	4	4	2	3		GHS Rating	4	4
	1,2,5	0	0	1	0.166667	2	3		1,3,6	0	0
	GHS Rating	4	4	4	4	2	3		GHS Rating	4	4
	1,2,6	0	0	1	0	2	3		1,4,5	0	0
	GHS Rating	4	4	4	4	2	3		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZQ	24	1	3	1	3	2	2	34	EPA
			48	1	1	1	2	1	1	18	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZQ	34	1	1.666667	0.666667	2	1.333333333	1	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.5	0.333333	1.5	0.833333	7	7	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,4	0.5	0.333333	1.666667	0.666667	7	7		1,3,5	0	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,5	0.5	0.333333	1.5	0.833333	7	7		1,3,6	0.166667	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,6	0.666667	0.333333	1.833333	1	7	7		1,4,5	0	0
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZP	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZP	2	0	0	0	0.666666667	0	0	0	3

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0.833333	0	2	3	Combina-	1,4,6	0.0	0.0	0.8	0.0	2	3
4	4	2	3	tion block	GHS Ratinç	4	4	4	4	2	3
1	0.166667	2	3	#3	1,5,6	0.0	0.0	1.0	0.2	2	3
4	4	2	3		GHS Ratinç	4	4	4	4	2	3
0.833333	0	2	3		2,3,4	0.0	0.0	0.8	0.0	2	3
4	4	2	3		GHS Ratinç	4	4	4	4	2	3
1	0.166667	2	3		2,3,5	0.0	0.0	1.0	0.2	2	3
4	4	2	3		GHS Ratinç	4	4	4	4	2	3

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZQ	24	0	0	0	2	1	1	8	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZQ	8	0	0	0	1	0.33333333	0.333333333	2	3

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.166667	0.166667	3	3	Combina-	1,4,6	0.2	0.0	1.5	0.3	3	7
4	4	3	3	tion block	GHS Ratinç	4	4	4	4	3	7
1	0.333333	2	3	#3	1,5,6	0.2	0.0	1.3	0.5	3	7
4	4	2	3		GHS Ratinç	4	4	4	4	3	7
1.333333	0.5	3	7		2,3,4	0.5	0.3	1.7	0.8	7	7
4	4	3	7		GHS Ratinç	4	4	4	4	7	7
1.166667	0.166667	3	3		2,3,5	0.5	0.3	1.5	0.8	7	7
4	4	3	3		GHS Ratinç	4	4	4	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZP	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	1	0	0	2	0
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZP	2	0	0	0	0.66666667	0	0	0	3

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.8	0.0	2	3	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	2	3	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	1.0	0.2	2	3	#5	3,4,6	0.0	0.0
	GHS Ratin	4	4	4	4	2	3		GHS Rating	4	4
	2,4,6	0.0	0.0	0.8	0.0	2	3		3,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	2	3		GHS Rating	4	4
	2,5,6	0.0	0.0	1.0	0.2	2	3		4,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	2	3		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZQ	24	0	0	0	2	0	0	4	EPA
			48	0	0	0	2	0	1	6	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZQ	6	0	0	0	1.33333333	0	0.333333333	3	3

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.7	0.3	1.8	1.0	7	7	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	7	7	tion block	GHS Rating	4	4
#4	2,4,5	0.5	0.3	1.7	0.8	7	7	#5	3,4,6	0.2	0.0
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,4,6	0.7	0.3	1.8	1.0	7	7		3,5,6	0.2	0.0
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,5,6	0.7	0.3	1.8	1.0	7	7		4,5,6	0.2	0.0
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	5	HZP	24	0	0	0	2	1	0	6	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZP	6	0	0	0	1	0.333333333	0	2	3

Redness	Chemosis	DtC EPA	DtC GHS
0.8	0.2	2	3
4	4	2	3
0.7	0.0	0	3
4	4	0	3
0.8	0.2	2	3
4	4	2	3
0.8	0.2	2	3
4	4	2	3

Summary			
HZP	1,2,3	4	3
	1,2,4	4	3
	1,2,5	4	3
	1,2,6	4	3
	1,3,4	4	3
	1,3,5	4	3
	1,3,6	4	3
	1,4,5	4	3
	1,4,6	4	3
	1,5,6	4	3

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	5	HZQ	24	0	0	0	2	1	0	6	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZQ	6	0	0	0	1	0.333333333	0	2	3

Redness	Chemosis	DtC EPA	DtC GHS
1.2	0.3	3	3
4	4	3	3
1.5	0.5	3	7
4	4	3	7
1.3	0.5	3	7
4	4	3	7
1.5	0.5	3	7
4	4	3	7

Summary			
HZQ	1,2,3	4	7
	1,2,4	4	7
	1,2,5	4	7
	1,2,6	4	7
	1,3,4	4	3
	1,3,5	4	3
	1,3,6	4	7
	1,4,5	4	3
	1,4,6	4	7
	1,5,6	4	7

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZP	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZP	2	0	0	0	0.5	0	0	0	2
2		2,3,4	4	3		2					
2		2,3,5	4	3		2					
2		2,3,6	4	3		2					
2		2,4,5	4	3		2					
2		2,4,6	4	3		2					
2		2,5,6	4	3		2					
2		3,4,5	4	3		2					
2		3,4,6	4	3		0					
2		3,5,6	4	3		2					
2		4,5,6	4	3		2					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZQ	24	1	1	0	2	1	0	11	EPA
			48	0	0	0	2	1	0	6	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZQ	11	0.333333	0.333333	0	1.66666667	0.666666667	0	3	7
7		2,3,4	4	7		7					
7		2,3,5	4	7		7					
7		2,3,6	4	7		7					
7		2,4,5	4	7		7					
3		2,4,6	4	7		7					
2		2,5,6	4	7		7					
3		3,4,5	4	3		3					
3		3,4,6	4	7		3					
3		3,5,6	4	7		3					
3		4,5,6	4	7		3					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZR*	24	1	3	1	3	3	1	34	EPA
			48	1	1	1	3	2	1	22	7
			72	0	0	0	2	1	0	6	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZR*	34	0.7	1.3	0.7	2.7	2.0	0.7	7	7
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZR*	34	0.7	1.3	0.7	2.7	2.0	0.7	7	7
	2	HZR*	23	0.7	1.0	0.3	1.7	1.0	0.3	3	7
	3	HZR*	13	0.3	0.3	0.0	1.7	0.7	1.0	3	7
	4	HZR*	31	1.0	1.7	1.0	2.0	2.3	2.0	22	22
	5	HZR*	29	0.7	1.0	0.3	1.7	1.0	1.0	3	7
	6	HZR*	34	1.0	1.7	0.7	2.0	1.3	1.0	7	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40824	HZS	24	1	4	1	3	2	2	39	EPA
			48	1	4	1	2	1	1	33	14
			72	1	2	1	2	1	1	23	GHS
			7 days	1	1	0	1	1	0	9	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	F40824	HZS	39	1.0	3.3	1.0	2.3	1.3	1.3	14	14
	ANIMAL ID										
Summary block used analysis of the twenty combinations	F40824	HZS	39	1.0	3.3	1.0	2.3	1.3	1.3	14	14
	F40863	HZS	39	1.0	3.3	1.0	2.3	1.7	1.3	22	22
	F40885	HZS	37	1.0	3.3	1.0	2.0	2.0	2.0	7	7
	F41368	HZS	41	1.0	4.0	1.0	2.0	2.0	2.3	22	22
	5	HZS	39	1.0	2.7	1.0	2.0	1.7	2.0	7	7
	6	HZS	39	1.0	3.0	1.0	2.0	2.0	2.3	22	22
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZR*	24	1	2	1	2	1	1	23	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	1	0	4	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZR*	23	0.666667	1	0.333333	1.666666667	1	0.333333333	3	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.666667	0.5	2.166667	1.5	7	7	Combination block #2	1,3,4	0.833333	0.833333
	GHS Rating	4	4	2	4	7	7		GHS Rating	4	4
	1,2,4	0.833333	0.833333	2.333333	2.166667	22	22		1,3,5	0.666667	0.5
	GHS Rating	4	4	2	2	22	22		GHS Rating	4	4
	1,2,5	0.666667	0.5	2.166667	1.5	7	7		1,3,6	0.833333	0.666667
	GHS Rating	4	4	2	4	7	7		GHS Rating	4	4
	1,2,6	0.833333	0.666667	2.333333	1.666667	7	7		1,4,5	0.833333	0.833333
	GHS Rating	4	4	2	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40863	HZS	24	1	4	1	2	2	3	39	EPA
			48	1	4	1	3	1	1	35	22
			72	1	2	1	2	2	0	23	GHS
			7 days	1	1	0	2	1	0	11	22
			14 days	1	1	0	2	1	1	13	
			21 days	0	0	0	1	1	1	6	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F40863	HZS	39	1	3.333333	1	2.333333333	1.666666667	1.333333333	22	22

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	1	2.333333	1.833333	22	22	Combination block #2	1,3,4	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2
	1,2,4	1	1	2.333333	1.833333	22	22		1,3,5	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2
	1,2,5	1	1	2.333333	1.666667	22	22		1,3,6	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2
	1,2,6	1	1	2.333333	1.833333	22	22		1,4,5	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZR*	24	1	1	0	2	1	1	13	EPA
			48	0	0	0	2	1	2	10	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZR*	13	0.333333	0.333333	0	1.666666667	0.66666667	1	3	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.333333	2.166667	22	22	Combina-	1,4,6	1.0	0.8	2.3	2.2	22	22
2	2	22	22	tion block	GHS Ratinç	2	4	2	2	22	22
2.166667	1.5	7	7	#3	1,5,6	0.8	0.7	2.3	1.7	7	7
2	4	7	7		GHS Ratinç	4	4	2	4	7	7
2.333333	1.666667	7	7		2,3,4	0.8	0.7	1.8	1.7	22	22
2	4	7	7		GHS Ratinç	4	4	4	4	22	22
2.333333	2.166667	22	22		2,3,5	0.7	0.3	1.7	1.0	3	7
2	2	22	22		GHS Ratinç	4	4	4	4	3	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F40885	HZS	24	1	4	1	2	2	2	37	EPA
			48	1	3	1	2	2	2	32	7
			72	1	3	1	2	2	2	32	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F40885	HZS	37	1	3.333333	1	2	2	2	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.166667	2	22	22	Combina-	1,4,6	1.0	1.0	2.2	2.0	22	22
2	2	22	22	tion block	GHS Ratinç	2	2	2	2	22	22
2.166667	1.833333	14	14	#3	1,5,6	1.0	1.0	2.2	1.8	22	22
2	4	14	14		GHS Ratinç	2	2	2	4	22	22
2.166667	2	22	22		2,3,4	1.0	1.0	2.2	2.0	22	22
2	2	22	22		GHS Ratinç	2	2	2	2	22	22
2.166667	1.833333	22	22		2,3,5	1.0	1.0	2.2	1.8	22	22
2	4	22	22		GHS Ratinç	2	2	2	4	22	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZR*	24	1	2	1	2	3	3	31	EPA
			48	1	2	1	2	2	2	27	22
			72	1	1	1	2	2	1	20	GHS
			7 days	1	1	0	2	1	1	13	22
			14 days	2	1	0	1	1	0	14	
			21 days	1	1	0	0	0	0	5	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZR*	31	1	1.666667	1	2	2.333333333	2	22	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.8	0.5	1.8	1.2	7	7	Combina-	3,4,5	0.8	0.7
tion block	GHS Ratin	4	4	4	4	7	7	tion block	GHS Rating	4	4
#4	2,4,5	0.8	0.7	1.8	1.7	22	22	#5	3,4,6	1.0	0.8
	GHS Ratin	4	4	4	4	22	22		GHS Rating	4	4
	2,4,6	1.0	0.8	2.0	1.8	22	22		3,5,6	0.8	0.5
	GHS Ratin	2	4	2	4	22	22		GHS Rating	4	4
	2,5,6	0.8	0.5	1.8	1.2	7	7		4,5,6	1.0	0.8
	GHS Ratin	4	4	4	4	7	7		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	F41368	HZS	24	1	4	1	2	3	3	41	EPA
			48	1	4	1	2	2	2	37	22
			72	1	4	1	2	1	2	35	GHS
			7 days	1	2	1	2	2	2	27	22
			14 days	3	1	0	3	2	3	31	
			21 days	2	1	0	2	2	1	20	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
F41368	HZS	41	1	4	1	2	2	2.333333333	22	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	1.0	2.2	2.0	22	22	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	2	2	2	22	22	tion block	GHS Rating	2	2
#4	2,4,5	1.0	1.0	2.2	1.8	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2
	2,4,6	1.0	1.0	2.2	2.0	22	22		3,5,6	1.0	1.0
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	2
	2,5,6	1.0	1.0	2.2	1.8	22	22		4,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZR*	24	1	2	1	2	2	3	29	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZR*	29	0.666667	1	0.333333	1.66666667	1	1	3	7

Redness	Chemosis	DtC EPA	DtC GHS
1.8	1.7	22	22
4	4	22	22
2.0	1.8	22	22
2	4	22	22
1.8	1.2	7	7
4	4	7	7
2.0	1.8	22	22
2	4	22	22

Summary			
HZR*	1,2,3	2	7
	1,2,4	2	22
	1,2,5	2	7
	1,2,6	2	7
	1,3,4	2	22
	1,3,5	2	7
	1,3,6	2	7
	1,4,5	2	22
	1,4,6	2	22
	1,5,6	2	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZS	24	1	4	1	2	2	3	39	EPA
			48	1	2	1	2	2	2	27	7
			72	1	2	1	2	1	1	23	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZS	39	1	2.666667	1	2	1.666666667	2	7	7

Redness	Chemosis	DtC EPA	DtC GHS
2.0	2.0	22	22
2	2	22	22
2.0	2.0	22	22
2	2	22	22
2.0	2.0	22	22
2	2	22	22
2.0	2.0	22	22
2	2	22	22

Summary			
HZS	1,2,3	2	22
	1,2,4	2	22
	1,2,5	2	22
	1,2,6	2	22
	1,3,4	2	22
	1,3,5	2	14
	1,3,6	2	22
	1,4,5	2	22
	1,4,6	2	22
	1,5,6	2	22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZR*	24	1	3	1	2	2	3	34	EPA
			48	1	1	1	2	1	0	16	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZR*	34	1	1.666667	0.666667	2	1.333333333	1	7	7
7		2,3,4	4	22		22					
22		2,3,5	4	7		3					
7		2,3,6	4	7		7					
7		2,4,5	4	22		22					
22		2,4,6	2	22		22					
7		2,5,6	4	7		7					
7		3,4,5	4	22		22					
22		3,4,6	2	22		22					
22		3,5,6	4	7		7					
7		4,5,6	2	22		22					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZS	24	1	4	1	2	2	3	39	EPA
			48	1	2	1	2	2	2	27	22
			72	1	3	1	2	2	2	32	GHS
			7 days	1	2	1	2	2	2	27	22
			14 days	3	1	1	2	2	3	34	
			21 days	4	1	0	2	2	3	34	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZS	39	1	3	1	2	2	2.333333333	22	22
22		2,3,4	2	22		22					
22		2,3,5	2	22		22					
22		2,3,6	2	22		22					
22		2,4,5	2	22		22					
22		2,4,6	2	22		22					
14		2,5,6	2	22		22					
22		3,4,5	2	22		22					
22		3,4,6	2	22		22					
22		3,5,6	2	22		22					
22		4,5,6	2	22		22					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	2	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	3	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	4	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	5	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	6	HZT	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	Dose Vol		0.1								

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZU*	24	1	2	1	3	2	2	29	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZU*	29	0.7	1.0	0.3	1.7	1.0	0.7	3	3

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZU*	29	0.7	1.0	0.3	1.7	1.0	0.7	3	3
	2	HZU*	31	1.0	2.0	0.3	1.7	1.0	0.0	7	7
	3	HZU*	22	1.0	1.3	0.0	2.0	1.3	0.7	7	7
	4	HZU*	25	0.7	1.0	0.3	1.3	0.7	0.7	3	3
	5	HZU*	24	0.7	0.7	0.3	1.7	1.0	1.0	3	7
	6	HZU*	13	0.3	0.3	0.0	1.3	0.3	0.3	3	3
	Dose Vol		0.1								

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZT	0	0	0	0	0	0	0	0	0

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0	0	0	0	0	0	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,4	0	0	0	0	0	0		1,3,5	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,5	0	0	0	0	0	0		1,3,6	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,6	0	0	0	0	0	0		1,4,5	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZU*	24	1	4	1	2	1	0	31	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	1	1	0	9	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZU*	31	1	2	0.333333	1.666666667	1	0	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	0.333333	1.833333	1.166667	7	7	Combination block #2	1,3,4	0.833333	0.333333
	GHS Rating	2	4	4	4	7	7		GHS Rating	4	4
	1,2,4	0.833333	0.333333	1.666667	1	7	7		1,3,5	0.833333	0.333333
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,5	0.833333	0.333333	1.666667	1	7	7		1,3,6	0.833333	0.166667
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,6	0.833333	0.333333	1.666667	1	7	7		1,4,5	0.666667	0.333333
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZT	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0	0	0	0	Combina-	1,4,6	0.0	0.0	0.0	0.0	0	0
4	4	0	0	tion block	GHS Ratinç	4	4	4	4	0	0
0	0	0	0	#3	1,5,6	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0
0	0	0	0		2,3,4	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0
0	0	0	0		2,3,5	0.0	0.0	0.0	0.0	0	0
4	4	0	0		GHS Ratinç	4	4	4	4	0	0

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZU*	24	1	2	0	2	2	2	22	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZU*	22	1	1.333333	0	2	1.33333333	0.666666667	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.833333	1.166667	7	7	Combina-	1,4,6	0.7	0.3	1.5	0.8	3	3
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	3	3
1.833333	1.166667	7	7	#3	1,5,6	0.7	0.3	1.7	1.0	3	7
4	4	7	7		GHS Ratinç	4	4	4	4	3	7
1.833333	1.166667	7	7		2,3,4	1.0	0.3	1.8	1.2	7	7
4	4	7	7		GHS Ratinç	2	4	4	4	7	7
1.666667	1	3	7		2,3,5	1.0	0.3	1.8	1.2	7	7
4	4	3	7		GHS Ratinç	2	4	4	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	4	HZT	0	0	0	0	0	0	0	0	0

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.0	0.0	0	0	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	0	0	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	0.0	0.0	0	0	#5	3,4,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4
	2,4,6	0.0	0.0	0.0	0.0	0	0		3,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4
	2,5,6	0.0	0.0	0.0	0.0	0	0		4,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	0		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZU*	24	1	2	1	2	1	2	25	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	4	HZU*	25	0.666667	1	0.333333	1.33333333	0.666666667	0.666666667	3	3

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.2	1.8	1.2	7	7	Combina-	3,4,5	0.8	0.3
tion block	GHS Ratin	2	4	4	4	7	7	tion block	GHS Rating	4	4
#4	2,4,5	0.8	0.3	1.7	1.0	7	7	#5	3,4,6	0.8	0.2
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,4,6	0.8	0.3	1.5	0.8	7	7		3,5,6	0.8	0.2
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4
	2,5,6	0.8	0.3	1.7	1.0	7	7		4,5,6	0.7	0.3
	GHS Ratin	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZT	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0
0.0	0.0	0	0
4	4	0	0

Summary			
HZT	1,2,3	4	0
	1,2,4	4	0
	1,2,5	4	0
	1,2,6	4	0
	1,3,4	4	0
	1,3,5	4	0
	1,3,6	4	0
	1,4,5	4	0
	1,4,6	4	0
	1,5,6	4	0

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZU*	24	1	1	1	2	2	3	24	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZU*	24	0.666667	0.666667	0.333333	1.66666667	1	1	3	7

Redness	Chemosis	DtC EPA	DtC GHS
1.8	1.2	7	7
4	4	7	7
1.7	1.0	7	7
4	4	7	7
1.8	1.2	7	7
4	4	7	7
1.5	0.8	3	7
4	4	3	7

Summary			
HZU*	1,2,3	2	7
	1,2,4	4	7
	1,2,5	4	7
	1,2,6	4	7
	1,3,4	4	7
	1,3,5	4	7
	1,3,6	4	7
	1,4,5	4	7
	1,4,6	4	3
	1,5,6	4	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZT	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZT	0	0	0	0	0	0	0	0	0
0		2,3,4	4	0		0					
0		2,3,5	4	0		0					
0		2,3,6	4	0		0					
0		2,4,5	4	0		0					
0		2,4,6	4	0		0					
0		2,5,6	4	0		0					
0		3,4,5	4	0		0					
0		3,4,6	4	0		0					
0		3,5,6	4	0		0					
0		4,5,6	4	0		0					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZU*	24	1	1	0	2	1	1	13	EPA
			48	0	0	0	2	0	0	4	3
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZU*	13	0.333333	0.333333	0	1.33333333	0.333333333	0.333333333	3	3
7		2,3,4	2	7		7					
7		2,3,5	2	7		7					
7		2,3,6	2	7		7					
7		2,4,5	4	7		7					
7		2,4,6	4	7		7					
7		2,5,6	4	7		7					
7		3,4,5	4	7		7					
3		3,4,6	4	7		7					
3		3,5,6	4	7		7					
3		4,5,6	4	7		3					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZV*	24	1	3	1	3	2	3	36	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZV*	36	1.0	1.7	0.3	2.3	1.3	1.0	7	7

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZV*	36	1.0	1.7	0.3	2.3	1.3	1.0	7	7
	2	HZV*	11	0.3	0.3	0.0	0.7	0.3	0.0	2	2
	3	HZV*	16	0.7	1.0	0.0	1.3	0.7	0.0	3	7
	4	HZV*	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	5	HZV*	20	0.7	0.7	0.3	1.7	1.0	0.3	3	7
	6	HZV*	7	0.3	0.3	0.0	0.7	0.0	0.0	2	3
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZW*	24	1	4	1	2	1	1	33	EPA
			48	1	2	1	2	1	0	21	7
			72	1	2	1	2	1	1	23	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZW*	33	1.0	2.7	1.0	2.0	1.0	0.7	7	7

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZW*	33	1.0	2.7	1.0	2.0	1.0	0.7	7	7
	2	HZW*	32	1.0	1.7	0.7	2.0	1.3	0.7	7	7
	3	HZW*	28	1.0	2.0	0.3	2.0	1.0	0.7	7	7
	4	HZW*	11	0.7	0.7	0.0	1.7	0.3	0.0	3	7
	5	HZW*	29	1.0	1.3	0.3	1.7	1.0	1.0	7	7
	6	HZW*	25	0.7	1.0	0.3	1.7	1.3	0.3	3	7
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZV*	24	1	1	0	2	1	0	11	EPA
			48	0	0	0	0	0	0	0	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZV*	11	0.333333	0.333333	0	0.666666667	0.333333333	0	2	2

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0.833333	0.166667	1.833333	1	7	7	Combination block #2	1,3,4	0.833333	0.166667
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,4	0.666667	0.166667	1.5	0.833333	7	7		1,3,5	0.833333	0.333333
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4
	1,2,5	0.833333	0.333333	2	1.166667	7	7		1,3,6	0.833333	0.166667
	GHS Rating	4	4	2	4	7	7		GHS Rating	4	4
	1,2,6	0.666667	0.166667	1.5	0.833333	7	7		1,4,5	0.833333	0.333333
	GHS Rating	4	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZW*	24	1	3	1	2	2	2	32	EPA
			48	1	1	1	2	1	0	16	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
2	HZW*	32	1	1.666667	0.666667	2	1.333333333	0.666666667	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	0.833333	2	1.166667	7	7	Combination block #2	1,3,4	1	0.666667
	GHS Rating	2	4	2	4	7	7		GHS Rating	2	4
	1,2,4	1	0.833333	2	1.166667	7	7		1,3,5	1	0.666667
	GHS Rating	2	4	2	4	7	7		GHS Rating	2	4
	1,2,5	1	0.833333	2	1.166667	7	7		1,3,6	1	0.666667
	GHS Rating	2	4	2	4	7	7		GHS Rating	2	4
	1,2,6	1	0.833333	2	1.333333	7	7		1,4,5	1	0.666667
	GHS Rating	2	4	2	4	7	7		GHS Rating	2	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZV*	24	1	2	0	2	1	0	16	EPA
			48	1	1	0	1	1	0	9	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZV*	16	0.666667	1	0	1.333333333	0.66666667	0	3	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
1.833333	1	7	7	Combina-	1,4,6	0.7	0.2	1.5	0.7	7	7
4	4	7	7	tion block	GHS Ratinç	4	4	4	4	7	7
2	1.166667	7	7	#3	1,5,6	0.8	0.3	2.0	1.2	7	7
2	4	7	7		GHS Ratinç	4	4	2	4	7	7
1.833333	1	7	7		2,3,4	0.5	0.0	1.0	0.5	3	7
4	4	7	7		GHS Ratinç	4	4	4	4	3	7
2	1.166667	7	7		2,3,5	0.7	0.2	1.5	0.8	3	7
2	4	7	7		GHS Ratinç	4	4	4	4	3	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	3	HZW*	24	1	3	1	2	1	1	28	EPA
			48	1	2	0	2	1	1	18	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
3	HZW*	28	1	2	0.333333	2	1	0.666666667	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2	1	7	7	Combina-	1,4,6	0.8	0.7	1.8	1.2	7	7
2	4	7	7	tion block	GHS Ratinç	4	4	4	4	7	7
2	1	7	7	#3	1,5,6	1.0	0.7	1.8	1.2	7	7
2	4	7	7		GHS Ratinç	2	4	4	4	7	7
2	1.166667	7	7		2,3,4	1.0	0.5	2.0	1.2	7	7
2	4	7	7		GHS Ratinç	2	4	2	4	7	7
1.833333	1	7	7		2,3,5	1.0	0.5	2.0	1.2	7	7
4	4	7	7		GHS Ratinç	2	4	2	4	7	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZV*	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZV*	2	0	0	0	0.5	0	0	0	2

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.5	0.0	1.0	0.5	3	7	Combina-	3,4,5	0.7	0.2
tion block	GHS Ratin	4	4	4	4	3	7	tion block	GHS Rating	4	4
#4	2,4,5	0.5	0.2	1.2	0.7	3	7	#5	3,4,6	0.5	0.0
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4
	2,4,6	0.3	0.0	0.7	0.2	2	3		3,5,6	0.7	0.2
	GHS Ratin	4	4	4	4	2	3		GHS Rating	4	4
	2,5,6	0.5	0.2	1.2	0.7	3	7		4,5,6	0.5	0.2
	GHS Ratin	4	4	4	4	3	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZW*	24	1	1	0	2	1	0	11	EPA
			48	1	1	0	2	0	0	9	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZW*	11	0.666667	0.666667	0	1.66666667	0.333333333	0	3	7

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.5	2.0	1.3	7	7	Combina-	3,4,5	1.0	0.3
tion block	GHS Ratin	2	4	2	4	7	7	tion block	GHS Rating	2	4
#4	2,4,5	1.0	0.5	1.8	1.2	7	7	#5	3,4,6	0.8	0.3
	GHS Ratin	2	4	4	4	7	7		GHS Rating	4	4
	2,4,6	0.8	0.5	1.8	1.3	7	7		3,5,6	1.0	0.3
	GHS Ratin	4	4	4	4	7	7		GHS Rating	2	4
	2,5,6	1.0	0.5	1.8	1.3	7	7		4,5,6	0.8	0.3
	GHS Ratin	2	4	4	4	7	7		GHS Rating	4	4

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZV*	24	1	1	1	2	2	1	20	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	0	0	2	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZV*	20	0.666667	0.666667	0.333333	1.66666667	1	0.333333333	3	7

Redness	Chemosis	DtC EPA	DtC GHS
1.5	0.8	3	7
4	4	3	7
1.0	0.3	3	7
4	4	3	7
1.5	0.8	3	7
4	4	3	7
1.2	0.5	3	7
4	4	3	7

Summary			
HZV*	1,2,3	4	7
	1,2,4	4	7
	1,2,5	2	7
	1,2,6	4	7
	1,3,4	4	7
	1,3,5	2	7
	1,3,6	4	7
	1,4,5	2	7
	1,4,6	4	7
	1,5,6	2	7

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZW*	24	1	2	1	2	2	3	29	EPA
			48	1	1	0	2	1	0	11	7
			72	1	1	0	1	0	0	7	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZW*	29	1	1.333333	0.333333	1.66666667	1	1	7	7

Redness	Chemosis	DtC EPA	DtC GHS
1.8	1.0	7	7
4	4	7	7
1.8	1.2	7	7
4	4	7	7
1.8	1.2	7	7
4	4	7	7
1.7	1.2	7	7
4	4	7	7

Summary			
HZW*	1,2,3	2	7
	1,2,4	2	7
	1,2,5	2	7
	1,2,6	2	7
	1,3,4	2	7
	1,3,5	2	7
	1,3,6	2	7
	1,4,5	2	7
	1,4,6	4	7
	1,5,6	2	7

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZV*	24	1	1	0	1	0	0	7	EPA
			48	0	0	0	1	0	0	2	2
			72	0	0	0	0	0	0	0	GHS
			7 days							0	3
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZV*	7	0.333333	0.333333	0	0.66666667	0	0	2	3
7		2,3,4	4	7		3					
7		2,3,5	4	7		3					
7		2,3,6	4	7		3					
7		2,4,5	4	7		3					
7		2,4,6	4	3		2					
7		2,5,6	4	7		3					
7		3,4,5	4	7		3					
7		3,4,6	4	7		3					
7		3,5,6	4	7		3					
7		4,5,6	4	7		3					

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZW*	24	1	2	1	2	2	1	25	EPA
			48	1	1	0	2	1	0	11	3
			72	0	0	0	1	1	0	4	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZW*	25	0.666667	1	0.333333	1.66666667	1.333333333	0.333333333	3	7
7		2,3,4	2	7		7					
7		2,3,5	2	7		7					
7		2,3,6	2	7		7					
7		2,4,5	2	7		7					
7		2,4,6	4	7		7					
7		2,5,6	2	7		7					
7		3,4,5	2	7		7					
7		3,4,6	4	7		7					
7		3,5,6	2	7		7					
7		4,5,6	4	7		7					

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZX	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	2	2	2	37	22
			72	1	4	1	2	2	2	37	GHS
			7 days	1	2	0	2	2	2	22	22
			14 days	1	1	0	2	2	2	17	
			21 days	1	1	0	2	1	0	11	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZX	37	1.0	4.0	1.0	2.0	2.0	2.0	22	22

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZX	37	1.0	4.0	1.0	2.0	2.0	2.0	22	22
	2	HZX	41	1.0	2.3	0.7	2.3	1.3	1.3	7	7
	3	HZX	39	1.0	2.7	0.7	2.7	1.3	1.3	7	7
	4	HZX	41	1.0	3.3	1.0	2.3	2.0	2.0	21	22
	5	HZX	39	1.0	4.0	1.0	2.3	2.0	2.0	22	22
	6	HZX	41	1.0	2.7	1.0	2.3	1.3	1.7	14	14
	Dose Vol		0.1								

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZY	24	1	4	1	2	3	3	41	EPA
			48	1	4	1	3	1	1	35	7
			72	1	3	1	2	1	1	28	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZY	41	1.0	3.7	1.0	2.3	1.7	1.7	7	7

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
Summary block used analysis of the twenty combinations	1	HZY	41	1.0	3.7	1.0	2.3	1.7	1.7	7	7
	2	HZY	37	1.0	4.0	1.0	2.7	1.7	1.7	14	14
	3	HZY	41	1.0	3.3	1.0	2.7	2.0	1.7	7	7
	4	HZY	37	1.0	3.7	1.0	2.3	2.0	1.7	21	21
	5	HZY	39	1.0	3.7	1.0	2.7	2.0	2.0	22	22
	6	HZY	31	1.0	4.0	0.3	2.0	1.0	1.0	22	22
	Dose Vol		0.1								

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZX	24	1	4	1	3	2	3	41	EPA
			48	1	2	1	2	1	1	23	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZX	41	1	2.333333	0.666667	2.333333333	1.333333333	1.333333333	7	7

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	0.833333	2.5	1.666667	22	22	Combination block #2	1,3,4	1	1
	GHS Rating	2	4	2	4	22	22		GHS Rating	2	2
	1,2,4	1	1	2.333333	2	22	22		1,3,5	1	1
	GHS Rating	2	2	2	2	22	22		GHS Rating	2	2
	1,2,5	1	1	2.333333	2	22	22		1,3,6	1	1
	GHS Rating	2	2	2	2	22	22		GHS Rating	2	2
	1,2,6	1	1	2.333333	1.666667	22	22		1,4,5	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZY	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	3	1	2	37	14
			72	1	4	1	3	2	1	37	GHS
			7 days	1	1	0	2	1	0	11	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZY	37	1	4	1	2.666666667	1.666666667	1.666666667	14	14

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	1	1	2.666667	1.833333	14	14	Combination block #2	1,3,4	1	1
	GHS Rating	2	2	2	4	14	14		GHS Rating	2	2
	1,2,4	1	1	2.5	1.833333	21	21		1,3,5	1	1
	GHS Rating	2	2	2	4	21	21		GHS Rating	2	2
	1,2,5	1	1	2.666667	1.833333	22	22		1,3,6	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2
	1,2,6	1	1	2.5	1.666667	22	22		1,4,5	1	1
	GHS Rating	2	2	2	4	22	22		GHS Rating	2	2

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	3	HZX	24	1	4	1	3	2	2	39	EPA
			48	1	3	1	3	1	2	32	7
			72	1	1	0	2	1	0	11	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZX	39	1	2.666667	0.666667	2.666666667	1.33333333	1.333333333	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.5	2	22	22	Combina-	1,4,6	1.0	1.0	2.3	2.0	22	22
2	2	22	22	tion block	GHS Ratin	2	2	2	2	22	22
2.5	2	22	22	#3	1,5,6	1.0	1.0	2.3	2.0	22	22
2	2	22	22		GHS Ratin	2	2	2	2	22	22
2.5	1.666667	22	22		2,3,4	1.0	0.8	2.5	1.7	21	22
2	4	22	22		GHS Ratin	2	4	2	4	21	22
2.333333	2	22	22		2,3,5	1.0	0.8	2.5	1.7	22	22
2	2	22	22		GHS Ratin	2	4	2	4	22	22

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	3	HZY	24	1	4	1	3	2	3	41	EPA
			48	1	4	1	3	2	2	39	7
			72	1	2	1	2	2	0	23	GHS
			7 days	0	0	0	0	0	0	0	7
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZY	41	1	3.333333	1	2.666666667	2	1.666666667	7	7

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
2.5	2	21	21	Combina-	1,4,6	1.0	1.0	2.3	1.8	22	22
2	2	21	21	tion block	GHS Ratin	2	2	2	4	22	22
2.666667	2	22	22	#3	1,5,6	1.0	1.0	2.5	1.8	22	22
2	2	22	22		GHS Ratin	2	2	2	4	22	22
2.5	1.833333	22	22		2,3,4	1.0	1.0	2.7	2.0	21	21
2	4	22	22		GHS Ratin	2	2	2	2	21	21
2.5	2	22	22		2,3,5	1.0	1.0	2.7	2.0	22	22
2	2	22	22		GHS Ratin	2	2	2	2	22	22

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZX	24	1	4	1	3	2	3	41	EPA
			48	1	3	1	2	2	1	30	21
			72	1	3	1	2	2	2	32	GHS
			7 days	1	2	1	2	2	2	27	22
			14 days	1	1	0	2	2	2	17	
			21 days	0	0	0	1	1	0	4	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZX	41	1	3.333333	1	2.33333333	2	2	21	22

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	0.8	2.5	1.3	14	14	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	4	2	4	14	14	tion block	GHS Rating	2	2
#4	2,4,5	1.0	1.0	2.3	2.0	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	2
	2,4,6	1.0	1.0	2.3	1.7	21	22		3,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	21	22		GHS Rating	2	2
	2,5,6	1.0	1.0	2.3	1.7	22	22		4,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZY	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	2	2	2	37	21
			72	1	3	1	3	2	1	32	GHS
			7 days	1	1	1	2	1	0	16	21
			14 days	1	1	0	1	1	1	11	
			21 days	0	0	0	0	0	0	0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
4	HZY	37	1	3.666667	1	2.33333333	2	1.666666667	21	21

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	1.0	1.0	2.7	1.8	22	22	Combina-	3,4,5	1.0	1.0
tion block	GHS Ratin	2	2	2	4	22	22	tion block	GHS Rating	2	2
#4	2,4,5	1.0	1.0	2.7	2.0	22	22	#5	3,4,6	1.0	1.0
	GHS Ratin	2	2	2	2	22	22		GHS Rating	2	2
	2,4,6	1.0	1.0	2.5	1.8	22	22		3,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2
	2,5,6	1.0	1.0	2.7	1.8	22	22		4,5,6	1.0	1.0
	GHS Ratin	2	2	2	4	22	22		GHS Rating	2	2

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZX	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	3	2	2	39	22
			72	1	4	1	2	2	2	37	GHS
			7 days	1	1	0	2	1	1	13	22
			14 days	1	1	0	1	1	0	9	
			21 days	1	1	0	0	0	0	5	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZX	39	1	4	1	2.33333333	2	2	22	22

Redness	Chemosis	DtC EPA	DtC GHS
2.5	2.0	22	22
2	2	22	22
2.5	1.7	21	22
2	4	21	22
2.5	1.7	22	22
2	4	22	22
2.3	2.0	22	22
2	2	22	22

Summary				
HZX	1,2,3	2	22	
	1,2,4	2	22	
	1,2,5	2	22	
	1,2,6	2	22	
	1,3,4	2	22	
	1,3,5	2	22	
	1,3,6	2	22	
	1,4,5	2	22	
	1,4,6	2	22	
	1,5,6	2	22	

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	5	HZY	24	1	4	1	2	2	2	37	EPA
			48	1	4	1	3	2	2	39	22
			72	1	3	1	3	2	2	34	GHS
			7 days	1	2	0	2	2	1	20	22
			14 days	2	1	0	2	2	1	20	
			21 days	2	1	0	2	2	1	20	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
5	HZY	39	1	3.666667	1	2.66666667	2	2	22	22

Redness	Chemosis	DtC EPA	DtC GHS
2.7	2.0	22	22
2	2	22	22
2.5	2.0	22	22
2	2	22	22
2.7	2.0	22	22
2	2	22	22
2.5	2.0	22	22
2	2	22	22

Summary				
HZY	1,2,3	2	14	
	1,2,4	2	21	
	1,2,5	2	22	
	1,2,6	2	22	
	1,3,4	2	21	
	1,3,5	2	22	
	1,3,6	2	22	
	1,4,5	2	22	
	1,4,6	2	22	
	1,5,6	2	22	

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZX	24	1	4	1	3	2	3	41	EPA
			48	1	2	1	2	1	2	25	14
			72	1	2	1	2	1	0	21	GHS
			7 days	1	1	0	0	0	0	5	14
			14 days	0	0	0	0	0	0	0	
			21 days							0	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
6	HZX	41	1	2.666667	1	2.33333333	1.333333333	1.666666667	14	14

22	2,3,4	2	22		21
22	2,3,5	2	22		22
22	2,3,6	2	14		14
22	2,4,5	2	22		22
22	2,4,6	2	22		21
22	2,5,6	2	22		22
22	3,4,5	2	22		22
22	3,4,6	2	22		21
22	3,5,6	2	22		22
22	4,5,6	2	22		22

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZY	24	1	4	0	2	1	2	30	EPA
			48	1	4	0	2	1	1	28	22
			72	1	4	1	2	1	0	31	GHS
			7 days	1	4	1	2	1	0	31	22
			14 days	1	4	0	1	1	0	24	
			21 days	1	1	0	1	0	0	7	

ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
6	HZY	31	1	4	0.333333	2	1	1	22	22

14	2,3,4	2	21		21
21	2,3,5	2	22		22
22	2,3,6	2	22		22
22	2,4,5	2	22		22
21	2,4,6	2	22		22
22	2,5,6	2	22		22
22	3,4,5	2	22		22
22	3,4,6	2	22		22
22	3,5,6	2	22		22
22	4,5,6	2	22		22

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	CONJUNCTIVAL IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	1	HZZ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
GHS Tissue	1	HZZ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	ANIMAL ID										
Summary block used analysis of the twenty combinations	1	HZZ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	2	HZZ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	3	HZZ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	4	HZZ	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	5	HZZ	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
	6	HZZ	2	0.0	0.0	0.0	0.5	0.0	0.0	0	2
	Dose Vol	0.1									

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	2	HZZ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	2	HZZ	0	0	0	0	0	0	0	0	0

	Combinations	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combination block #1	1,2,3	0	0	0	0	0	0	Combination block #2	1,3,4	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,4	0	0	0.25	0	0	2		1,3,5	0	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4
	1,2,5	0	0	0	0	0	0		1,3,6	0	0
	GHS Rating	4	4	4	4	0	0		GHS Rating	4	4
	1,2,6	0	0	0.25	0	0	2		1,4,5	0	0
	GHS Rating	4	4	4	4	0	2		GHS Rating	4	4

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	3	HZZ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	3	HZZ	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS		Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS
0.25	0	0	2	Combina-	1,4,6	0.0	0.0	0.5	0.0	0	2
4	4	0	2	tion block	GHS Ratin	4	4	4	4	0	2
0	0	0	0	#3	1,5,6	0.0	0.0	0.3	0.0	0	2
4	4	0	0		GHS Ratin	4	4	4	4	0	2
0.25	0	0	2		2,3,4	0.0	0.0	0.3	0.0	0	2
4	4	0	2		GHS Ratin	4	4	4	4	0	2
0.25	0	0	2		2,3,5	0.0	0.0	0.0	0.0	0	0
4	4	0	2		GHS Ratin	4	4	4	4	0	0

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	4	HZZ	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	4	HZZ	2	0	0	0	0.5	0	0	0	2

	Combinatio	Opacity	Iris	Redness	Chemosis	DtC EPA	DtC GHS		Combinations	Opacity	Iris
Combina-	2,3,6	0.0	0.0	0.3	0.0	0	2	Combina-	3,4,5	0.0	0.0
tion block	GHS Ratin	4	4	4	4	0	2	tion block	GHS Rating	4	4
#4	2,4,5	0.0	0.0	0.3	0.0	0	2	#5	3,4,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4
	2,4,6	0.0	0.0	0.5	0.0	0	2		3,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4
	2,5,6	0.0	0.0	0.3	0.0	0	2		4,5,6	0.0	0.0
	GHS Ratin	4	4	4	4	0	2		GHS Rating	4	4

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

		TEST		CORNEAL			CONJUNCTIVAL				DAYS-TO-
Volume	ANIMAL ID	MATL	TIME	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DRAIZE	CLEAR
0.1	5	HZZ	24	0	0	0	0	0	0	0	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	0
			14 days							0	
			21 days							0	

	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	5	HZZ	0	0	0	0	0	0	0	0	0

Redness	Chemosis	DtC EPA	DtC GHS
0.3	0.0	0	2
4	4	0	2
0.5	0.0	0	2
4	4	0	2
0.3	0.0	0	2
4	4	0	2
0.5	0.0	0	2
4	4	0	2

Summary			
HZZ	1,2,3	4	0
	1,2,4	4	2
	1,2,5	4	0
	1,2,6	4	2
	1,3,4	4	2
	1,3,5	4	0
	1,3,6	4	2
	1,4,5	4	2
	1,4,6	4	2
	1,5,6	4	2

IIVS Submission - In Vivo Data and Analysis for the Gettings et al. (1996) Study

Volume	ANIMAL ID	TEST MATL	TIME	CORNEAL OPACITY	CORNEAL AREA	IRIS	CONJUNCTIVAL REDNESS	CONJUNCTIVAL CHEMOSIS	CONJUNCTIVAL DISCHARGE	DRAIZE	DAYS-TO-CLEAR
0.1	6	HZZ	24	0	0	0	1	0	0	2	EPA
			48	0	0	0	0	0	0	0	0
			72							0	GHS
			7 days							0	2
			14 days							0	
			21 days							0	

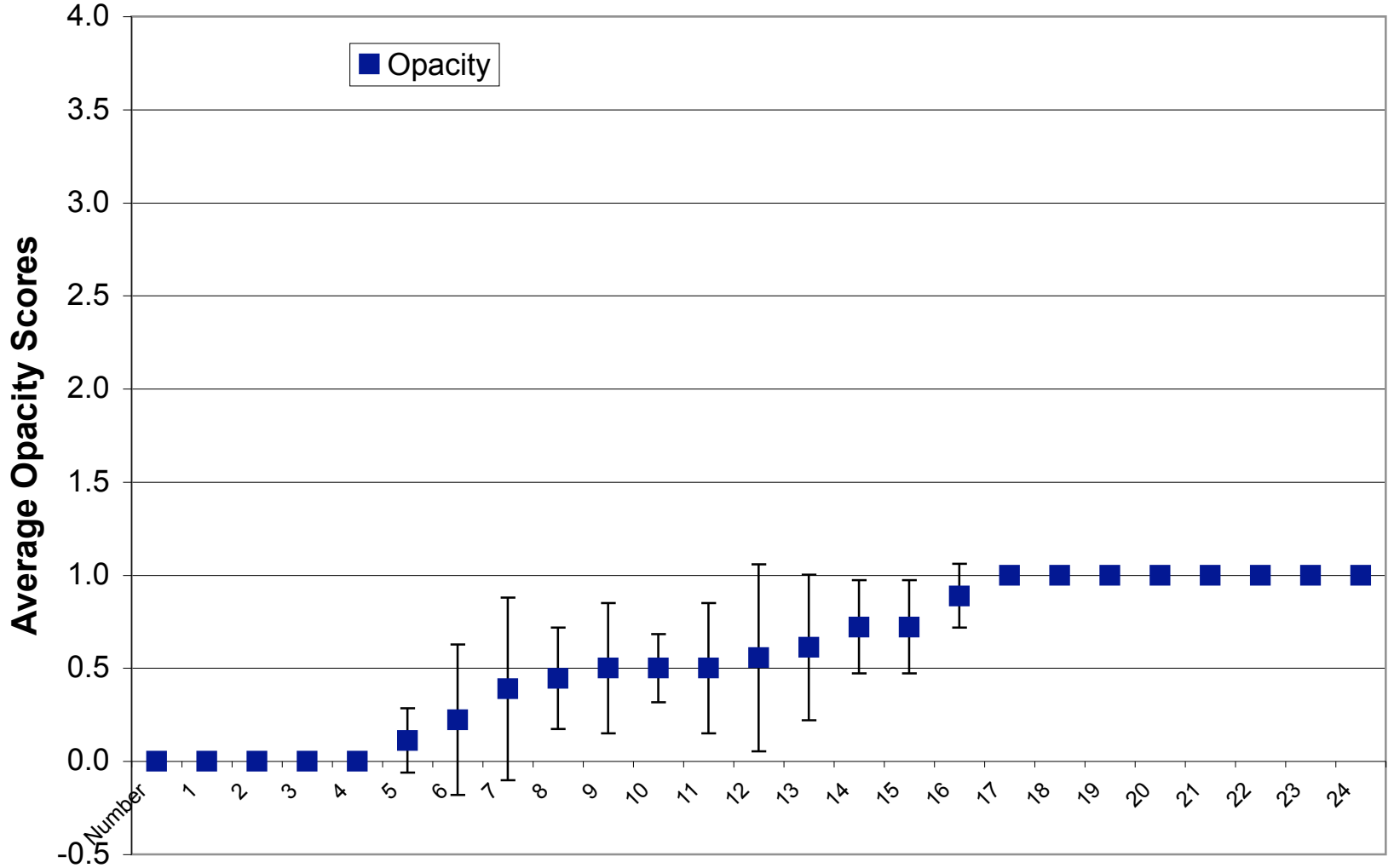
	ANIMAL ID	MATL	MAS	OPACITY	AREA	IRIS	REDNESS	CHEMOSIS	DISCHARGE	DtC EPA	DtC GHS
	6	HZZ	2	0	0	0	0.5	0	0	0	2
0		2,3,4	4	2		0					
0		2,3,5	4	0		0					
0		2,3,6	4	2		0					
0		2,4,5	4	2		0					
0		2,4,6	4	2		0					
0		2,5,6	4	2		0					
0		3,4,5	4	2		0					
0		3,4,6	4	2		0					
0		3,5,6	4	2		0					
0		4,5,6	4	2		0					

Summary of Boot Strap Analysis for the Gettings et al. (1996) Study

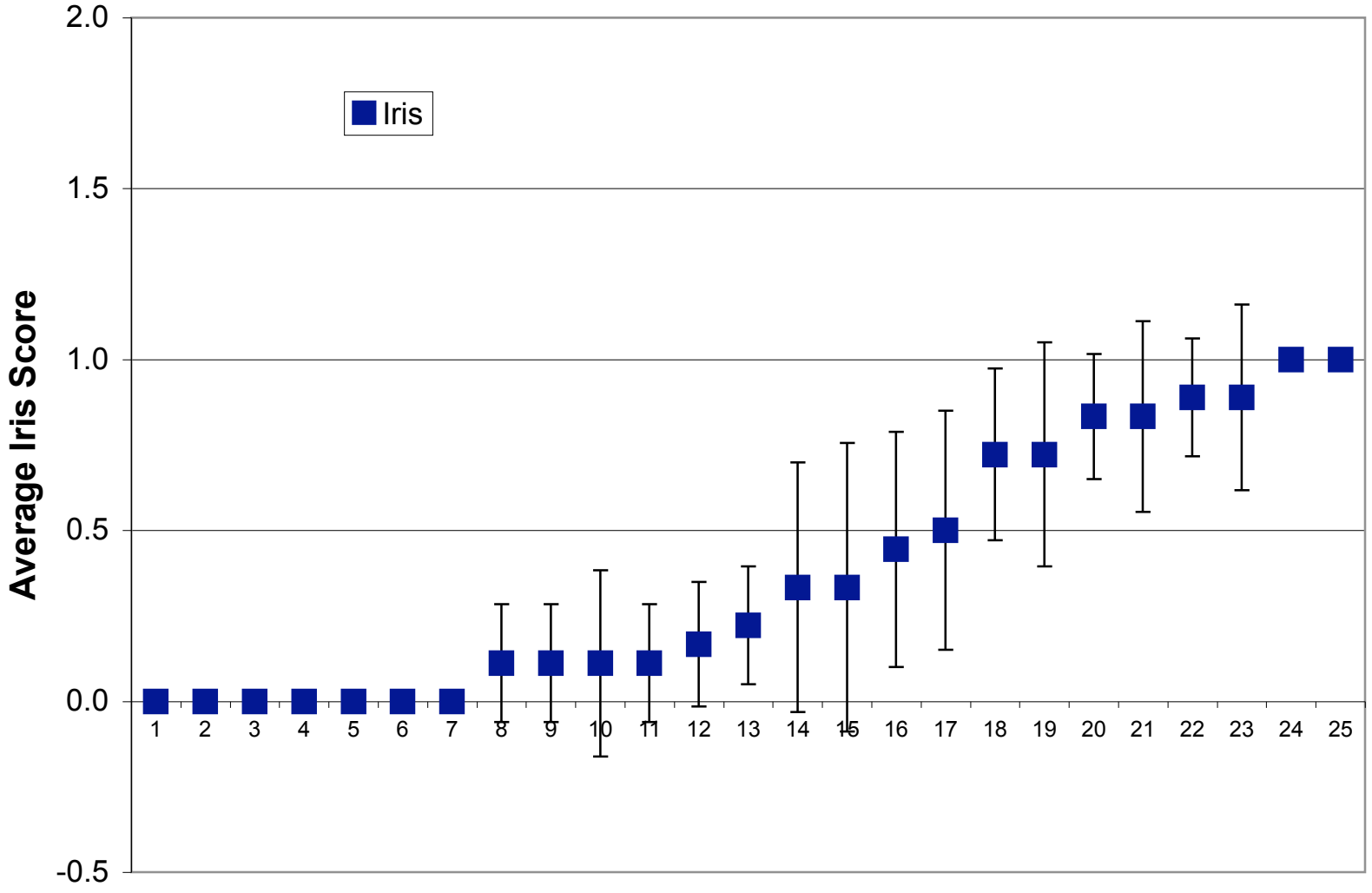
	Summary of the Animal Data									
Name	Material	GHS 1	GHS 2a	GHS 2b	GHS NI	EPA 1	EPA 2	EPA 3	EPA 4	Mean OD490	SD OD490
Shampoo 7	HZA	16	4	0	0	16	4	0	0	0.41	0.16
Liquid Soap 1	HZB*	0	0	4	16	0	0	20	0	0.20	0.02
Shampoo 1	HZC*	0	0	10	10	0	0	20	0	0.96	0.31
Shampoo 5	HZD*	0	0	0	20	0	0	20	0	0.23	0.08
Gel Cleaner	HZE	10	0	0	10	10	0	10	0	0.19	0.05
Baby Shampoo 2	HZF	16	4	0	0	16	4	0	0	0.42	0.08
Shampoo 8	HZG*	0	0	0	20	0	0	20	0	0.20	0.06
Eye Makeup re.	HZH	0	0	0	20	0	0	0	20	0.02	0.02
Skin Cleaner	HZI	16	3	1	0	16	3	1	0	0.77	0.04
Mild Shampoo	HZJ	0	0	0	20	0	0	0	20	0.05	0.03
Bubble bath	HZK	20	0	0	0	20	0	0	0	0.96	0.32
Foam Bath	HZL	19	0	1	0	16	0	4	0	0.91	0.26
Shampoo 3	HZM*	0	0	10	10	0	0	10	10	0.21	0.05
Shampoo 6	HZN*	0	0	0	20	0	0	20	0	0.27	0.08
Baby Shampoo 1	HZP	0	0	0	20	0	0	19	1	0.26	0.05
Cleaning Gel	HZQ	0	0	0	20	0	0	20	0	0.16	0.05
Facial Cl Foam	HZR*	10	0	6	4	10	0	10	0	0.24	0.02
Shower Gel	HZS	19	1	0	0	19	1	0	0	1.55	0.09
Polishing Scrub	HZT	0	0	0	20	0	0	0	20	0.00	0.00
Hand Soap	HZU*	0	0	4	16	0	0	20	0	0.29	0.09
Shampoo 4	HZV*	0	0	4	16	0	0	20	0	0.27	0.04
Liquid Soap 2	HZW*	0	0	16	4	0	0	20	0	0.35	0.10
Shampoo 2	HZX	19	1	0	0	16	4	0	0	0.71	0.29
Shampoo AntiD	HZY	16	4	0	0	16	4	0	0	0.85	0.20
Facial Cleaner	HZZ	0	0	0	20	0	0	0	20	0.00	0.00

Name	Material	GHS 1	GHS 2a	GHS 2b	GHS NI	EPA 1	EPA 2	EPA 3	EPA 4	Mean OD490	SD OD490
Polishing Scrub	HZT	0	0	0	20	0	0	0	20	0.001	0.001
Facial Cleaner	HZZ	0	0	0	20	0	0	0	20	0.004	0.004
Eye Makeup re.	HZH	0	0	0	20	0	0	0	20	0.020	0.016
Mild Shampoo	HZJ	0	0	0	20	0	0	0	20	0.050	0.025
Cleaning Gel	HZQ	0	0	0	20	0	0	20	0	0.164	0.050
Gel Cleaner	HZE	10	0	0	10	10	0	10	0	0.194	0.048
Shampoo 8	HZG*	0	0	0	20	0	0	20	0	0.197	0.058
Liquid Soap 1	HZB*	0	0	4	16	0	0	0	0	0.199	0.024
Shampoo 3	HZM*	0	0	10	10	0	0	10	10	0.214	0.049
Shampoo 5	HZD*	0	0	0	20	0	0	20	0	0.231	0.084
Facial Cl Foam	HZR*	10	0	6	4	10	0	10	0	0.239	0.022
Baby Shampoo 1	HZP	0	0	0	20	0	0	19	1	0.261	0.051
Shampoo 6	HZN*	0	0	0	20	0	0	20	0	0.267	0.076
Shampoo 4	HZV*	0	0	4	16	0	0	20	0	0.268	0.045
Hand Soap	HZU*	0	0	4	16	0	0	20	0	0.293	0.092
Liquid Soap 2	HZW*	0	0	16	4	0	0	20	0	0.352	0.100
Shampoo 7	HZA	16	4	0	0	16	4	0	0	0.406	0.156
Baby Shampoo 2	HZF	16	4	0	0	16	4	0	0	0.425	0.082
Shampoo 2	HZX	19	1	0	0	16	4	0	0	0.705	0.289
Skin Cleaner	HZI	16	3	1	0	16	3	1	0	0.769	0.036
Shampoo AntiD	HZY	16	4	0	0	16	4	0	0	0.847	0.199
Foam Bath	HZL	19	0	1	0	16	0	4	0	0.912	0.261
Bubble bath	HZK	20	0	0	0	20	0	0	0	0.956	0.324
Shampoo 1	HZC*	0	0	10	10	0	0	20	0	0.957	0.306
Shower Gel	HZS	19	1	0	0	19	1	0	0	1.548	0.093

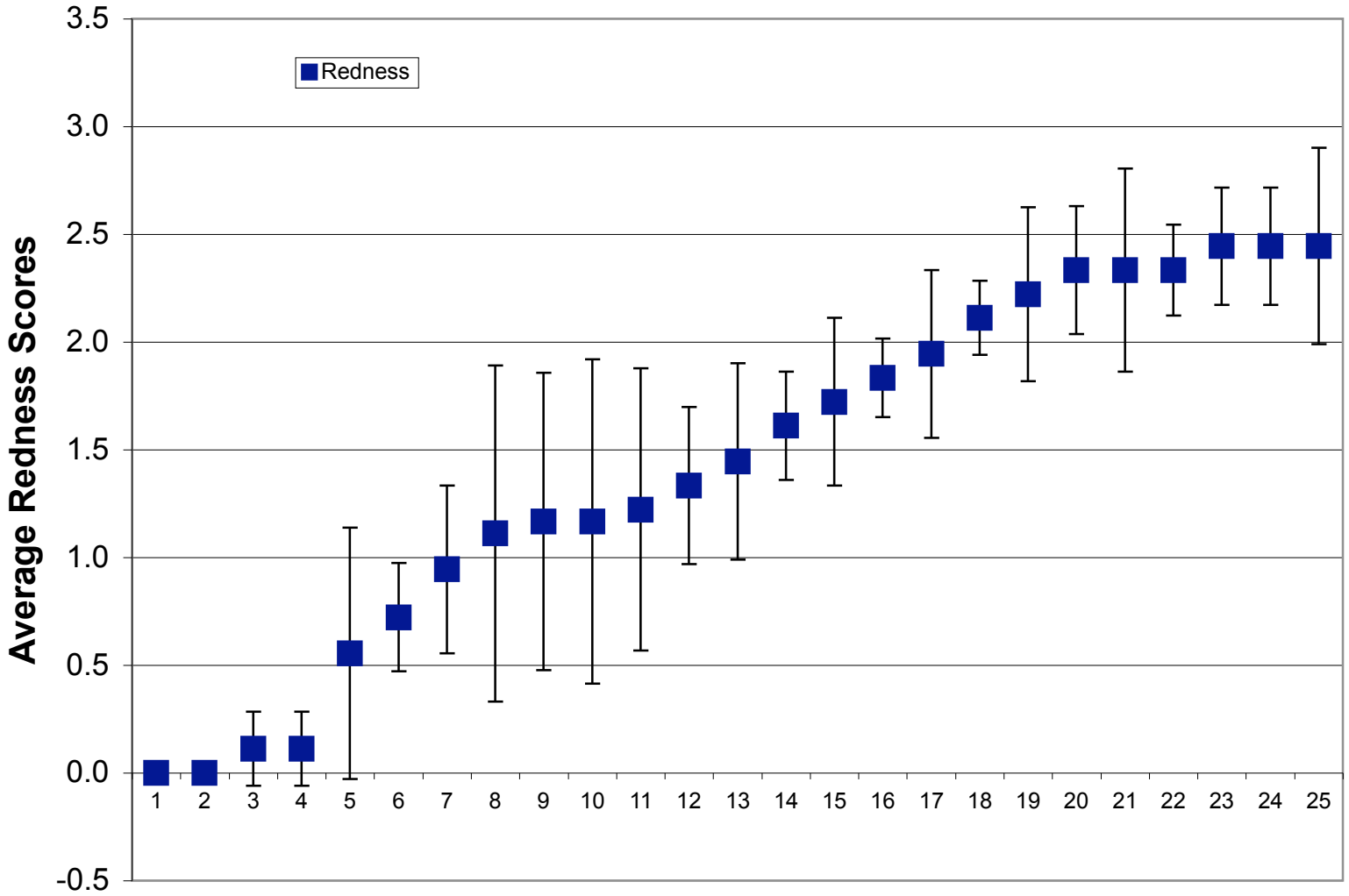
IIVS Submission - Analysis of In Vivo Data for Gettings et al. (1996)



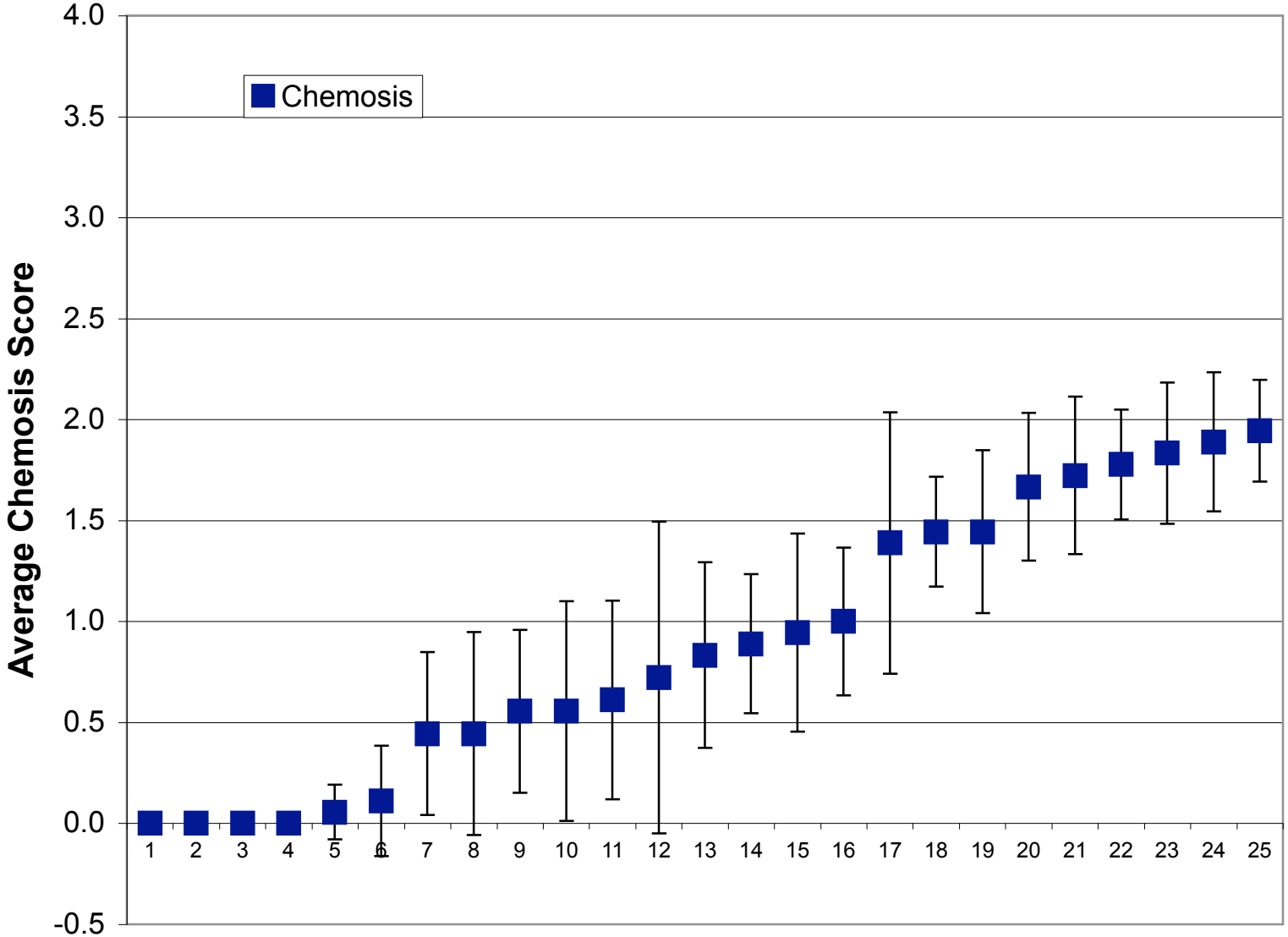
IIVS Submission - Analysis of In Vivo Data for Gettings et al. (1996)



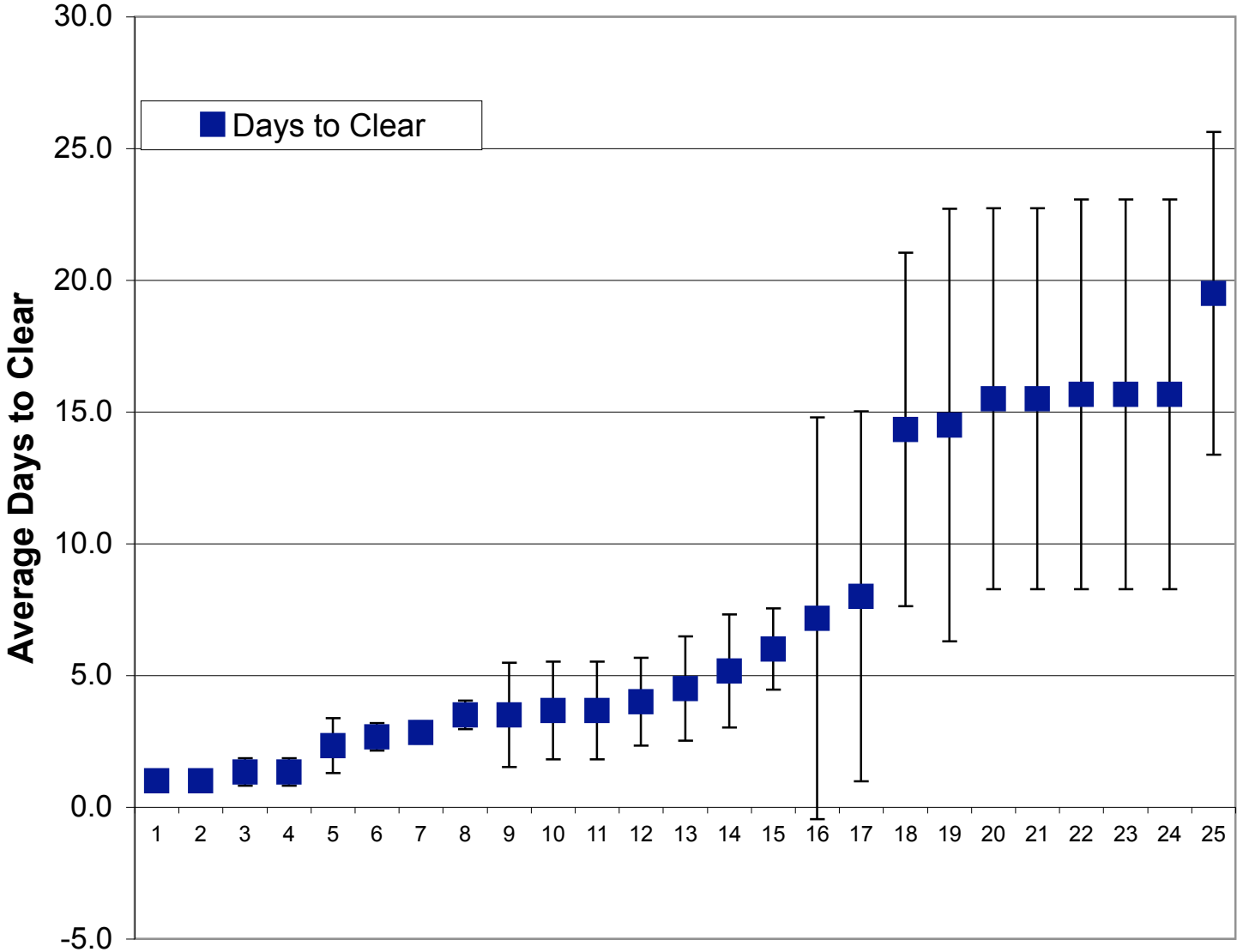
IIVS Submission - Analysis of In Vivo Data for Gettings et al. (1996)



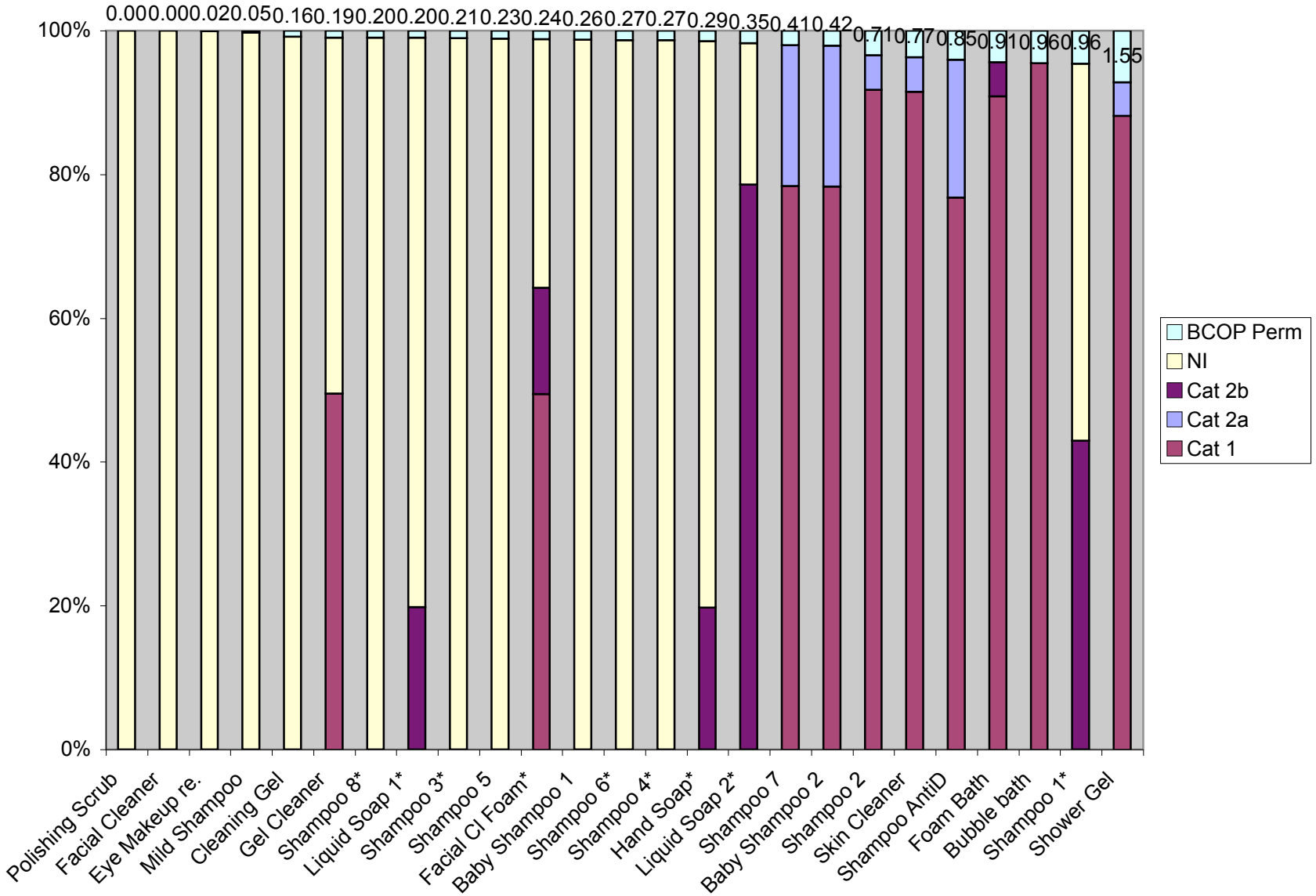
IIVS Submission - Analysis of In Vivo Data for Gettings et al. (1996)



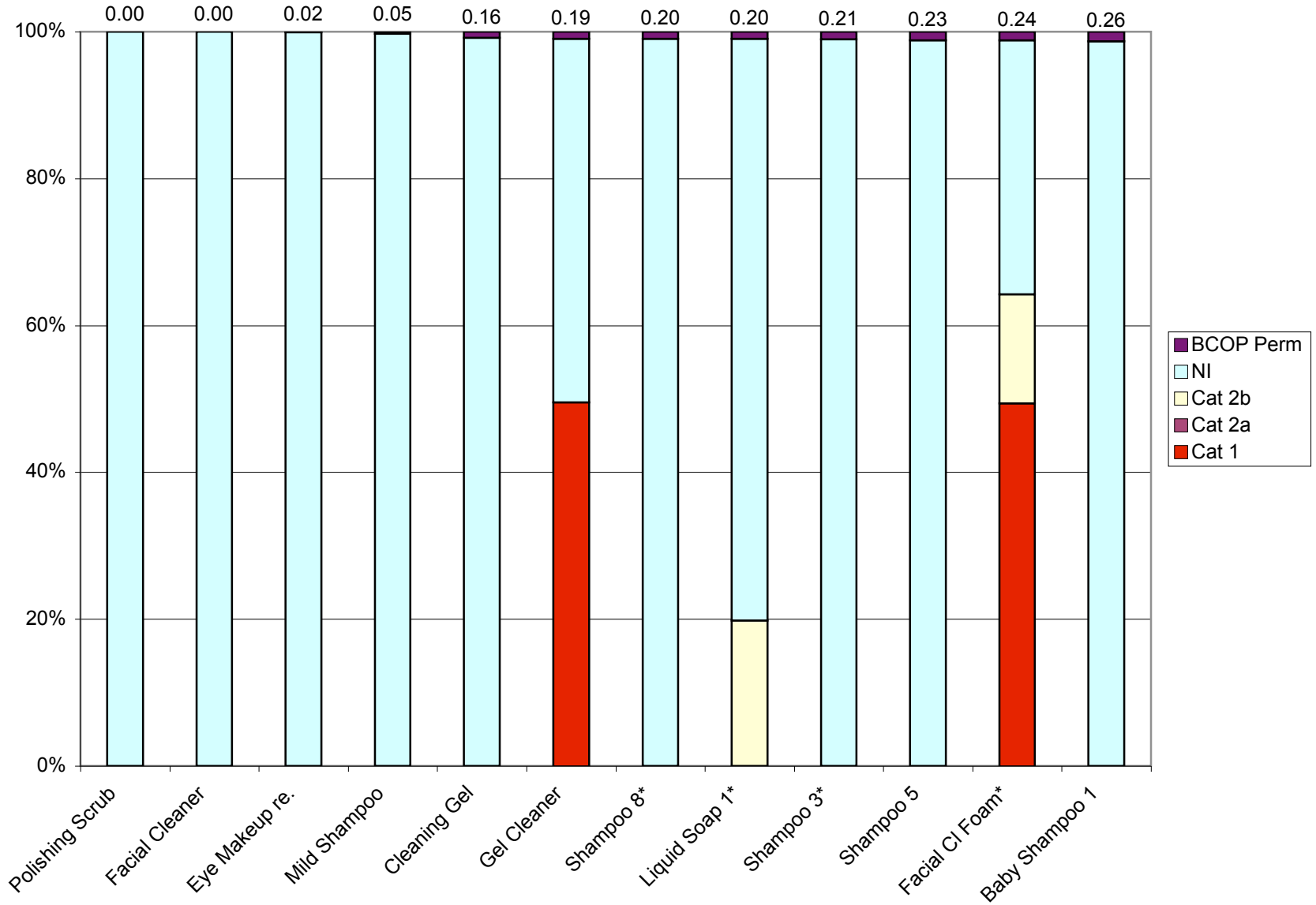
IIVS Submission - Analysis of In Vivo Data for Gettings et al. (1996)



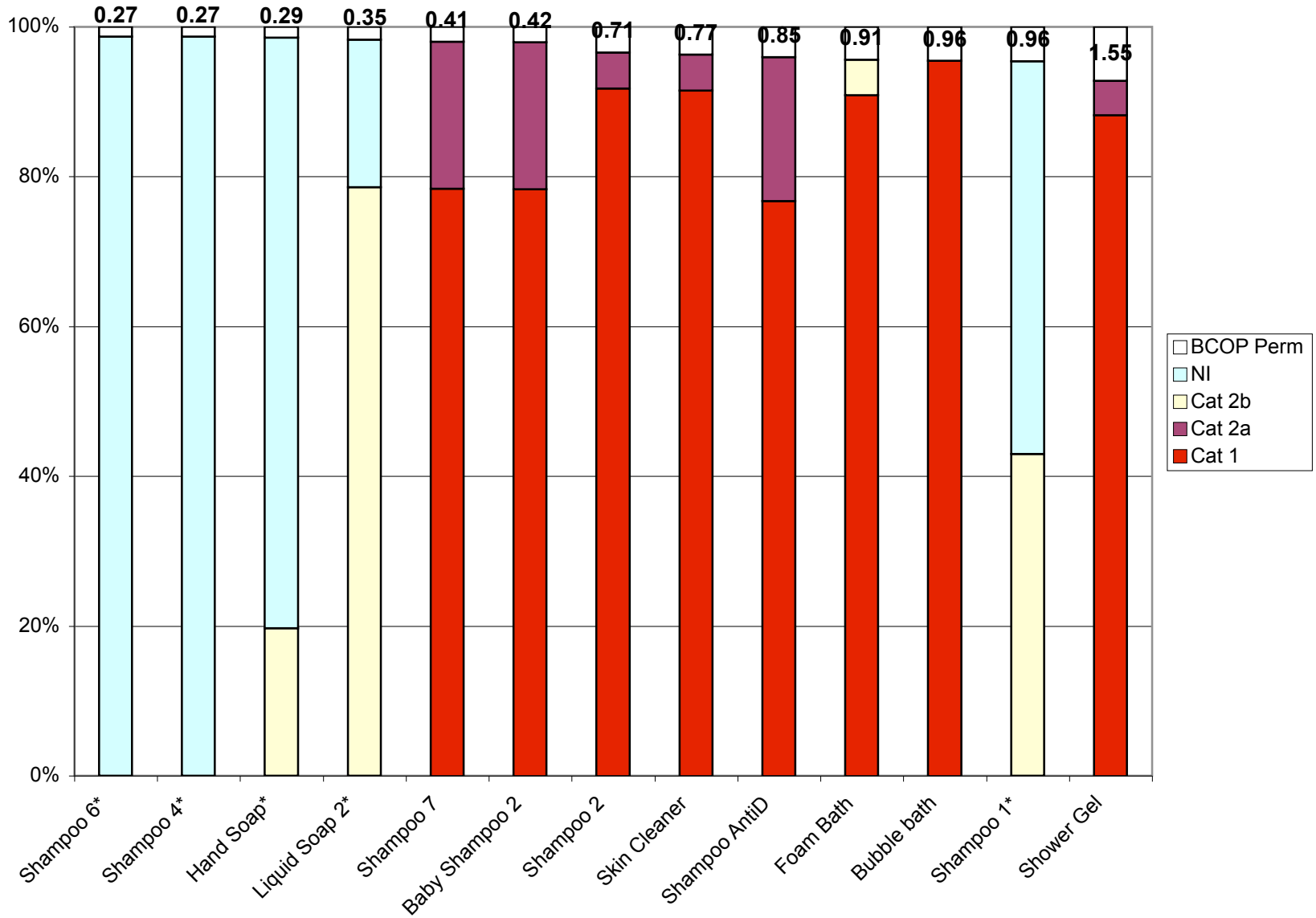
IIVS Submission - Comparison of BCOP Permeability Data with GHS Categories for In Vivo Data



IIVS Submission - Comparison of BCOP Permeability Data with GHS Categories for In Vivo Data



IIVS Submission - Comparison of BCOP Permeability Data with GHS Categories for In Vivo Data



[This Page Intentionally Left Blank]

Appendix G6

Dataset Received from Johnson & Johnson Pharmaceutical Research and Development – A Division of Janssen Pharmaceutica N.V. (Laboratory No. 9 in Gautheron et al. 1994)

[This Page Intentionally Left Blank]

Johnson & Johnson
PHARMACEUTICAL RESEARCH
& DEVELOPMENT
DIVISION OF JANSSEN PHARMACEUTICA N.V.

Pergamon

0887-2333(94)E0010-Q

Toxic. in Vitro Vol. 8, No. 3, pp. 381–392, 1994
Elsevier Science Ltd. Printed in Great Britain

INTERLABORATORY ASSESSMENT OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY*

P. GAUTHERON[1]†, J. GIROUX[2], M. COTTIN[3], L. AUDEGOND[4], A. MORILLA[5], L. MAYORDOMO-BLANCO[6], A. TORTAJADA[6], G. HAYNES[7], J. A. VERICAT[7], R. PIROVANO[8], E. GILLIO TOS[8], C. HAGEMANN[9], P. VANPARYS[10], G. DEKNUDT[10], G. JACOBS[11], M. PRINSEN[12], S. KALWEIT[13] and H. SPIELMANN[13]

[1]MSD-Chibret, Centre de Recherche, BP 134, 63203 Riom, [2]Agence du Médicament, Unité Pharmacologie-Toxicologie, 34000 Montpellier, [3]L'Oréal, Centre de Recherche Fondamentale, BP 22, 93601 Aulnay sous Bois, [4]Roussel-Uclaf, Laboratorie de Toxicologie, 93230 Romainville, France, [5]Laboratorios Cusi PO Box No. 2, 08320 El Masnou, Barcelona, [6]Cida, Centro Industrial Santiga, Argenters 6, 08130 Sta. Perpetua de Mogoda, Barcelona, Spain, [7]Research Toxicology Centre SpA, 00040 Pomezia (Roma), [8]Istituto di Richerche Biomediche "A. Marxer" RBM SpA, C.P. 226, 10015 Ivrea (TO), Italy, [9]Ciba-Geigy, Toxicology Services, CH-4332 Stein/AG, Switzerland, [10]Janssen Pharmaceutica, Toxicology Department, 2340 Beerse, [11]Institute of Hygiene and Epidemiology, B-1050 Brussels, Belgium, [12]TNO Nutrition and Food Research, PO Box 360, 3704 HE Zeist, The Netherlands, [13]ZEBET, Robert von Ostertag Institut, Postfach 48 04 47, 1000 Berlin 48, Germany

This document contains the individual cornea data collected in laboratory no. 09 (Janssen Pharmaceutica) => 52 compounds were tested.

Compounds tested

Table 1. Test chemicals and suppliers

Chemical			
Code no.	Name	CAS no.*	Supplier: catalogue no.
1	2-Ethoxyethanol	110-80-5	Aldrich: 25,637-4
2	Anthracene	120-12-7	Aldrich: 14,106-2
3	Allyl alcohol	107-18-6	Aldrich: 24,053-2
4	EDTA, di-potassium salt	25102-12-9	Aldrich: 22,600-9
5	Hexane	110-54-3	Aldrich: 13,936-6
6	2,4-Pentanedione	123-54-6	Aldrich: P 775-4
7	Phenylbutazone	50-33-9	Aldrich: 21,186-9
8	1-Nitropropane	108-03-2	Aldrich: N2,285-1
9	3-Glycidoxypropyltrimethoxysilane	2530-83-8	Aldrich: 23,578-4
10	Aluminium hydroxide	21645-51-2	Aldrich: 23,918-6
11	1,2,4-Trimethylbenzene	95-63-6	Aldrich: 24,027-3
12	2-Mercaptopyrimidine	1450-85-7	Aldrich: 12,962-3
13	Betaine monohydrate	590-47-6	Aldrich: 21,913-4
14	Sodium oxalate	62-76-0	Aldrich: 22,343-3
15	DL-Glutamic acid	19285-83-7	Aldrich: G 279-6
16	Petroleum ether	8032-32-4	Aldrich: 26,173-4
17	Butyrolactone	96-48-0	Aldrich: B10 360-84
18	2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	Aldrich: 14,739-7
19	Furan	110-00-9	Aldrich: 18,592-2
20	Imidazole	288-32-4	Aldrich: I 20-2
21	1-phenyl-3-pyrazolidone	92-43-3	Aldrich: 12,791-4
22	2-Aminophenol	95-55-6	Aldrich: A7 130-1
23	Gluconolactone	90-80-2	Aldrich: G200-1
24	2-Methoxyethanol	109-86-4	Aldrich: 27,048-2
25	Dimethyl sulfoxide	67-68-5	Aldrich: 27,043-1
26	Magnesium carbonate	56378-72-4	Aldrich: 22,766-8
27	Propyl-4-Hydroxybenzoate	94-13-3	Aldrich: P5 335-7
28	Iminodibenzyl	494-19-9	Aldrich: I 130-8
29	Octanol	111-87-5	Aldrich: 29,324-5
30	Methylisobutyl ketone	108-10-1	Aldrich: 29,326-1
31	Dibenzoyl-L-tartaric acid	2743-38-6	Aldrich: 34,584-9
32	Ethanol	64-17-5	local vendor
33	Methanol	67-56-1	local vendor
34	Ethyl acetoacetate	141-97-9	Aldrich: 24,070-2
35	Promethazine hydrochloride	58-33-3	Aldrich: 28,411-4
36	Deoxycholic acid, sodium salt	302-95-4	Sigma: D-6750
37	MYRJ-45		ICI: A-8563
38	BRIJ-35	9002-92-0	Sigma: P-1254
39	Tetraaminopyrimidine sulfate	5392-28-9	Aldrich: T 380-7
40	*N*-Lauroylsarcosine, sodium salt	7631-98-3	Sigma: L-5125
41	Quinacrine	69-05-6	Sigma: Q-3251
42	Hexadecyltrimethylammonium bromide	57-09-0	Sigma: H-5882
43	Thiourea	62-56-6	Aldrich: 24,025-7
44	Dimethylbiguanide	657-24-9	Sigma: D-5035
45	Benzethonium chloride	121-54-0	Sigma: B-8879
46	Triton X-155	9010-44-0	Sigma: X-155
47	1,2,3-Trichloropropane	96-18-4	Aldrich: 11,012-4
48	Cyclohexanone	108-94-1	Aldrich: C10 218-0
49	Diacetone alcohol	123-42-2	Aldrich: H4 154-4
50	Laurylsulfobetaine	14933-08-5	Sigma: D-4516
51	Pyridine	110-86-1	Aldrich: 27,040-7
52	Triethanolamine	102-71-6	Aldrich: T5 830-0

*Chemical Abstracts Service registry no.

EEC VALIDATION STUDY ON THE BOVINE CORNEA OPACITY-PERMEABILITY ASSAY

BLIND CODE	CHEMICAL	L Su So	OPACITY			PERMEABILITY		IN VITRO SCORE	EXP. Nr.	Re-marks
			10 min	120 min	240 min	O.D.	µg/ml			
1	2-ethoxyethanol	L	62.3 ± 2.3	61.7 ± 1.9		1.515 ± 0.134	19.3	84.4 ± 1.2	5	I
2	anthracene	So			1.4 ± 1.2	0.003 ± 0.007	0.0	1.4 ± 1.3	42	C
3	allyl alcohol	L	97.6 ± 18.9	94.1 ± 18.9		1.948 ± 0.455	24.7	123.3 ± 14.4	5	I
4	ethylenediaminetetraacetate DiK	So			0.8 ± 0.5	0.010 ± 0.014	0.1	0.9 ± 0.6	42	C
5	hexane	L	1.3 ± 0.8	1.3 ± 1.8		0.002 ± 0.002	0	1.4 ± 1.8	6	I
6	2,4-pentadione	L	54.6 ± 4.7	49.1 ± 3.4		0.084 ± 0.036	1.1	50.3 ± 3.4	6	I
7	phenylbutazone	So			0.7 ± 0.4	-0.008 ± 0.008	0	0.5 ± 0.4	42	C
8	1-nitropropane	L	1.5 ± 1.1	16.5 ± 1.7		0.008 ± 0.018	0.1	16.6 ± 1.9	7	I
9	3-glycidoxypropyltrimethoxysilane	L	16.1 ± 5.2	16.6 ± 4.5		0.065 ± 0.082	0.8	17.6 ± 4.7	7	I
10	aluminium hydroxide	So			9.7 ± 2.3	0.012 ± 0.007	0.1	9.9 ± 2.3	43	C
11	1,2,4,-trimethylbenzene	L	4.6 ± 0.9	12.5 ± 1.5		0.579 ± 0.369	7.3	21.2 ± 4.5	8	I
12	2-mercaptopyrimidine	So			-0.2 ± 0.4	-0.004 ± 0.002	0	-0.2 ± 0.4	43	C
13	betaine monohydrate	So			3.1 ± 2.3	0.029 ± 0.014	0.1	3.5 ± 2.2	43	C
14	sodium oxalate	So			1.7 ± 0.9	0.103 ± 0.042	0.6	3.2 ± 1.3	22	C
15	DL-glutamic acid	So			-0.2 ± 0.5	-0.005 ± 0.005	0	-0.2 ± 0.5	22	C
16	petroleum ether	L	0.7 ± 1.1	1.4 ± 1.9		0.015 ± 0.011	0.2	2.1 ± 1.9	8	I
17	butyrolactone	L	32.3 ± 3.9	34.2 ± 3.1		0.495 ± 0.199	6.3	41.6 ± 5.0	9	I
18	2,4-dichloro-5-sulfamoyl-benzoic acid	So			19.3 ± 4.8	-0.010 ± 0.004	0	19.2 ± 4.7	25	C
19	furan	L	15.2 ± 2.1	20.6 ± 2.5		1.970 ± 0.197	24.9	50.2 ± 4.0	9	I
20	imidazole	So			40.3 ± 9.9	1.598 ± 0.271	9.2	64.3 ± 11.2	25	C

EEC VALIDATION STUDY ON THE BOVINE CORNEA OPACITY-PERMEABILITY ASSAY

BLIND CODE	CHEMICAL	L Su So	OPACITY			PERMEABILITY		IN VITRO SCORE	EXP. Nr.	Re-marks
			10 min	120 min	240 min	O.D.	µg/ml			
21	1-phenyl-3-pyrazolidone	So			11.1 ± 1.0	0.143 ± 0.052	0.8	**13.2 ± 1.6**	40	C
22	2-aminophenol	So			10.9 ± 1.4	0.144 ± 0.188	0.8	**13.0 ± 2.5**	40	C
23	gluconolactone	So			85.2 ± 5.6	0.154 ± 0.041	0.8	**87.5 ± 5.3**	26	C
24	2-methoxyethanol	L	46.6 ± 8.4	45.1 ± 7.1		0.800 ± 0.137	10.1	**57.1 ± 8.9**	10	I
25	DMSO	L	8.7 ± 2.6	6.3 ± 1.7		0.204 ± 0.056	2.6	**9.4 ± 1.4**	10	I
26	magnesium carbonate	So			0.5 ± 0.5	0.016 ± 0.004	0.1	**0.7 ± 0.5**	26	C
27	propyl-4-hydroxybenzoate	So			5.2 ± 1.7	0.066 ± 0.059	0.4	**6.2 ± 1.5**	27	C
28	iminodibenzyl	So			0.2 ± 0.4	-0.001 ± 0.003	0	**0.2 ± 0.4**	27	C
29	octanol	L	22.4 ± 2.2	27.7 ± 5.0		2.212 ± 0.377	28.0	**60.9 ± 6.9**	11	I
30	methyl isobutyl ketone	L	10.6 ± 1.0	11.2 ± 2.7		0.546 ± 0.244	6.8	**19.4 ± 3.1**	11	I
31	dibenzoyl-L-tartaric acid	So			75.2 ± 14.2	0.416 ± 0.116	2.4	**81.5 ± 13.7**	27	C
32	ethanol	L	23.9 ± 4.0	22.3 ± 4.1		1.560 ± 0.316	19.8	**45.7 ± 6.6**	12	I
33	methanol	L	81.7 ± 5.6	73.7 ± 6.0		1.698 ± 0.560	9.8	**99.2 ± 12.8**	13b	C
34	ethyl acetoacetate	L	30.7 ± 2.8	24.0 ± 2.9		0.117 ± 0.007	0.6	**25.7 ± 3.8**	14	C
35	promethazine HCl	So			134.9 ± 9.7	0.287 ± 0.216	1.6	**139.2 ± 10.2**	38	C
36	deoxycholic acid sodium salt	Su	10.8 ± 1.5	13.9 ± 2.6		5.718 ± 0.511	32.6	**99.6 ± 8.0**	17	C
37	MYRJ 45	Su	-0.3 ± 0.4	0.4 ± 1.4		0.005 ± 0.004	0	**0.5 ± 1.4**	17	C
38	polyoxyethylene 23 lauryl ether	Su	0.7 ± 0.0	1.1 ± 0.6		-0.002 ± 0.008	0	**1.0 ± 0.7**	18	C
39	tetraaminopyrimidine sulfate	So			2.6 ± 1.4	-0.003 ± 0.006	0	**2.5 ± 1.4**	38	C
40	N-lauroylsarcosine sodium salt	Su	3.4 ± 1.2	7.8 ± 0.9		3.653 ± 0.496	21.0	**62.6 ± 7.3**	18	C

EEC VALIDATION STUDY ON THE BOVINE CORNEA OPACITY-PERMEABILITY ASSAY

BLIND CODE	CHEMICAL	L Su So	OPACITY			PERMEABILITY		IN VITRO SCORE	EXP. Nr.	Re-marks
			10 min	120 min	240 min	O.D.	µg/ml			
41	quinacrine	So			57.0 ± 5.4	0.063 ± 0.040	0.4	57.9 ± 5.8	38	C
42	hexadecyltrimethylammonium bromide	Su	11.3 ± 1.8	18.3 ± 3.6		3.438 ± 0.562	19.8	69.9 ± 6.9	18	C
43	thiourea	So			85.8 ± 9.2	4.373 ± 1.028	25.0	151.4 ± 20.7	39	C
44	dimethyl biguanide	So			0.7 ± 1.8	0.097 ± 0.176	0.6	2.1 ± 2.6	39	C
45	benzethonium chloride	Su	53.1 ± 3.8	84.6 ± 3.3		5.420 ± 0.949	30.9	165.9 ± 14.5	19	C
46	Triton X-155	Su	1.4 ± 1.3	3.0 ± 1.6		0.008 ± 0.014	0	3.1 ± 1.7	19	C
47	1,2,3-trichloropropane	L	4.1 ± 1.3	7.7 ± 1.9		5.561 ± 1.398	31.7	91.1 ± 20.0	14	C
48	cyclohexanone	L	57.9 ± 3.4	76.6 ± 3.9		4.341 ± 0.551	24.9	141.7 ± 8.2	16	C
49	diacetone alcohol	L	24.1 ± 3.6	31.1 ± 3.2		4.119 ± 1.341	23.6	92.2 ± 22.0	17	C
50	laurylsulfobetaine	Su	11.8 ± 2.6	16.2 ± 4.3		5.742 ± 1.462	32.7	102.4 ± 24.8	41	C
51	pyridine	L	39.3 ± 4.1	44.4 ± 3.3		4.015 ± 0.849	23.0	104.7 ± 15.7	16	C
52	triethanolamine	L	0.8 ± 0.7	2.6 ± 0.9		0.025 ± 0.011	1.4	3.0 ± 1.0	16	C

L = Liquid (100%)

Su = Surfactant (10%)

So = Solid (20%)

I = Incomplete medium (MEM with only 1% FBS) ----> Exp. Nr. 5 to 13a

C = Complete medium

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 09 Mar 92

Name: Ph. Vanparys

Experiment no. 5

Compound of the same pair no. 3

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	9	9	9.0	C1	13	13	13.0		0.003		13.0
2		-9	C2	0	-4.5	-14	C2	0	-7.0		0.002		-7.0
3		-9	0	C3	-4.5	-14	0	C3	-7.0		0.004		-6.9
mean					0.0				-0.3		0.003		-0.3
± S.D.					7.8				11.5		0.001		11.5
4	compound no.	56	65	66	62.3	51	66	66	61.0	61.3	1.447	1.444	83.0
5	1	59	68	68	65.0	54	69	68	63.7	64.0	1.502	1.498	86.5
6		54	64	64	60.7	50	64	65	59.7	60.0	1.669	1.666	85.0
7	concentration	58	68	68	64.7	54	68	69	63.7	64.0	1.307	1.304	83.6
8	100%	56	65	65	62.0	51	66	66	61.0	61.3	1.537	1.534	84.3
9		53	62	62	59.0	50	64	64	59.3	59.7	1.649	1.646	84.3
mean					62.3				61.4	61.7	1.518	1.515	**84.4**
± S.D.					2.3				1.9	1.9	0.134	0.134	**1.2**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.
5	1
6	
7	concentration
8	100%
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	71
2	B	152
	A	
3	B	223
	A	

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 25 Jun 92

Experiment no. 42

Compound of the same pair no. 4 + 7

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.016		0.2
2		-1	C2	0	-0.5		0.023		-0.2
3		-1	0	C3	-0.5		0.018		-0.2
mean					-0.3		0.019		-0.1
± S.D.					0.3		0.004		0.3
4	compound no. 2	2	3	3	2.7	3.0	0.020	0.001	3.0
5		0	0	0	0.0	0.3	0.010	-0.009	0.2
6		1	1	2	1.3	1.7	0.024	0.005	1.7
7	concentration 20%	0	0	0	0.0	0.3	0.023	0.004	0.4
8		2	2	3	2.3	2.7	0.026	0.007	2.8
9		0	0	0	0.0	0.3	0.029	0.010	0.5
mean					1.1	1.4	0.022	0.003	1.4
± S.D.					1.2	1.2	0.007	0.007	1.3

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no. 2	pH: 8.12
5		
6		
7	concentration 20%	
8		
9		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	158
	A	170
3	B	248
	A	259

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 09 Mar 92

Experiment no. 5

Compound of the same pair no. 1

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	9	9	9.0	C1	13	13	13.0		0.003		13.0
2		-9	C2	0	-4.5	-14	C2	0	-7.0		0.002		-7.0
3		-9	0	C3	-4.5	-14	0	C3	-7.0		0.004		-6.9
mean					0.0				-0.3		0.003		-0.3
± S.D.					7.8				11.5		0.001		11.5
10	compound no. 3	115	123	123	120.3	106	122	121	116.3	116.7	1.555	1.552	139.9
11		109	118	119	115.3	102	117	117	112.0	112.3	1.957	1.953	141.6
12		88	97	97	94.0	80	95	95	90.0	90.3	1.859	1.856	118.2
13	concentration	96	105	105	102.0	88	102	102	97.3	97.7	1.581	1.578	121.3
14	100%	77	86	86	83.0	69	85	85	79.7	80.0	1.948	1.945	109.2
15		65	74	74	71.0	57	72	72	67.0	67.3	2.807	2.804	109.4
mean					97.6				93.7	94.1	1.951	1.948	123.3
± S.D.					18.9				18.9	18.9	0.455	0.455	14.4

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no. 3
11	
12	
13	concentration 100%
14	
15	
mean	
± S.D	

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	71
2	B	152
	A	
3	B	223
	A	

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 25 Jun 92

Experiment no. 42

Compound of the same pair no. 2 + 7

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.016		0.2
2		• -1	C2	0	-0.5		0.023		-0.2
3		-1	0	C3	-0.5		0.018		-0.2
mean					-0.3		0.019		-0.1
± S.D.					0.3		0.004		0.3
10	compound no. 4	0	0	0	0.0	0.3	0.043	0.024	0.7
11		0	1	1	0.7	1.0	0.039	0.020	1.3
12		0	0	0	0.0	0.3	0.014	-0.004	0.3
13	concentration 20%	0	0	0	0.0	0.3	0.015	-0.004	0.3
14		0	1	1	0.7	1.0	0.043	0.024	1.4
15		1	1	2	1.3	1.7	0.019	0.000	1.7
mean					0.4	0.8	0.029	0.010	**0.9**
± S.D.					0.5	0.5	0.014	0.014	**0.6**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no. 4	pH: 5.2
11		
12		
13	concentration 20%	
14		
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	75
2	B	158
	A	170
3	B	248
	A	259

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 19 Mar 92

Experiment no. 6

Compound of the same pair no. 6

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	4	4	4.0	C1	4	4	4.0		0.003		4.0
2		-2	C2	0	-1.0	-2	C2	0	-1.0		0.003		-1.0
3		-2	0	C3	-1.0	-2	0	C3	-1.0		0.001		-1.0
mean					0.7				0.7		0.002		0.7
± S.D.					2.9				2.9		0.001		2.9
4	compound no.	0	2	2	1.3	0	2	2	1.3	0.7	0.004	0.002	0.7
5	5	0	2	2	1.3	3	6	6	5.0	4.3	0.004	0.002	4.4
6		1	3	3	2.3	1	4	4	3.0	2.3	0.002	0.000	2.3
7	concentration	-1	1	1	0.3	-1	1	1	0.3	-0.3	0.009	0.007	-0.2
8	100%	-1	1	1	0.3	-1	1	1	0.3	-0.3	0.005	0.003	-0.3
9		0	3	3	2.0	0	3	3	2.0	1.3	0.004	0.002	1.4
mean					1.3				2.0	1.3	0.005	0.002	1.4
± S.D.					0.8				1.8	1.8	0.002	0.002	1.8

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
4	compound no.
5	5
6	
7	concentration
8	100%
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-68
2	B	158
	A	-152
3	B	254
	A	-235

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 19 Mar 92

Experiment no. 6

Compound of the same pair no. 5

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	4	4	4.0	C1	4	4	4.0		0.003		4.0
2		-2	C2	0	-1.0	-2	C2	0	-1.0		0.003		-1.0
3		-2	0	C3	-1.0	-2	0	C3	-1.0		0.001		-1.0
mean					0.7				0.7		0.002		0.7
± S.D.					2.9				2.9		0.001		2.9
10	compound no.	47	50	49	48.7	43	46	46	45.0	44.3	0.077	0.075	45.5
11	6	51	54	54	53.0	46	49	49	48.0	47.3	0.048	0.046	48.0
12		52	55	55	54.0	50	52	52	51.3	50.7	0.135	0.133	52.7
13	concentration	50	53	53	52.0	47	50	50	49.0	48.3	0.125	0.123	50.2
14	100%	60	63	63	62.0	53	56	56	55.0	54.3	0.057	0.055	55.2
15		56	59	58	57.7	48	51	51	50.0	49.3	0.076	0.074	50.4
mean					54.6				49.7	49.1	0.086	0.084	50.3
± S.D.					4.7				3.4	3.4	0.036	0.036	3.4

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no.
11	6
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	68
2	B	158
	A	152
3	B	254
	A	235

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 25 Jun 92

Experiment no. 42

Compound of the same pair no. 2 + 4

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.016		0.2
2		•-1	C2	0	-0.5		0.023		-0.2
3		-1	0	C3	-0.5		0.018		-0.2
mean					-0.3		0.019		-0.1
± S.D.					0.3		0.004		0.3
16	compound no. 7	0	0	0	0.0	0.3	0.014	-0.005	0.3
17		0	0	0	0.0	0.3	0.006	-0.012	0.1
18		0	0	0	0.0	0.3	0.003	-0.015	0.1
19	concentration 20%	0	1	1	0.7	1.0	0.011	-0.007	0.9
20		0	0	1	0.3	0.7	0.024	0.005	0.7
21		1	1	1	1.0	1.3	0.006	-0.013	1.1
mean					0.3	0.7	0.011	-0.008	0.5
± S.D.					0.4	0.4	0.008	0.008	0.4

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no. 7	pH: not enough compound for a determination.
17		
18		
19	concentration 20%	Compound no. 7 was washed away 4 times instead of 3 times.
20		
21		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-170
3	B	248
	A	-259

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 20 Mar 92

Experiment no. 7

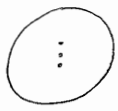
Compound of the same pair no. 9

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	-4	-2.0	C1	-2	-4	-3.0		0.004		-2.9
2		0	C2	-4	-2.0	1	C2	-2	-0.5		0.002		-0.5
3		3	3	C3	3.0	3	1	C3	2.0		0.003		2.0
mean					-0.3				-0.5		0.003		-0.5
± S.D.					2.9				2.5		0.001		2.5
4	compound no.	3	4	0	2.3	20	18	16	18.0	18.5	0.001	-0.002	18.5
5	8	3	3	0	2.0	16	14	12	14.0	14.5	0.005	0.002	14.5
6		3	4	0	2.3	18	16	15	16.3	16.8	0.002	-0.001	16.8
7	concentration	0	1	-2	-0.3	20	18	16	18.0	18.5	0.048	0.045	19.2
8	100%	3	3	0	2.0	17	15	13	15.0	15.5	0.007	0.004	15.6
9		1	2	-1	0.7	17	14	13	14.7	15.2	0.003	0.000	15.2
mean					1.5				16.0	16.5	0.011	0.008	**16.6**
± S.D.					1.1				1.7	1.7	0.018	0.018	**1.9**

cor-nea	treatment
1	
2	MEM
3	→ 3 very small air-bubbles
mean	
± S.D.	



4	compound no.
5	8
6	
7	concentration
8	100%
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-152
3	B	254
	A	-235

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

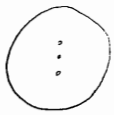
Date: 20 Mar 92
Experiment no. 7
Compound of the same pair no. 8

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	-4	-2.0	C1	-2	-4	-3.0		0.004		-2.9
2		0	C2	-4	-2.0	1	C2	-2	-0.5		0.002		-0.5
3		3	3	C3	3.0	3	1	C3	2.0		0.003		2.0
mean					-0.3				-0.5		0.003		-0.5
± S.D.					2.9				2.5		0.001		2.5
10	compound no.	21	21	18	20.0	21	19	17	19.0	19.5	0.216	0.213	22.7
11	9	20	20	17	19.0	23	20	19	20.7	21.2	0.018	0.015	21.4
12		21	22	18	20.3	22	20	18	20.0	20.5	0.008	0.005	20.6
13	concentration	16	16	12	14.7	14	12	10	12.0	12.5	0.114	0.111	14.2
14	100%	8	8	4	6.7	12	10	8	10.0	10.5	0.026	0.023	10.8
15		17	17	13	15.7	17	15	13	15.0	15.5	0.028	0.025	15.9
mean					16.1				16.1	16.6	0.068	0.065	17.6
± S.D.					5.2				4.5	4.5	0.082	0.082	4.7

cor-nea	treatment
1	
2	MEM
3	→ 3 very small air-bubbles
mean	
± S.D.	



10	compound no.
11	9
12	
13	concentration
14	100% → small area which is not opace
15	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	158
	A	152
3	B	254
	A	235

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 26 Jun 92

Experiment no. 43

Compound of the same pair no. 12 + 13

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min			correc.	Permeability (OD) correc.		in-vitro score
1	MEM	C1	0	0	0.0	0.012		0.2
2		0	C2	0	0.0	0.010		0.2
3		0	0	C3	0.0	0.022		0.3
mean					0.0	0.015		0.2
± S.D.					0.0	0.006		0.1
4	compound no.	9	9	8	8.7	0.027	0.012	8.9
5	10	10	10	9	9.7	0.019	0.004	9.7
6		10	10	10	10.0	0.030	0.015	10.2
7	concentration	13	13	13	13.0	0.026	0.011	13.2
8	20%	6	6	6	6.0	0.037	0.022	6.3
9		11	11	11	11.0	0.020	0.006	11.1
mean					9.7	0.026	0.012	**9.9**
± S.D.					2.3	0.007	0.007	**2.3**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.	pH: not enough compound for a pH determination.
5	10	
6		
7	concentration	
8	20%	
9		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-262

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 23 Mar 92

Experiment no. 8

Compound of the same pair no. 16

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	0	-0.5	C1	-3	-2	-2.5		-0.003		-2.5
2		0	C2	0	0.0	2	C2	0	1.0		-0.005		0.9
3		0	-1	C3	-0.5	1	-1	C3	0.0		-0.002		0.0
mean					-0.3				-0.5		-0.003		-0.6
± S.D.					0.3				1.8		0.002		1.8
4	compound no.	6	6	6	6.0	14	10	11	11.7	12.2	0.337	0.340	17.3
5	11	5	4	5	4.7	15	12	13	13.3	13.8	0.529	0.532	21.8
6		4	3	4	3.7	14	11	11	12.0	12.5	0.380	0.383	18.3
7	concentration	5	4	5	4.7	14	11	12	12.3	12.8	0.783	0.786	24.6
8	100%	4	3	4	3.7	11	8	9	9.3	9.8	1.213	1.216	28.1
9		5	4	5	4.7	15	12	13	13.3	13.8	0.209	0.212	17.0
mean					4.6				12.0	12.5	0.575	0.579	**21.2**
± S.D.					0.9				1.5	1.5	0.369	0.369	**4.5**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.
5	11
6	
7	concentration
8	100%
9	
mean	
± S.D.	

rinsed 4 times instead off 3 times

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	75
2	B	158
	A	168
3	B	253
	A	260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 26 Jun 92

Experiment no. 43

Compound of the same pair no. 10 + 13

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.012		0.2
2		0	C2	0	0.0		0.010		0.2
3		0	0	C3	0.0		0.022		0.3
mean					0.0		0.015		0.2
± S.D.					0.0		0.007		0.1
10	compound no.	0	0	0	0.0	0.0	0.010	-0.005	-0.1
11	12	0	0	0	0.0	0.0	0.011	-0.004	-0.1
12		0	0	0	0.0	0.0	0.008	-0.006	-0.1
13	concentration	0	0	0	0.0	0.0	0.016	0.001	0.0
14	20%	0	0	0	0.0	0.0	0.010	-0.005	-0.1
15		-1	-1	-1	-1.0	-1.0	0.011	-0.004	-1.1
mean					-0.2	-0.2	0.011	-0.004	-0.2
± S.D.					0.4	0.4	0.002	0.002	0.4

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.	pH: 6.84
11	12	
12		
13	concentration	Compound No.12 was washed away 4 times instead of 3 times.
14	20%	
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-262

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 26 Jun 92

Experiment no. 43

Compound of the same pair no. 10 + 12

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.012		0.2
2		0	C2	0	0.0		0.010		0.2
3		0	0	C3	0.0		0.022		0.3
mean					0.0		0.015		0.2
± S.D.					0.0		0.006		0.1
16	compound no. 13	7	7	6	6.7	6.7	0.030	0.015	6.9
17		1	1	1	1.0	1.0	0.041	0.026	1.4
18		1	1	1	1.0	1.0	0.041	0.027	1.4
19	concentration 20%	5	5	5	5.0	5.0	0.034	0.020	5.3
20		2	2	1	1.7	1.7	0.070	0.055	2.5
21		3	3	3	3.0	3.0	0.043	0.029	3.4
mean					3.1	3.1	0.043	0.029	3.5
± S.D.					2.3	2.3	0.014	0.014	2.2

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no. 13	pH: 8.24
17		
18		
19	concentration 20%	
20		
21		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	75
2	B	159
	A	170
3	B	250
	A	262

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 27 Apr 92

Experiment no. 22

Compound of the same pair no. 15

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	In-vitro score
1	MEM	C1	0	2	1.0		0.016		1.2
2		0	C2	1	0.5		0.014		0.7
3		-3	-2	C3	-2.5		0.015		-2.3
mean					-0.3		0.015		-0.1
± S.D.					1.9		0.001		1.9
4	compound no. 14	-1	0	1	0.0	0.3	0.106	0.092	1.7
5		0	0	2	0.7	1.0	0.042	0.028	1.4
6		1	1	4	2.0	2.3	0.122	0.108	3.9
7	concentration 20%	0	1	3	1.3	1.7	0.143	0.128	3.6
8		1	1	3	1.7	2.0	0.164	0.150	4.2
9		1	2	4	2.3	2.7	0.129	0.115	4.4
mean					1.3	1.7	0.118	0.103	**3.2**
± S.D.					0.9	0.9	0.042	0.042	**1.3**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
4	compound no. 14
5	
6	
7	concentration 20%
8	
9	
mean	
± S.D.	

Compound 14 was warmed up to 32°C and stirred on a magnetic stirrer.

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	248
	A	-257

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 27 Apr 92

Experiment no. 22

Compound of the same pair no. 14

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	2	1.0		0.016		1.2
2		0	C2	1	0.5		0.014		0.7
3		-3	-2	C3	-2.5		0.015		-2.3
mean					-0.3		0.015		-0.1
± S.D.					1.9		0.001		1.9
10	compound no.	-1	0	1	0.0	0.3	0.011	-0.004	0.3
11	15	-2	-1	0	-1.0	-0.7	0.018	0.003	-0.6
12		-1	0	1	0.0	0.3	0.003	-0.012	0.2
13	concentration	-2	-1	0	-1.0	-0.7	0.010	-0.005	-0.7
14	20%	-1	0	1	0.0	0.3	0.008	-0.006	0.2
15		-2	-1	0	-1.0	-0.7	0.006	-0.008	-0.8
mean					-0.5	-0.2	0.009	-0.005	**-0.2**
± S.D.					0.5	0.5	0.005	0.005	**0.5**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no.
11	15
12	
13	concentration
14	20%
15	
mean	
± S.D.	

Compound 15 was warmed up to 32°C and stirred on a magnetic stirrer.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	248
	A	-257

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 23 Mar 92

Experiment no. 8

Compound of the same pair no. 11

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	0	-0.5	C1	-3	-2	-2.5		-0.003		-2.5
2		0	C2	0	0.0	2	C2	0	1.0		-0.005		0.9
3		0	-1	C3	-0.5	1	-1	C3	0.0		-0.002		0.0
mean					-0.3				-0.5		-0.003		-0.6
± S.D.					0.3				1.8		0.002		1.8
10	compound no.	1	0	0	0.3	5	1	2	2.7	3.2	0.003	0.006	3.3
11	16	0	0	0	0.0	1	-1	0	0.0	0.5	0.017	0.020	0.8
12		3	2	3	2.7	5	2	3	3.3	3.8	0.032	0.035	4.4
13	concentration	0	0	0	0.0	4	0	1	1.7	2.2	0.005	0.008	2.3
14	100%	2	1	1	1.3	4	1	2	2.3	2.8	0.003	0.006	2.9
15		0	-1	0	-0.3	0	-3	-2	-1.7	-1.2	0.009	0.012	-1.0
mean					0.7				1.4	1.9	0.012	0.015	2.1
± S.D.					1.1				1.9	1.9	0.011	0.011	1.9

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.
11	16
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	253
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 26 Mar 92

Experiment no. 9

Compound of the same pair no. 19

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	-1	0	-0.5		0.000		-0.5
2		0	C2	1	0.5	1	C2	2	1.5		0.005		1.6
3		0	-1	C3	-0.5	0	-2	C3	-1.0		0.038		-0.4
mean					0.0				0.0		0.014		0.2
± S.D.					0.5				1.3		0.021		1.2
4	compound no. 17	31	31	32	31.3	35	34	36	35.0	35.0	0.394	0.380	40.7
5		32	31	32	31.7	34	32	34	33.3	33.3	0.520	0.506	40.9
6		27	26	28	27.0	31	30	32	31.0	31.0	0.305	0.291	35.4
7	concentration 100%	32	31	32	31.7	32	30	33	31.7	31.7	0.761	0.747	42.9
8		39	38	40	39.0	40	38	41	39.7	39.7	0.735	0.721	50.5
9		33	32	34	33.0	35	33	36	34.7	34.7	0.340	0.326	39.6
mean					32.3				34.2	34.2	0.509	0.495	41.6
± S.D.					3.9				3.1	3.1	0.199	0.199	5.0

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	
4	compound no. 17
5	
6	
7	concentration 100%
8	
9	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-166
3	B	244
	A	-254

EEC VALIDATION OF THE BCOP ASSAY

Data sheet

Lab. no. 09

Date: 07 May 92

Experiment no. 25

Compound of the same pair no. 20 + 21

Name: Ph. Vanparys

Signature:

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

opacity at 240 min				correc.
C1	0	0	0.0	
0	C2	0	0.0	
0	0	C3	0.0	
			0.0	
			0.0	

Permeability (OD)	correc.	in-vitro score
0.021		0.3
0.012		0.2
0.010		0.1
0.014		0.2
0.006		0.1

cornea	compound no. / concentration
4	compound no.
5	18
6	
7	concentration
8	20%
9	
mean	
± S.D	

24	24	24	24.0	24.0
21	21	21	21.0	21.0
15	16	15	15.3	15.3
12	12	11	11.7	11.7
22	22	22	22.0	22.0
22	22	22	22.0	22.0
			19.3	19.3
			4.8	4.8

0.004	-0.011	23.8
0.000	-0.014	20.8
0.003	-0.011	15.2
0.011	-0.003	11.6
0.002	-0.012	21.8
0.006	-0.008	21.9
0.004	-0.010	**19.2**
0.004	0.004	**4.7**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

cornea	compound no. / concentration
4	compound no.
5	18
6	
7	concentration
8	20%
9	
mean	
± S.D.	

Compound 18 was warmed up to 32 °C and stirred on a magnetic stirrer.

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	159
	A	170
3	B	250
	A	260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date:	26 Mar 92
Experiment no.	9
Compound of the same pair no.	17

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	-1	0	-0.5		0.000		-0.5
2		0	C2	1	0.5	1	C2	2	1.5		0.005		1.6
3		0	-1	C3	-0.5	0	-2	C3	-1.0		0.038		-0.4
mean					0.0				0.0		0.014		0.2
± S.D.					0.5				1.3		0.021		1.2
10	compound no. 19	18	17	19	18.0	24	23	25	24.0	24.0	1.900	1.886	52.3
11		17	16	18	17.0	23	21	24	22.7	22.7	2.288	2.274	56.8
12		15	14	15	14.7	20	18	21	19.7	19.7	1.829	1.815	46.9
13	concentration 100%	13	13	14	13.3	20	18	20	19.3	19.3	1.785	1.771	45.9
14		16	15	16	15.7	21	20	22	21.0	21.0	1.944	1.930	49.9
15		13	12	13	12.7	17	16	18	17.0	17.0	2.157	2.143	49.1
mean					15.2				20.6	20.6	1.984	1.970	50.2
± S.D.					2.1				2.5	2.5	0.197	0.197	4.0

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no. 19
11	
12	
13	concentration 100%
14	
15	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-166
3	B	244
	A	-254

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 07 May 92

Experiment no. 25

Compound of the same pair no. 18 + 21

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.021		0.3
2		0	C2	0	0.0		0.012		0.2
3		0	0	C3	0.0		0.010		0.1
mean					0.0		0.014		0.2
± S.D.					0.0		0.006		0.1
10	compound no.	43	41	41	41.7	41.7	2.110	2.096	73.1
11	20	33	33	32	32.7	32.7	1.430	1.416	53.9
12		28	28	27	27.7	27.7	1.427	1.412	48.9
13	concentration	52	53	51	52.0	52.0	1.400	1.386	72.8
14	20%	37	37	36	36.7	36.7	1.694	1.680	61.9
15		51	52	51	51.3	51.3	1.612	1.597	75.3
mean					40.3	40.3	1.612	1.598	64.3
± S.D.					9.9	9.9	0.271	0.271	11.2

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.	Compound 20 was warmed up to 32 °C en stirred on a magnetic stirrer.
11	20	
12		
13	concentration	
14	20%	
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 18 Jun 92

Name: Ph. Vanparys

Experiment no. 40

Compound of the same pair no. 14 + 22

Signature:

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

opacity at 240 min				correc.
C1	0	0	0.0	
-1	C2	0	-0.5	
0	0	C3	0.0	
			-0.2	
			0.3	

Permeability (OD)	correc.	in-vitro score
0.025		0.4
0.019		-0.2
0.018		0.3
0.020		0.1
0.004		0.3

10	compound no.	10	12	12	11.3	11.5	0.176	0.156	13.8
11	21	10	12	12	11.3	11.5	0.113	0.093	12.9
12		9	10	10	9.7	9.8	0.131	0.110	11.5
13	concentration	10	12	11	11.0	11.2	0.134	0.114	12.9
14	20%	9	10	10	9.7	9.8	0.165	0.145	12.0
15		12	13	12	12.3	12.5	0.258	0.237	16.1
mean					10.9	11.1	0.163	0.143	**13.2**
± S.D.					1.0	1.0	0.052	0.052	**1.6**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.	pH: 7.41
11	21	
12		
13	concentration	Compound No. 21 was washed away 4 times instead of 3 times.
14	20%	
15		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-170
3	B	249
	A	-260

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 18 Jun 92

Name: Ph. Vanparys

Experiment no. 40

Compound of the same pair no. 14 + 21

Signature:

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

opacity at 240 min				correc.
C1	0	0	0.0	
-1	C2	0	-0.5	
0	0	C3	0.0	
			-0.2	
			0.3	

Permeability (OD)	correc.
0.025	
0.019	
0.018	
0.020	
0.004	

in-vitro score
0.4
-0.2
0.3
0.1
0.3

16	compound no.	8	10	9	9.0	9.2	0.113	0.093	10.6
17	22	10	11	11	10.7	10.8	0.076	0.056	11.7
18		12	12	12	12.0	12.2	0.086	0.065	13.1
19	concentration	10	11	11	10.7	10.8	0.059	0.039	11.4
20	20%	12	13	13	12.7	12.8	0.104	0.083	14.1
21		9	9	10	9.3	9.5	0.546	0.526	17.4
mean					10.7	10.9	0.164	0.144	**13.0**
± S.D.					1.4	1.4	0.188	0.188	**2.5**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no.	pH: 7.88
17	22	
18		
19	concentration	
20	20%	
21		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-		0
1	B	75
	A	75
2	B	158
	A	170
3	B	249
	A	260

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 08 May 92

Experiment no. 26

Compound of the same pair no. 22 + 26

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.017		0.3
2		0	C2	0	0.0		0.031		0.5
3		0	0	C3	0.0		0.012		0.2
mean					0.0		0.020		0.3
±S.D.					0.0		0.010		0.1
10	compound no. 23	87	89	90	88.7	88.7	0.192	0.171	91.2
11		85	87	88	86.7	86.7	0.201	0.181	89.4
12		76	78	80	78.0	78.0	0.163	0.143	80.1
13	concentration 20%	77	79	80	78.7	78.7	0.230	0.210	81.8
14		85	87	92	88.0	88.0	0.120	0.100	89.5
15		89	91	94	91.3	91.3	0.138	0.118	93.1
mean					85.2	85.2	0.174	0.154	87.5
±S.D.					5.6	5.6	0.041	0.041	5.3

cornea	treatment
1	MEM
2	
3	
mean	
±S.D.	

Fluorescein leakage into the waterbath

10	compound no. 23
11	
12	
13	concentration 20%
14	
15	
mean	
±S.D.	

Compound No. 23 was warmed up to 32°C and stirred on a magnetic stirrer.

O.D. versus C1 measured 8 min. after first measurement

106
101
92
91
107
104

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-171
3	B	251
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 27 Mar 92

Experiment no. 10

Compound of the same pair no. 25

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	1	2	1.5	C1	1	1	1.0		0.005		1.1
2		-1	C2	1	0.0	-2	C2	0	-1.0		0.001		-1.0
3		-2	0	C3	-1.0	-2	0	C3	-1.0		0.004		-0.9
mean					0.2				-0.3		0.003		-0.3
± S.D.					1.3				1.2		0.002		1.2
4	compound no.	49	51	52	50.7	47	49	49	48.3	48.7	0.882	0.879	61.8
5	24	40	42	43	41.7	38	40	40	39.3	39.7	0.688	0.685	49.9
6		52	53	55	53.3	51	53	53	52.3	52.7	0.754	0.751	63.9
7	concentration	36	38	39	37.7	36	38	38	37.3	37.7	0.735	0.732	48.6
8	100%	56	58	59	57.7	51	53	53	52.3	52.7	1.047	1.044	68.3
9		37	39	40	38.7	37	40	39	38.7	39.0	0.716	0.713	49.7
mean					46.6				44.7	45.1	0.804	0.800	**57.1**
± S.D.					8.4				7.1	7.1	0.137	0.137	**8.6**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	
4	compound no.
5	24
6	
7	concentration
8	100%
9	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-166
3	B	249
	A	-255

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 27 Mar 92

Experiment no. 10

Compound of the same pair no. 24

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	1	2	1.5	C1	1	1	1.0		0.005		1.1
2		-1	C2	1	0.0	-2	C2	0	-1.0		0.001		-1.0
3		-2	0	C3	-1.0	-2	0	C3	-1.0		0.004		-0.9
mean					0.2				-0.3		0.003		-0.3
± S.D.					1.3				1.2		0.002		1.2
10	compound no.	10	11	12	11.0	5	7	7	6.3	6.7	0.221	0.218	9.9
11	25	5	7	7	6.3	4	6	5	5.0	5.3	0.162	0.159	7.7
12		10	12	12	11.3	7	9	9	8.3	8.7	0.199	0.196	11.6
13	concentration	4	5	6	5.0	2	5	4	3.7	4.0	0.294	0.291	8.4
14	100%	8	10	11	9.7	4	6	6	5.3	5.7	0.233	0.230	9.1
15		8	9	10	9.0	6	8	8	7.3	7.7	0.137	0.134	9.7
mean					8.7				6.0	6.3	0.208	0.204	9.4
± S.D.					2.6				1.7	1.7	0.056	0.056	1.4

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.
11	25
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-		0
1	B	75
	A	-75
2	B	159
	A	-166
3	B	249
	A	-255

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 08 May 92

Name: Ph. Vanparys

Experiment no. 26

Compound of the same pair no. 22 + 23

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.017		0.3
2		0	C2	0	0.0		0.031		0.5
3		0	0	C3	0.0		0.012		0.2
mean					0.0		0.020		0.3
± S.D.					0.0		0.010		0.1
16	compound no. 26	1	1	1	1.0	1.0	0.040	0.020	1.3
17		0	0	0	0.0	0.0	0.035	0.015	0.2
18		0	0	0	0.0	0.0	0.044	0.023	0.4
19	concentration 20%	1	1	1	1.0	1.0	0.032	0.012	1.2
20		0	0	0	0.0	0.0	0.035	0.015	0.2
21		1	1	1	1.0	1.0	0.035	0.014	1.2
mean					0.5	0.5	0.037	0.016	**0.7**
± S.D.					0.5	0.5	0.004	0.004	**0.5**

cornea	treatment
1	
2	MEM
3	
mean	
± S.D.	

Fluorescein leakage into the waterbath.

16	compound no. 26
17	
18	
19	concentration 20%
20	
21	
mean	
± S.D.	

Compound No. 26 was warmed up to 32°C and stirred on a magnetic stirrer.

Anterior chambers were opened for treatment and washing.

Glass of the anterior chamber No. 17 was found to be cracked at the end of the fluorescein incubation period.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-171
3	B	251
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 11 May 92

Experiment no. 27

Compound of the same pair no. 28 + 31

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0		0.020		0.3
3		0	0	C3	0.0		0.010		0.2
mean					0.0		0.014		0.2
± S.D.					0.0		0.005		0.1
4	compound no.	8	8	8	8.0	8.0	0.023	0.008	8.1
5	27	4	4	4	4.0	4.0	0.183	0.169	6.5
6		6	6	7	6.3	6.3	0.077	0.063	7.3
7	concentration	4	4	4	4.0	4.0	0.028	0.014	4.2
8	20%	4	5	5	4.7	4.7	0.103	0.089	6.0
9		4	4	4	4.0	4.0	0.067	0.053	4.8
mean					5.2	5.2	0.080	0.066	6.2
± S.D.					1.7	1.7	0.059	0.059	1.5

cornea	treatment
1	
2	MEM
3	
mean	
± S.D.	

Glass of the anterior chamber No. 3 was found to be cracked at the end of the fluorescein incubation period.

4	compound no.
5	27
6	
7	concentration
8	20%
9	
mean	
± S.D.	

Fluorescein leakage from chamber No. 5.

Compound No. 27 was warmed up to 32°C and stirred on a magnetic stirrer.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-171
3	B	249
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 11 May 92

Experiment no. 27

Compound of the same pair no. 27 + 31

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0		0.020		0.3
3		0	0	C3	0.0		0.010		0.2
mean					0.0		0.014		0.2
± S.D.					0.0		0.005		0.1
10	compound no. 28	0	0	0	0.0	0.0	0.016	0.002	0.0
11		0	0	0	0.0	0.0	0.017	0.002	0.0
12		0	0	0	0.0	0.0	0.012	-0.002	0.0
13	concentration 20%	0	0	0	0.0	0.0	0.012	-0.003	0.0
14		1	1	1	1.0	1.0	0.015	0.001	1.0
15		0	0	0	0.0	0.0	0.010	-0.004	-0.1
mean					0.2	0.2	0.014	-0.001	0.2
± S.D.					0.4	0.4	0.003	0.003	0.4

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

Glass of the anterior chamber No. 3 was found to be cracked at the end of the fluorescein incubation period.

10	compound no. 28
11	
12	
13	concentration 20%
14	
15	
mean	
± S.D.	

Compound No. 28 was warmed up to 32°C and stirred on a magnetic stirrer

Fluorescein leakage from chamber No.13.

Compound No. 28 was washed away 6 times instead of 3 times

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-171
3	B	249
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 30 Mar 92
Experiment no. 11
Compound of the same pair no. 30

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-2	-3	-2.5	C1	-2	-4	-3.0		0.003		-3.0
2		2	C2	0	1.0	1	C2	-2	-0.5		0.002		-0.5
3		3	0	C3	1.5	3	1	C3	2.0		0.003		2.0
mean					0.0				-0.5		0.003		-0.5
± S.D.					2.2				2.5		0.001		2.5
4	compound no.	26	24	23	24.3	20	19	17	18.7	19.2	2.585	2.582	57.9
5	29	28	25	24	25.7	34	32	30	32.0	32.5	1.949	1.946	61.7
6		24	22	21	22.3	29	28	26	27.7	28.2	2.268	2.265	62.1
7	concentration	23	21	20	21.3	32	31	29	30.7	31.2	2.688	2.685	71.4
8	100%	22	20	19	20.3	32	30	28	30.0	30.5	2.094	2.091	61.9
9		22	20	19	20.3	26	24	23	24.3	24.8	1.703	1.700	50.3
mean					22.4				27.2	27.7	2.215	2.212	**60.9**
± S.D.					2.2				5.0	5.0	0.377	0.377	**6.9**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.
5	29
6	
7	concentration
8	100%
9	
mean	
± S.D	

the anterior compartment was rinsed 5-times instead of 3-times

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	157
	A	166
3	B	247
	A	256

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 30 Mar 92

Experiment no. 11

Compound of the same pair no. 29

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-2	-3	-2.5	C1	-2	-4	-3.0		0.003		-3.0
2		2	C2	0	1.0	1	C2	-2	-0.5		0.002		-0.5
3		3	0	C3	1.5	3	1	C3	2.0		0.003		2.0
mean					0.0				-0.5		0.003		-0.5
± S.D.					2.2				2.5		0.001		2.5
10	compound no.	13	11	10	11.3	13	11	9	11.0	11.5	0.507	0.504	19.1
11	30	13	11	10	11.3	16	14	12	14.0	14.5	0.398	0.395	20.4
12		11	9	8	9.3	10	8	6	8.0	8.5	0.362	0.359	13.9
13	concentration	11	9	8	9.3	9	7	5	7.0	7.5	0.943	0.940	21.6
14	100%	13	10	10	11.0	15	13	11	13.0	13.5	0.338	0.335	18.5
15		13	10	10	11.0	13	11	9	11.0	11.5	0.743	0.740	22.6
mean					10.6				10.7	11.2	0.549	0.546	19.4
± S.D.					1.0				2.7	2.7	0.244	0.244	3.1

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.
11	30
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	.75
2	B	157
	A	.166
3	B	247
	A	.256

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 11 May 92

Experiment no. 27

Compound of the same pair no. 27 +28

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0		0.020		0.3
3		0	0	C3	0.0		0.010		0.2
mean					0.0		0.014		0.2
± S.D.					0.0		0.005		0.1
16	compound no. 31	93	90	87	90.0	90.0	0.538	0.523	97.9
17		84	81	78	81.0	81.0	0.439	0.425	87.4
18		57	53	51	53.7	53.7	0.467	0.453	60.5
19	concentration 20%	67	63	61	63.7	63.7	0.499	0.485	70.9
20		87	88	88	87.7	87.7	0.209	0.194	90.6
21		78	75	73	75.3	75.3	0.430	0.416	81.6
mean					75.2	75.2	0.430	0.416	81.5
± S.D.					14.2	14.2	0.116	0.116	13.7

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

Glass of the anterior chamber No. 3 was found to be cracked at the end of the fluorescein incubation period.

		O.D. versus C1 measured 4 min. after first measurement.
16	compound no. 31	82
17		73
18		45
19	concentration 20%	57
20		87
21		69
mean		
± S.D.		

Compound No. 31 was warmed up to 32°C and stirred on a magnetic stirrer.

Compound No. 31 was washed away 6 times instead of 3 times.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-171
3	B	249
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 02 Apr 92

Experiment no. 12

Compound of the same pair no. -

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-2	0	-1.0	C1	-2	0	-1.0		0.001		-1.0
2		1	C2	2	1.5	1	C2	2	1.5		0.000		1.5
3		-1	-3	C3	-2.0	-2	-4	C3	-3.0		0.001		-3.0
mean					-0.5				-0.8		0.001		-0.8
± S.D.					1.8				2.3		0.001		2.2
10	compound no.	32	30	33	31.7	29	27	31	29.0	29.8	1.628	1.627	54.2
11	32	23	21	24	22.7	20	19	22	20.3	21.2	1.908	1.907	49.8
12		23	22	24	23.0	21	19	22	20.7	21.5	1.687	1.686	46.8
13	concentration	24	23	26	24.3	23	21	25	23.0	23.8	1.395	1.394	44.7
14	100%	21	20	22	21.0	19	17	20	18.7	19.5	1.012	1.011	34.7
15		21	19	22	20.7	17	16	19	17.3	18.2	1.731	1.730	44.1
mean					23.9				21.5	22.3	1.560	1.560	**45.7**
± S.D.					4.0				4.1	4.1	0.316	0.316	**6.6**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.	
11	32	
12		
13	concentration	
14	100%	
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-		0
1	B	75
	A	-75
2	B	158
	A	-167
3	B	248
	A	-256

EEC VALIDATION OF THE BCOP ASSAY

Data sheet

Lab. no. 09

Date: 03 Apr 92

Experiment no. 13 b

Compound of the same pair no. -

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM with	C1	-3	-2	-2.5	C1	-2	-2	-2.0		0.002		-2.0
2	I-Glutamine	2	C2	0	1.0	0	C2	0	0.0		0.003		0.0
3	Bicarbonate	1	-1	C3	0.0	1	0	C3	0.5		0.000		0.5
mean	pH 7.4				-0.5				-0.5		0.002		-0.5
± S.D.					1.8				1.3		0.002		1.3
10	compound no.	87	83	84	84.7	77	76	76	76.3	76.8	1.554	1.552	100.1
11	33 (2)	74	71	72	72.3	65	63	64	64.0	64.5	0.919	0.917	78.3
12		83	80	80	81.0	71	70	71	70.7	71.2	2.090	2.088	102.5
13	concentration	81	77	78	78.7	71	70	70	70.3	70.8	1.797	1.795	97.8
14	100%	87	84	85	85.3	79	77	77	77.7	78.2	1.335	1.333	98.2
15		90	87	87	88.0	81	80	80	80.3	80.8	2.502	2.500	118.3
mean					81.7				73.2	73.7	1.700	1.698	**99.2**
± S.D.					5.6				6.0	6.0	0.560	0.560	**12.8**

cornea	treatment
1	MEM with
2	I-Glutamine
3	Bicarbonate
mean	pH 7.4
± S.D.	

10	compound no.
11	33 (2)
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-		
1	B	
	A	
2	B	
	A	
3	B	
	A	

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 06 Apr 92

Experiment no. 14

Compound of the same pair no. 47

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	-1	-1.0	C1	0	0	0.0		0.014		0.2
2		0	C2	0	0.0	0	C2	0	0.0		0.007		0.1
3		0	0	C3	0.0	0	0	C3	0.0		0.005		0.1
mean					-0.3				0.0		0.009		0.1
± S.D.					0.6				0.0		0.005		0.1
4	compound no. 34	34	34	35	34.3	28	29	28	28.3	28.3	0.224	0.215	31.6
5		33	33	33	33.0	25	26	25	25.3	25.3	0.052	0.043	26.0
6		30	30	30	30.0	20	21	21	20.7	20.7	0.069	0.060	21.6
7	concentration 100%	28	29	29	28.7	23	24	23	23.3	23.3	0.316	0.307	27.9
8		31	32	31	31.3	25	26	25	25.3	25.3	0.032	0.023	25.7
9		26	27	27	26.7	21	21	21	21.0	21.0	0.058	0.049	21.7
mean					30.7				24.0	24.0	0.125	0.117	25.7
± S.D.					2.8				2.9	2.9	0.117	0.117	3.8

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no. 34
5	
6	
7	concentration 100%
8	
9	
mean	
± S.D.	

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	158
	A	167
3	B	248
	A	255

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 12 Jun 92

Experiment no. 38

Compound of the same pair no. 39 + 41

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.009		0.1
2		0	C2	0	0.0		0.019		0.3
3		0	0	C3	0.0		0.005		0.1
mean					0.0		0.011		0.2
± S.D.					0.0		0.007		0.1
4	compound no.	118	119	120	119.0	119.0	0.272	0.261	122.9
5	35	132	134	137	134.3	134.3	0.575	0.564	142.8
6		142	145	145	144.0	144.0	0.062	0.051	144.8
7	concentration	139	140	143	140.7	140.7	0.550	0.539	148.8
8	20%	141	144	144	143.0	143.0	0.190	0.179	145.7
9		128	128	130	128.7	128.7	0.139	0.128	130.6
mean					134.9	134.9	0.298	0.287	**139.2**
± S.D.					9.7	9.7	0.216	0.216	**10.2**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.	pH: 5.80
5	35	
6		
7	concentration	
8	20%	
9		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 16 Apr 92

Name: Ph. Vanparys

Experiment no. 17

Compound of the same pair no. 37 + 49

Signature:

cornea	treatment	opacity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	1	0	0.5	C1	2	1	1.5		0.006		1.6
2		-2	C2	-1	-1.5	-3	C2	-2	-2.5		0.010		-2.4
3		0	0	C3	0.0	-2	1	C3	-0.5		0.015		-0.3
mean					-0.3				-0.5		0.010		-0.3
± S.D.					1.0				2.0		0.005		2.0
4	compound no. 36	9	11	9	9.7	10	13	12	11.7	12.2	5.191	5.181	89.9
5		10	12	11	11.0	13	16	14	14.3	14.8	5.500	5.490	97.2
6		9	11	10	10.0	10	13	11	11.3	11.8	6.107	6.097	103.3
7	concentration 10%	12	14	13	13.0	15	18	16	16.3	16.8	5.214	5.204	94.9
8		11	13	12	12.0	14	18	16	16.0	16.5	6.454	6.444	113.2
9		8	10	9	9.0	9	12	10	10.3	10.8	5.905	5.895	99.3
mean					10.8				13.3	13.8	5.729	5.718	99.6
± S.D.					1.5				2.6	2.6	0.511	0.511	8.0

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no. 36
5	
6	
7	concentration 10%
8	
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-263

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 16 Apr 92

Name: Ph. Vanparys

Experiment no. 17

Compound of the same pair no. 36 + 49

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	In-vitro score
1	MEM	C1	1	0	0.5	C1	2	1	1.5		0.006		1.6
2		-2	C2	-1	-1.5	-3	C2	-2	-2.5		0.010		-2.4
3		0	0	C3	0.0	-2	1	C3	-0.5		0.015		-0.3
mean					-0.3				-0.5		0.010		-0.3
± S.D.					1.0				2.0		0.005		2.0
10	compound no.	0	1	0	0.3	1	4	2	2.3	2.8	0.022	0.012	3.0
11	37	-2	0	-1	-1.0	-3	0	-2	-1.7	-1.2	0.012	0.001	-1.1
12		-1	0	0	-0.3	-2	1	0	-0.3	0.2	0.016	0.005	0.2
13	concentration	-1	0	0	-0.3	-2	0	-1	-1.0	-0.5	0.013	0.002	-0.5
14	10%	-1	0	0	-0.3	-1	1	0	0.0	0.5	0.011	0.001	0.5
15		-1	0	0	-0.3	-1	1	0	0.0	0.5	0.019	0.009	0.6
mean					-0.3				-0.1	0.4	0.015	0.005	0.5
± S.D.					0.4				1.4	1.4	0.004	0.004	1.4

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no.
11	37
12	
13	concentration
14	10%
15	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-263

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 17 Apr 92

Name: Ph. Vanparys

Experiment no. 18

Compound of the same pair no. 40 + 42

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0	0	C2	0	0.0		0.011		0.2
3		1	1	C3	1.0	0	0	C3	0.0		0.008		0.1
mean					0.3				0.0		0.011		0.2
± S.D.					0.6				0.0		0.003		0.0
4	compound no. 38	1	1	0	0.7	2	2	1	1.7	1.7	0.022	0.012	1.8
5		1	1	0	0.7	1	1	0	0.7	0.7	0.012	0.001	0.7
6		1	1	0	0.7	1	1	0	0.7	0.7	0.011	0.000	0.7
7	concentration 10%	1	1	0	0.7	2	2	2	2.0	2.0	0.005	-0.006	1.9
8		1	1	0	0.7	1	1	0	0.7	0.7	0.003	-0.008	0.6
9		1	1	0	0.7	1	1	0	0.7	0.7	0.002	-0.009	0.5
mean					0.7				1.1	1.1	0.009	-0.002	**1.0**
± S.D.					0.0				0.6	0.6	0.008	0.008	**0.7**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no. 38
5	
6	
7	concentration 10%
8	
9	
mean	
± S.D.	

Compound No. 38 was warmed up to 32°C and stirred on a magnetic stirrer

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	158
	A	169
3	B	249
	A	258

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 12 Jun 92

Name: Ph. Vanparys

Experiment no. 38

Compound of the same pair no. 35 + 41

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.009		0.1
2		•0	C2	0	0.0		0.019		0.3
3		0	0	C3	0.0		0.005		0.1
mean					0.0		0.011		0.2
± S.D.					0.0		0.007		0.1
10	compound no. 39	3	3	2	2.7	2.7	0.002	-0.009	2.5
11		3	3	3	3.0	3.0	0.013	0.002	3.0
12		3	3	3	3.0	3.0	0.002	-0.009	2.9
13	concentration	1	1	1	1.0	1.0	0.018	0.007	1.1
14	20%	1	1	1	1.0	1.0	0.004	-0.007	0.9
15		5	5	4	4.7	4.7	0.007	-0.004	4.6
mean					2.6	2.6	0.008	-0.003	2.5
± S.D.					1.4	1.4	0.006	0.006	1.4

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no. 39
11	
12	
13	concentration 20%
14	
15	
mean	
± S.D.	

pH: 2.97

The anterior chamber was opened for washing.

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	159
	A	170
3	B	250
	A	260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 17 Apr 92

Experiment no. 18

Compound of the same pair no. 38 + 42

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0	0	C2	0	0.0		0.011		0.2
3		1	1	C3	1.0	0	0	C3	0.0		0.008		0.1
mean					0.3				0.0		0.011		0.2
± S.D.					0.6				0.0		0.003		0.0
10	compound no. 40	3	4	3	3.3	9	9	8	8.7	8.7	3.128	3.117	55.4
11		3	3	2	2.7	9	9	8	8.7	8.7	3.775	3.764	65.1
12		5	5	4	4.7	8	9	8	8.3	8.3	3.992	3.981	68.1
13	concentration 10%	2	2	1	1.7	7	7	6	6.7	6.7	4.426	4.415	72.9
14		5	5	4	4.7	7	7	6	6.7	6.7	3.238	3.227	55.1
15		4	4	3	3.7	8	8	7	7.7	7.7	3.422	3.411	58.8
mean					3.4				7.8	7.8	3.664	3.653	**62.6**
± S.D.					1.2				0.9	0.9	0.496	0.496	**7.3**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no. 40
11	
12	
13	concentration 10%
14	
15	
mean	
± S.D.	

Compound No. 40 was warmed up to 32°C and stirred on a magnetic stirrer.

Membranes released from the corneas:

very small air-bubbles behind the corneas.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-169
3	B	249
	A	-258

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 12 Jun 92

Experiment no. 38

Compound of the same pair no. 35 + 39

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.009		0.1
2		0	C2	0	0.0		0.019		0.3
3		0	0	C3	0.0		0.005		0.1
mean					0.0		0.011		0.2
± S.D.					0.0		0.007		0.1
16	compound no. 41	53	52	52	52.3	52.3	0.035	0.024	52.7
17		58	58	58	58.0	58.0	0.052	0.041	58.6
18		64	64	64	64.0	64.0	0.108	0.097	65.4
19	concentration 20%	54	53	53	53.3	53.3	0.029	0.018	53.6
20		52	52	51	51.7	51.7	0.096	0.085	52.9
21		63	63	62	62.7	62.7	0.123	0.112	64.3
mean					57.0	57.0	0.074	0.063	**57.9**
± S.D.					5.4	5.4	0.040	0.040	**5.8**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no. 41	pH : 7.26
17		
18		Compound no. 41 was washed away 4 times.
19	concentration 20%	
20		
21		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 17 Apr 92

Name: Ph. Vanparys

Experiment no. 18

Compound of the same pair no. 38 + 40

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	0	0	0.0		0.013		0.2
2		0	C2	0	0.0	0	C2	0	0.0		0.011		0.2
3		1	1	C3	1.0	0	0	C3	0.0		0.008		0.1
mean					0.3				0.0		0.011		0.2
± S.D.					0.6				0.0		0.003		0.0
16	compound no.	12	12	11	11.7	18	18	17	17.7	17.7	3.170	3.159	65.1
17	42	13	13	12	12.7	19	19	18	18.7	18.7	3.968	3.957	78.0
18		11	12	11	11.3	25	25	24	24.7	24.7	2.638	2.627	64.1
19	concentration	8	8	7	7.7	14	14	13	13.7	13.7	3.449	3.438	65.2
20	10%	12	12	12	12.0	19	19	18	18.7	18.7	3.282	3.271	67.7
21		12	13	12	12.3	17	17	16	16.7	16.7	4.187	4.176	79.3
mean					11.3				18.3	18.3	3.449	3.438	**69.9**
± S.D.					1.8				3.6	3.6	0.562	0.562	**6.9**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no.
17	42
18	
19	concentration
20	10%
21	
mean	
± S.D.	

Compound No. 42 was warmed up to 32°C and stirred on a magnetic stirrer.

CALIBRATION		
Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	158
	A	169
3	B	248
	A	258

EEC VALIDATION OF THE BCOP ASSAY

Data sheet

Lab. no. 09

Date: 15 Jun 92

Experiment no. 39

Compound of the same pair no. 44

Name: Ph. Vanparys

Signature:

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.036		0.5
2		0	C2	0	0.0		0.022		0.3
3		0	0	C3	0.0		0.028		0.4
mean					0.0		0.029		0.4
± S.D.					0.0		0.007		0.1
4	compound no. 43	86	86	86	86.0	86.0	4.402	4.373	151.6
5		71	71	71	71.0	71.0	3.408	3.379	121.7
6		79	79	79	79.0	79.0	4.386	4.357	144.4
7	concentration 20%	94	94	94	94.0	94.0	4.370	4.342	159.1
8		91	91	91	91.0	91.0	6.292	6.263	184.9
9		95	93	93	93.7	93.7	3.553	3.524	146.5
mean					85.8	85.8	4.402	4.373	**151.4**
± S.D.					9.2	9.2	1.028	1.028	**20.7**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no. 43	pH: 8.66
5		
6		
7	concentration 20%	
8		
9		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	75
2	B	159
	A	170
3	B	249
	A	260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

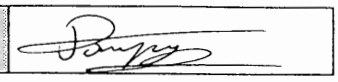
Lab. no. 09

Date: 15 Jun 92

Name: Ph. Vanparys

Experiment no. 39

Compound of the same pair no. 43

Signature: 

cornea	treatment	opacity at 240 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0		0.036		0.5
2		0	C2	0	0.0		0.022		0.3
3		0	0	C3	0.0		0.028		0.4
mean					0.0		0.029		0.4
± S.D.					0.0		0.007		0.1
10	compound no. 44	0	0	0	0.0	0.0	0.045	0.016	0.2
11		2	2	2	2.0	2.0	0.043	0.014	2.2
12		1	1	1	1.0	1.0	0.064	0.036	1.5
13	concentration 20%	-1	-2	-2	-1.7	-1.7	0.062	0.034	-1.2
14		0	-1	-1	-0.7	-0.7	0.485	0.457	6.2
15		4	3	3	3.3	3.3	0.055	0.026	3.7
mean					0.7	0.7	0.126	0.097	2.1
± S.D.					1.8	1.8	0.176	0.176	2.6

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no. 44	pH: 8.50
11		
12		
13	concentration 20%	
14		
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	249
	A	-260

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 21 Apr 92

Name: Ph. Vanparys

Experiment no. 19

Compound of the same pair no. 46

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	-1	0	-0.5		0.012		-0.3
2		0	C2	0	0.0	1	C2	1	1.0		0.009		1.1
3		0	0	C3	0.0	0	-1	C3	-0.5		0.015		-0.3
mean					0.0				0.0		0.012		0.2
± S.D.					0.0				0.9		0.003		0.8
4	compound no.	52	52	54	52.7	84	83	84	83.7	83.7	6.949	6.937	187.7
5	45	57	58	59	58.0	88	87	88	87.7	87.7	4.728	4.716	158.4
6		54	55	56	55.0	86	85	86	85.7	85.7	4.464	4.452	152.5
7	concentration	50	50	51	50.3	84	83	84	83.7	83.7	5.923	5.911	172.3
8	10%	47	47	48	47.3	79	78	80	79.0	79.0	4.781	4.769	150.5
9		54	55	56	55.0	89	87	88	88.0	88.0	5.748	5.736	174.0
mean					53.1				84.6	84.6	5.432	5.420	**165.9**
± S.D.					3.8				3.3	3.3	0.949	0.949	**14.5**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.
5	45
6	
7	concentration
8	10%
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-171
3	B	252
	A	-263

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 21 Apr 92

Name: Ph. Vanparys

Experiment no. 19

Compound of the same pair no. 45

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	-1	0	-0.5		0.012		-0.3
2		0	C2	0	0.0	1	C2	1	1.0		0.009		1.1
3		0	0	C3	0.0	0	-1	C3	-0.5		0.015		-0.3
mean					0.0				0.0		0.012		0.2
± S.D.					0.0				0.9		0.003		0.8
10	compound no.	1	1	1	1.0	3	2	3	2.7	2.7	0.014	0.003	2.7
11	46	2	1	1	1.3	4	3	4	3.7	3.7	0.005	-0.006	3.6
12		3	3	3	3.0	5	4	5	4.7	4.7	0.011	0.000	4.7
13	concentration	0	0	0	0.0	1	0	1	0.7	0.7	0.016	0.004	0.7
14	10%	1	0	0	0.3	2	1	2	1.7	1.7	0.031	0.020	2.0
15		3	3	3	3.0	5	4	5	4.7	4.7	0.043	0.032	5.1
mean					1.4				3.0	3.0	0.020	0.008	**3.1**
± S.D.					1.3				1.6	1.6	0.014	0.014	**1.7**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.	
11	46	
12		
13	concentration	
14	10%	
15		
mean		
± S.D.		

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-171
3	B	252
	A	-263

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date: 06 Apr 92
Experiment no. 14
Compound of the same pair no. 34

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	-1	-1.0	C1	0	0	0.0		0.014		0.2
2		0	C2	0	0.0	0	C2	0	0.0		0.007		0.1
3		0	0	C3	0.0	0	0	C3	0.0		0.005		0.1
mean					-0.3				0.0		0.009		0.1
± S.D.					0.6				0.0		0.005		0.1
10	compound no.	4	4	4	4.0	6	6	6	6.0	6.0	6.160	6.151	98.3
11	47	5	6	6	5.7	11	12	11	11.3	11.3	4.200	4.191	74.2
12		3	4	3	3.3	6	7	6	6.3	6.3	4.500	4.491	73.7
13	concentration	4	5	5	4.7	7	7	7	7.0	7.0	7.760	7.751	123.3
14	100%	2	2	2	2.0	8	9	8	8.3	8.3	4.520	4.511	76.0
15		4	5	5	4.7	7	8	7	7.3	7.3	6.280	6.271	101.4
mean					4.1				7.7	7.7	5.570	5.561	**91.1**
± S.D.					1.3				1.9	1.9	1.398	1.398	**20.0**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

10	compound no.
11	47
12	
13	concentration
14	100%
15	
mean	
± S.D.	

Na-fluorescein concentration in posterior compartment diluted with a factor 4. The OD values were multiplied with 4 to obtain the final OD value indicated in the table.

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	.75
2	B	158
	A	.167
3	B	248
	A	.255

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 13 Apr 92
Experiment no. 16
Compound of the same pair no. 51 + 52

REPEAT OF EXP. NR. 15

Name: Ph. Vanparys

Signature:

cor-nea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	0	-0.5	C1	-1	0	-0.5		0.009		-0.4
2		0	C2	1	0.5	0	C2	1	0.5		0.009		0.6
3		0	-1	C3	-0.5	-1	-2	C3	-1.5		0.010		-1.4
mean					-0.2				-0.5		0.009		-0.4
± S.D.					0.6				1.0		0.001		1.0
16	compound no. 48 (2)	63	62	63	62.7	80	80	81	80.3	80.8	3.963	3.954	140.1
17		57	57	58	57.3	76	75	76	75.7	76.2	5.402	5.393	157.1
18		52	52	53	52.3	70	69	70	69.7	70.2	4.382	4.373	135.8
19	concentration 100%	57	57	59	57.7	75	74	76	75.0	75.5	3.912	3.903	134.0
20		59	59	60	59.3	80	79	81	80.0	80.5	4.090	4.081	141.7
21		58	57	59	58.0	76	75	77	76.0	76.5	4.350	4.341	141.6
mean					57.9				76.1	76.6	4.350	4.341	**141.7**
± S.D.					3.4				3.9	3.9	0.551	0.551	**8.2**

cor-nea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no. 48 (2)
17	
18	
19	concentration 100%
20	
21	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	249
	A	-259

EEC VALIDATION OF THE BC0-P ASSAY

Data sheet

Lab. no. 09

Date:	16 Apr 92
Experiment no.	17
Compound of the same pair no.	36 + 37

REPEAT OF EXP. NR. 15

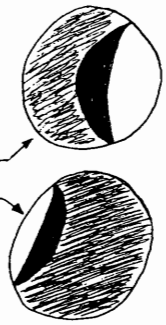
Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	1	0	0.5	C1	2	1	1.5		0.006		1.6
2		-2	C2	-1	-1.5	-3	C2	-2	-2.5		0.010		-2.4
3		0	0	C3	0.0	-2	1	C3	-0.5		0.015		-0.3
mean					-0.3				-0.5		0.010		-0.3
± S.D.					1.0				2.0		0.005		2.0
16	compound no.	21	24	23	22.7	30	34	32	32.0	32.5	4.517	4.507	100.1
17	49 (2)	23	25	24	24.0	29	33	30	30.7	31.2	4.129	4.119	92.9
18		29	31	30	30.0	33	36	34	34.3	34.8	6.519	6.509	132.5
19	concentration	18	20	19	19.0	23	27	25	25.0	25.5	3.785	3.775	82.1
20	100%	24	27	26	25.7	31	35	31	32.3	32.8	3.074	3.064	78.8
21		22	24	23	23.0	28	31	29	29.3	29.8	2.750	2.740	70.9
mean					24.1				30.6	31.1	4.129	4.119	**92.9**
± S.D.					3.6				3.2	3.2	1.341	1.341	**22.0**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

16	compound no.
17	49 (2)
18	
19	concentration
20	100%
21	
mean	
± S.D.	



Fine opace membrane is released from the corneas

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	159
	A	-170
3	B	250
	A	-263

EEC VALIDATION OF THE BCOP ASSAY

Data sheet

Lab. no. 09

Date: 22 Jun 92

Experiment no. 41

Compound of the same pair no.

Name: Ph. Vanparys

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	0	0	0.0	C1	0	0	0.0		0.005		0.1
2		0	C2	0	0.0	0	C2	0	0.0		0.009		0.1
3		0	1	C3	0.5	0	0	C3	0.0		0.012		0.2
mean					0.2				0.0		0.009		0.1
± S.D.					0.3				0.0		0.004		0.1
4	compound no.	9	11	10	10.0	15	14	14	14.3	14.3	6.268	6.259	108.2
5	50	12	14	14	13.3	20	19	19	19.3	19.3	4.413	4.404	85.4
6		13	14	13	13.3	19	19	19	19.0	19.0	7.409	7.400	130.0
7	concentration	9	10	9	9.3	10	10	10	10.0	10.0	3.752	3.743	66.2
8	10%	9	10	9	9.3	14	13	13	13.3	13.3	5.552	5.543	96.5
9		15	16	16	15.7	22	21	21	21.3	21.3	7.111	7.102	127.9
mean					11.8				16.2	16.2	5.751	5.742	**102.4**
± S.D.					2.6				4.3	4.3	1.462	1.462	**24.8**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.	pH: 9.06
5	50	
6		
7	concentration	Cornea no. 7 with transparant area in the middle.
8	10%	
9		
mean		
± S.D.		

CALIBRATION		
Filter paper	Holder	Opacity
-	-	
1	B	
	A	
2	B	
	A	
3	B	
	A	

EEC VALIDATION OF THE BCO-P ASSAY

Data sheet

Lab. no. 09

Date: 13 Apr 92

Name: Ph. Vanparys

Experiment no. 16

Compound of the same pair no. 48 + 52

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	0	-0.5	C1	-1	0	-0.5		0.009		-0.4
2		0	C2	1	0.5	0	C2	1	0.5		0.009		0.6
3		0	-1	C3	-0.5	-1	-2	C3	-1.5		0.010		-1.4
mean					-0.2				-0.5		0.009		-0.4
± S.D.					0.6				1.0		0.001		1.0
4	compound no.	41	41	42	41.3	47	46	48	47.0	47.5	5.154	5.145	124.7
5	51	37	37	38	37.3	43	43	44	43.3	43.8	3.662	3.653	98.6
6		36	35	37	36.0	40	40	41	40.3	40.8	3.053	3.044	86.5
7	concentration	36	36	37	36.3	42	41	42	41.7	42.2	3.216	3.207	90.3
8	100%	38	38	39	38.3	42	42	43	42.3	42.8	4.318	4.309	107.5
9		47	46	47	46.7	49	48	50	49.0	49.5	4.742	4.733	120.5
mean					39.3				43.9	44.4	4.024	4.015	104.7
± S.D.					4.1				3.3	3.3	0.849	0.849	15.7

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	

4	compound no.
5	51
6	
7	concentration
8	100%
9	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	249
	A	-259

EEC VALIDATION OF THE BCOP ASSAY

Data sheet

Lab. no. 09

Date: 13 Apr 92

Name: Ph. Vanparys

Experiment no. 16

Compound of the same pair no. 48 + 51

Signature:

cornea	treatment	opactity at 10 min				opacity at 120 min				correc.	Permeability (OD)	correc.	in-vitro score
1	MEM	C1	-1	0	-0.5	C1	-1	0	-0.5		0.009		-0.4
2		0	C2	1	0.5	0	C2	1	0.5		0.009		0.6
3		0	-1	C3	-0.5	-1	-2	C3	-1.5		0.010		-1.4
mean					-0.2				-0.5		0.009		-0.4
± S.D.					0.6				1.0		0.001		1.0
10	compound no.	1	0	2	1.0	3	2	3	2.7	3.2	0.038	0.029	3.6
11	52	2	1	3	2.0	3	2	3	2.7	3.2	0.039	0.030	3.6
12		0	0	1	0.3	1	1	2	1.3	1.8	0.025	0.016	2.1
13	concentration	1	0	1	0.7	3	2	4	3.0	3.5	0.052	0.043	4.1
14	100%	1	0	1	0.7	2	2	3	2.3	2.8	0.027	0.018	3.1
15		0	0	0	0.0	1	0	1	0.7	1.2	0.025	0.016	1.4
mean					0.8				2.1	2.6	0.034	0.025	**3.0**
± S.D.					0.7				0.9	0.9	0.011	0.011	**1.0**

cornea	treatment
1	MEM
2	
3	
mean	
± S.D.	
10	compound no.
11	52
12	
13	concentration
14	100%
15	
mean	
± S.D.	

CALIBRATION

Filter paper	Holder	Opacity
-	-	0
1	B	75
	A	-75
2	B	158
	A	-168
3	B	249
	A	-259

[This Page Intentionally Left Blank]

Appendix G7

Dataset Received from Johnson & Johnson Pharmaceutical Research and Development – A Division of Janssen Pharmaceutica N.V. (BCOP Tests With Young vs. Old Corneas)

[This Page Intentionally Left Blank]

The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

Freddy Van Goethem, Marc Sysmans and Philippe Vanparys

Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica N.V., Genetic and In Vitro Toxicology, Turnhoutseweg 30, B-2340 Beerse, Belgium.

BCOP results obtained with corneas from:

1) adult animals (> 24 months)

2) young animals (6 - 8 months)

Methodology

After background opacity measurement, medium was removed from the anterior compartment and corneas were treated with 0.75 ml of the test solution. Corneas (3 per group) were treated for 10 minutes followed by a 120 minutes recovery period. Medium was removed from the anterior compartment and replaced by 1 ml of a 0.4% sodium-fluorescein solution. Corneas were incubated in a horizontal position for 90 minutes at 32°C in a water-bath. After incubation, medium from the posterior chamber was removed and its optical density (OD) determined with a spectrophotometer at 490 nm. In Vitro Score = opacity + [15 x permeability]

Code of each compound is recorded on each raw data sheet

››› compound **17** (acetone) need to be repeated since results did not comply with previously collected data in our laboratory. Due to the high vapor pressure of acetone (201.57 mmHg @ 22.0 °C), a technical artefact could have occurred…

The use of corneas from animals of different age in the Bovine Corneal Opacity and Permeability (BCOP) assay.

Code	Compound	CAS No.	In vivo EU	In vivo GHS	In Vitro BCOP (>24 months)				In Vitro BCOP (6 - 8 months)			
					Opacity	Perm.	IVS	Class	Opacity	Perm.	IVS	Class
1	3,3-dimethylpentane	562-49-2	NI	NI	0.6	0.01	0.8	NON	0.0	0.02	0.3	NON
2	3-methoxy-1,2-propanediol	623-39-2	NI	NI	-0.3	0.00	0.2	NON	0.6	0.02	0.9	NON
3	polyethylene glycol 400	25322-68-3	NI	NI	-0.3	0.00	-0.3	NON	0.0	0.08	1.1	NON
4	glycerol	56-81-5	NI	NI	-1.0	0.01	-0.9	NON	-0.7	-0.01	-0.8	NON
5	methyl cyclopentane	96-37-7	NI	NI	1.0	0.43	7.5	MILD	1.3	0.26	5.2	MILD
6	tween 20	9005-64-5	NI	NI	0.0	0.01	0.1	NON	0.0	-0.01	-0.1	NON
7	methyl *iso*-butyl ketone	108-10-1	NI	NI	6.6	1.07	22.7	MILD	5.7	0.83	18.1	MILD
8	toluene	108-88-3	NI	NI	6.3	3.18	54	MOD	6.0	1.46	28.0	MOD
9	methyl amyl ketone	110-43-0	NI	NI	5.3	1.80	32.3	MOD	4.0	0.99	18.8	MILD
10	2-methyl-1-pentanol	105-30-6	NI	2B	12.0	4.30	76.6	SEV	8.6	1.94	37.7	MOD
11	ethanol	64-17-5	NI	2B	16.0	2.34	51	MOD	16.3	1.83	43.8	MOD
12	sodium hydroxide (1%)	1310-73-2	R36	2B	99.7	4.16	162	SEV	135.7	3.74	191.8	SEV
13	triton X-100 (5%)	9002-93-1	R36	2B	4.3	3.81	61.5	SEV	4.7	3.70	60.1	SEV
14	1-octanol	111-87-5	R36	2B	10.0	5.24	88.6	SEV	10.3	1.53	33.3	MOD
15	2-ethyl-1-hexanol	104-76-7	R36	2B	4.3	1.76	30.6	MOD	2.3	0.86	15.3	MILD
16	n-hexanol	111-27-3	R36	2A	15.3	3.73	71.2	SEV	14.0	3.62	68.2	SEV
17	acetone	67-64-1	R36	2A	39**	2.95	83.2	SEV	91.3	2.86	134.2	SEV
18	cyclohexanol	108-93-0	R41	1	15.3	5.04	90.7	SEV	11.6	2.13	43.6	MOD
19	cetylpyridinium bromide (6%)	140-72-7	R41	1	11.7	1.01	26.8	MOD	15.0	1.66	39.9	MOD
20	benzalkonium chloride (10%)	8001-54-5	R41	1	92.2	4.22	155.4	SEV	105.7	4.05	166.5	SEV

Prediction Model

BCOP In Vitro Score	Class
≤ 3	NON
3.1-25	MILD
25.1-55	MOD
> 55.1	SEV

** to be repeated (technical artefact probably occurred)

Johnson and Johnson Pharmaceutical Research and Development (Belgium)

- Compounds 1 → 20
- Adult animals (≥ 24 months)

Calculation of the in vitro eye irritation score for liquids

Test article	3,3-Dimethylpentane [562-49-2]
Batch No.	14502CN
Concentration	100%
Code	A1 (1)
Sequence	Intern 8B

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	2	2	0		0.006		0.1
2	MEM	1	1	0		0.012		0.2
3	100%	1	3	2		0.009		2.1
	Mean ± S.D.			0.7 ± 1.2		0.009 ± 0.003		0.8 ± 1.1
4	Test article	1	3	2	1.3	0.023	0.014	1.5
5		0	1	1	0.3	0.018	0.009	0.4
6	100%	1	2	1	0.3	0.014	0.005	0.4
	Mean ± S.D.				0.6 ± 0.6		0.009 ± 0.005	0.8 ± 0.6

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	154	B	-157
	3	A	250	B	-255

Paraph	
Date	18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	3-methoxy-1,2-propanediol 98%
Batch No.	05307-078
Concentration	100%
Code	B1 (2)
Sequence	Intern 10A

Treatment time	2 hours

OP-KIT

No. Comea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	MEM	0	0	0		0.008		0.1
3	100%	0	1	1		0.010		1.2
	Mean ± S.D.			0.3 ± 0.6		0.007 ± 0.003		0.5 ± 0.6
4	Test article	0	0	0	-0.3	0.019	0.012	-0.1
5		0	0	0	-0.3	0.066	0.059	0.6
6	100%	0	0	0	-0.3	0.032	0.025	0.1
	Mean ± S.D.				-0.3 ± 0.0		0.032 ± 0.024	0.2 ± 0.4

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph	
Date	31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Polyethylene glycol 400
Batch No.	3H0110
Concentration	100%
Code	C1 (3)
Sequence	11A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.002		0.0
2	NaCl 0.9%	0	0	0		0.003		0.0
3	100%	1	1	0		0.001		0.0
	Mean ± S.D.			0.0 ± 0.0		0.002 ± 0.001		0.0 ± 0.0
4	Test article	0	0	0	0.0	0.000	-0.002	0.0
5		0	0	0	0.0	0.003	0.001	0.0
6	100%	1	0	-1	-1.0	0.010	0.008	-0.9
	Mean ± S.D.				-0.3 ± 0.6		0.002 ± 0.005	-0.3 ± 0.5

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph

Date 28-Feb-00

Calculation of the in vitro eye irritation score for liquids

Test article	Glycerol
Batch No.	HS03116BS
Concentration	100%
Code	B2 (4)
Sequence	Intern 10A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	MEM	0	0	0		0.008		0.1
3	100%	0	1	1		0.010		1.2
	Mean ± S.D.			0.3 ± 0.6		0.007 ± 0.003		0.5 ± 0.6
7	Test article	1	0	-1	-1.3	0.017	0.010	-1.2
8		1	1	0	-0.3	0.015	0.008	-0.2
9	100%	1	0	-1	-1.3	0.017	0.010	-1.2
	Mean ± S.D.				-1.0 ± 0.6		0.009 ± 0.001	-0.9 ± 0.6

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl cyclopentane
Batch No.	09817PS-089
Concentration	100%
Code	D5 (5)
Sequence	12A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	NaCl 0.9%	1	1	0		0.006		0.1
3	100%	1	1	0		0.005		0.1
	Mean ± S.D.			0.0 ± 0.0		0.005 ± 0.001		0.1 ± 0.0
16	Test article	0	1	1	1.0	0.262	0.257	4.9
17		0	2	2	2.0	0.839	0.834	14.5
18	100%	0	0	0	0.0	0.212	0.207	3.1
	Mean ± S.D.				1.0 ± 1.0		0.433 ± 0.348	7.5 ± 6.1

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph	20-Mar-00
Date	

Calculation of the in vitro eye irritation score for liquids

Test article	Tween 20
Batch No.	A010055102
Concentration	100%
Code	C2 (6)
Sequence	11A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.002		0.0
2	NaCl 0.9%	0	0	0		0.003		0.0
3	100%	1	1	0		0.001		0.0
	Mean ± S.D.			0.0 ± 0.0		0.002 ± 0.001		0.0 ± 0.0
7	Test article	0	0	0	0.0	0.010	0.008	0.1
8		0	0	0	0.0	0.023	0.021	0.3
9	100%	0	0	0	0.0	0.004	0.002	0.0
	Mean ± S.D.				0.0 ± 0.0		0.010 ± 0.010	0.1 ± 0.2

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	28-Feb-00

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl iso-butyl ketone (4 methyl-2-pentanone) [108-10-1]
Batch No.	CU 10369BU
Concentration	100%
Code	A2 (7)
Sequence	Intern 8B

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	2	2	0		0.006		0.1
2	MEM	1	1	0		0.012		0.2
3	100%	1	3	2		0.009		2.1
	Mean ± S.D.			0.7 ± 1.2		0.009 ± 0.003		0.8 ± 1.1
7	Test article	1	8	7	6.3	1.909	1.900	34.8
8		0	7	7	6.3	0.621	0.612	15.5
9	100%	1	9	8	7.3	0.706	0.697	17.8
	Mean ± S.D.				6.6 ± 0.6		1.070 ± 0.720	22.7 ± 10.5

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	18-Jan-00

RDF/BCO/18

Calculation of the in vitro eye irritation score for liquids

Test article	Toluene [108-88-3]
Batch No.	990281O001
Concentration	100%
Code	D4 (8)
Sequence	12A

Treatment time	

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	NaCl 0.9%	1	1	0		0.006		0.1
3	100%	1	1	0		0.005		0.1
	Mean ± S.D.			0.0 ± 0.0		0.005 ± 0.001		0.1 ± 0.0
13	Test article	0	6	6	6.0	3.480	3.475	58.1
14		0	7	7	7.0	2.832	2.827	49.4
15	100%	0	6	6	6.0	3.236	3.231	54.5
	Mean ± S.D.				6.3 ± 0.6		3.178 ± 0.327	54.0 ± 4.4

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	methyl amyl ketone (2 heptanone) [110-43-0]
Batch No.	66400-104
Concentration	100%
Code	A3
Sequence	Intern 8B

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	2	2	0		0.006		0.1
2	MEM	1	1	0		0.012		0.2
3	100%	1	3	2		0.009		2.1
	Mean ± S.D.			0.7 ± 1.2		0.009 ± 0.003		0.8 ± 1.1
10	Test article	1	8	7	6.3	1.095	1.086	22.6
11		0	6	6	5.3	1.924	1.915	34.0
12	100%	2	7	5	4.3	2.404	2.395	40.2
	Mean ± S.D.				5.3 ± 1.0		1.799 ± 0.662	32.3 ± 8.9

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	2-methyl-1-pentanol
Batch No.	05002PG
Concentration	100%
Code	B3 (10)
Sequence	Intern 10A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	MEM	0	0	0		0.008		0.1
3	100%	0	1	1		0.010		1.2
	Mean ± S.D.			0.3 ± 0.6		0.007 ± 0.003		0.5 ± 0.6
10	Test article	0	10	10	9.7	3.336	3.329	59.6
11		0	13	13	12.7	4.916	4.909	86.3
12	100%	0	14	14	13.7	4.680	4.673	83.8
	Mean ± S.D.				12.0 ± 2.1		4.304 ± 0.852	76.6 ± 14.7

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Ethanol [64-17-5]
Batch No.	993O710002
Concentration	100%
Code	D1 (11)
Sequence	12A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	NaCl 0.9%	1	1	0		0.006		0.1
3	100%	1	1	0		0.005		0.1
	Mean ± S.D.			0.0 ± 0.0		0.005 ± 0.001		0.1 ± 0.0
4	Test article	0	16	16	16.0	2.340	2.335	51.0
5		0	17	17	17.0	2.164	2.159	49.4
6	100%	0	15	15	15.0	2.520	2.515	52.7
	Mean ± S.D.				16.0 ± 1.0		2.336 ± 0.178	51.0 ± 1.7

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	A	B
	2	A	B
	3	A	B

Paraph	
Date	20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Sodium hydroxide 1%
Batch No.	66H0320
Concentration	1%
Code	D3 (12)
Sequence	12A

Treatment time:

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	NaCl 0.9%	1	1	0		0.006		0.1
3	100%	1	1	0		0.005		0.1
	Mean ± S.D.			0.0 ± 0.0		0.005 ± 0.001		0.1 ± 0.0
10	Test article	0	101	101	101.0	3.952	3.947	160.2
11		0	111	111	111.0	4.276	4.271	175.1
12	100%	0	87	87	87.0	4.256	4.251	150.8
	Mean ± S.D.				99.7 ± 12.1		4.156 ± 0.182	162.0 ± 12.3

NC: Negative Control

REMARKS	Filter	OPACITY	
	1	A	B
	2	A	B
	3	A	B

Paraph

Date 20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Triton X-100 (5%)
Batch No.	28H2536
Concentration	100%
Code	C4 (13)
Sequence	11A

Treatment time	10 min
	OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.002		0.0
2	NaCl 0.9%	0	0	0		0.003		0.0
3	100%	1	1	0		0.001		0.0
	Mean ± S.D.			0.0 ± 0.0		0.002 ± 0.001		0.0 ± 0.0
13	Test article	1	6	5	5.0	4.268	4.266	69.0
14		0	4	4	4.0	3.384	3.382	54.7
15	100%	2	6	4	4.0	3.792	3.790	60.9
	Mean ± S.D.				4.3 ± 0.6		3.813 ± 0.442	61.5 ± 7.2

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	A:	B:
	2	A:	B:
	3	A:	B:

Paraph	
Date	28-Feb-00

Calculation of the in vitro eye irritation score for liquids

Test article	n-octanol
Batch No.	27336-019
Concentration	100%
Code	B4 (19)
Sequence	Intern 10A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	MEM	0	0	0		0.008		0.1
3	100%	0	1	1		0.010		1.2
	Mean ± S.D.			0.3 ± 0.6		0.007 ± 0.003		0.5 ± 0.6
13	Test article	1	7	6	5.7	5.180	5.173	83.3
14		0	15	15	14.7	5.828	5.821	102.0
15	100%	1	11	10	9.7	4.724	4.717	80.5
	Mean ± S.D.				10.0 ± 4.5		5.237 ± 0.555	88.6 ± 11.7

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	2-ethyl-1-hexanol [107-76-7]
Batch No.	26812-019
Concentration	100%
Code	A4 (15)
Sequence	Intern 8B

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	2	2	0		0.006		0.1
2	MEM	1	1	0		0.012		0.2
3	100%	1	3	2		0.009		2.1
	Mean ± S.D.			0.7 ± 1.2		0.009 ± 0.003		0.8 ± 1.1
13	Test article	2	6	4	3.3	1.763	1.754	29.6
14		1	7	6	5.3	2.196	2.187	38.1
15	100%	2	7	5	4.3	1.337	1.328	24.2
	Mean ± S.D.				4.3 ± 1.0		1.756 ± 0.430	30.6 ± 7.0

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	1-Hexanol [111-27-3]
Batch No.	381949/1
Concentration	100%
Code	D2 (16)
Sequence	12A

Treatment time	

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	NaCl 0.9%	1	1	0		0.006		0.1
3	100%	1	1	0		0.005		0.1
	Mean ± S.D.			0.0 ± 0.0		0.005 ± 0.001		0.1 ± 0.0
7	Test article	0	17	17	17.0	3.700	3.695	72.4
8		0	13	13	13.0	4.060	4.055	73.8
9	100%	0	16	16	16.0	3.440	3.435	67.5
	Mean ± S.D.				15.3 ± 2.1		3.728 ± 0.311	71.2 ± 3.3

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	20-Mar-00

Calculation of the in vitro eye irritation score for liquids

Test article	Acetone [67-64-1]
Batch No.	39H3430
Concentration	100%
Code	A5 (17)
Sequence	Intern 8B

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	2	2	0		0.006		0.1
2	MEM	1	1	0		0.012		0.2
3	100%	1	3	2		0.009		2.1
	Mean ± S.D.			0.7 ± 1.2		0.009 ± 0.003		0.8 ± 1.1
16	Test article	1	36	35	34.3	1.688	1.679	59.5
17		1	42	41	40.3	2.888	2.879	83.5
18	100%	1	44	43	42.3	4.304	4.295	106.7
	Mean ± S.D.				39.0 ± 4.2		2.951 ± 1.309	83.2 ± 23.6

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	18-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Cyclohexanol
Batch No.	18285-049
Concentration	100%
Code	B5 (18)
Sequence	Intern 10A

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.004		0.1
2	MEM	0	0	0		0.008		0.1
3	100%	0	1	1		0.010		1.2
	Mean ± S.D.			0.3 ± 0.6		0.007 ± 0.003		0.5 ± 0.6
16	Test article	0	16	16	15.7	6.180	6.173	108.3
17		0	16	16	15.7	3.288	3.281	64.9
18	100%	0	14	14	13.7	5.680	5.673	98.8
	Mean ± S.D.				15.0 ± 1.2		5.042 ± 1.546	90.7 ± 22.8

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	A	B
	2	A	B
	3	A	B

Paraph	
Date	31-Jan-00

Calculation of the in vitro eye irritation score for liquids

Test article	Cetylpyridinium bromide (6%)
Batch No.	105H0915
Concentration	100%
Code	C5 (19)
Sequence	11A

Treatment time	
OP-KIT	

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.002		0.0
2	NaCl 0.9%	0	0	0		0.003		0.0
3	100%	1	1	0		0.001		0.0
	Mean ± S.D.			0.0 ± 0.0		0.002 ± 0.001		0.0 ± 0.0
16	Test article	1	15	14	14.0	1.659	1.657	38.9
17		1	12	11	11.0	0.657	0.655	20.8
18	100%	0	10	10	10.0	0.714	0.712	20.7
	Mean ± S.D.				11.7 ± 2.1		1.008 ± 0.563	26.8 ± 10.5

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A		B	
	2	A		B	
	3	A		B	

Paraph	
Date	28-Jan-00

Benzalkonium chloride (10%)

Exp.	Opacity	Permeability	In Vitro Score
1	88.0	4.426	154.4
2	94.6	4.148	156.9
3	87.0	4.252	150.8
4	93.0	4.278	157.2
5	98.3	3.972	157.9
mean	**92.2**	**4.2**	**155.4**
SD	**4.7**	**0.17**	**2.9**

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)
Batch No.	76H2520
Concentration	10 g/g%
Prevalidation phase	II
Sequence	A
Treatment time	10 min

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
16	NC	1	2	1		0.005		1.1
17	0.9 % NaCl	1	2	1		0.018		1.3
18	100%	1	2	1		0.002		1.0
	Mean ± S.D.			1.0 ± 0.0		0.008 ± 0.009		1.1 ± 0.2
19	Test article	1	97	96	95.0	3.927	3.919	153.8
20		1	82	81	80.0	4.245	4.237	143.6
21	10g/g%	1	91	90	89.0	5.130	5.122	165.8
	Mean ± S.D.				88.0 ± 7.5		4.426 ± 0.623	154.4 ± 11.1

NC: Negative control
PC: Positive control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	153	B	-159
	3	A	236	B	-253

Paraph	
Date	13-Feb-97

Janssen Pharmaceutica N.V. - Dept. of Toxicology - Section In Vitro Toxicology

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)
Batch No.	76H2520
Concentration	10 g/g%
Prevalidation phase	II
Sequence	D
Treatment time	10 min

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
16	NC	1	1	0		0.005		0.1
17	0.9 % NaCl	1	2	1		0.007		1.1
18	100%	0	1	1		0.004		1.1
	Mean ± S.D.			0.7 ± 0.6		0.005 ± 0.002		0.8 ± 0.6
19	Test article	1	108	107	106.3	4.785	4.780	178.0
20		0	92	92	91.3	3.464	3.459	143.2
21	10g/g%	0	87	87	86.3	4.210	4.205	149.4
	Mean ± S.D.				94.6 ± 10.4		4.148 ± 0.662	156.9 ± 18.6

NC: Negative control
PC: Positive control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	153	B	-158
	3	A	235	B	-252

Paraph

Date

RDF/BCO/19

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)
Batch No.	76H2520
Concentration	10 g/g%
Prevalidation phase	II
Sequence	F
Treatment time	10 min

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
10	NC	0	1	1		0.009		1.1
11	0.9 % NaCl	1	2	1		0.001		1.0
12	100%	0	1	1		0.018		1.3
	Mean ± S.D.			1.0 ± 0.0		0.009 ± 0.009		1.1 ± 0.2
19	Test article	1	88	87	86.0	4.333	4.324	150.9
20		1	82	81	80.0	4.255	4.246	143.7
21	10g/g%	1	97	96	95.0	4.196	4.187	157.8
	Mean ± S.D.				87.0 ± 7.5		4.252 ± 0.069	150.8 ± 7.1

NC: Negative control
PC: Positive control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	152	B	-158
	3	A	234	B	-252

Paraph	
Date	13-Mar-97

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)
Batch No.	76H2520
Concentration	10 g/g%
Prevalidation phase	II
Sequence	G
Treatment time	10 min

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
19	NC	3	3	0		0.008		0.1
20	0.9 % NaCl	0	0	0		0.038		0.6
21	100%	1	1	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.019 ± 0.016		0.3 ± 0.3
25	Test article	0	96	96	96.0	4.531	4.512	163.7
26		0	93	93	93.0	5.219	5.200	171.0
27	10g/g%	2	92	90	90.0	3.142	3.123	136.8
	Mean ± S.D.				93.0 ± 3.0		4.278 ± 1.058	157.2 ± 18.0

NC: Negative control
PC: Positive control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	152	B	-158
	3	A	231	B	-249

Paraph	
Date	20-Mar-97

BCOP PREVALIDATION 1997

Calculation of in vitro eye irritation score for surfactants (10% w/w)

Test article	1 (BAK)
Batch No.	76H2520
Concentration	10 g/g%
Prevalidation phase	II
Sequence	F H

Treatment time	10 min

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
10	NC	1	1	0		0.013		0.2
11	0.9 % NaCl	1	4	3		0.013		3.2
12	100%	1	1	0		0.021		0.3
	Mean ± S.D.			1.0 ± 1.7		0.016 ± 0.005		1.2 ± 1.7
19	Test article	1	99	98	97.0	4.051	4.035	157.5
20		1	99	98	97.0	4.312	4.296	161.4
21	10g/g%	1	103	102	101.0	3.600	3.584	154.8
	Mean ± S.D.				98.3 ± 2.3		3.972 ± 0.360	157.9 ± 3.3

NC: Negative control
PC: Positive control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	152	B	-161
	3	A	236	B	-253

Paraph	
Date	21-Mar-97

• Compounds 1 → 20

• young animals (6-8 months)

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	3,3 Dimethylpentane [562-49-2]		
Batch No.	14602CN		
Concentration	99%	Treatment time	10 min
Code	A1		
Sequence	2005/ Interlab3 kalveren		**OP-KIT**

No. Comea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.008		0.1
2	MEM	0	0	0		0.026		0.4
3	100%	0	0	0		0.006		0.1
	Mean ± S.D.			0.0 ± 0.0		0.013 ± 0.011		0.2 ± 0.2
4	Test article	0	0	0	0.0	0.046	0.033	0.5
5		0	0	0	0.0	0.028	0.015	0.2
6	100%	0	0	0	0.0	0.023	0.010	0.2
	Mean ± S.D.				0.0 ± 0.0		0.019 ± 0.012	0.3 ± 0.2

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	158	B	-160
	3	A	256	B	-258

Paraph	
Date	07-Mar-05

Filter	
0.1	1
0.3	15
0.6	50
0.8	90
1	145

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	3-methoxy-1,2-propanediol [623-39-2]
Batch No.	A0155893001
Concentration	100%
Code	B1 (2)
Sequence	2005/ intern2 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.084		1.3
2	NaCl 0.9%	0	2	2		0.085		3.3
3	100%	1	1	0		0.036		0.5
	Mean ± S.D.			0.7 ± 1.2		0.068 ± 0.028		1.7 ± 1.4
4	Test article	0	0	0	-0.7	0.090	0.022	-0.4
5		2	6	4	3.3	0.096	0.028	3.7
6	100%	0	0	0	-0.7	0.070	0.002	-0.7
	Mean ± S.D.				0.6 ± 2.3		0.017 ± 0.014	0.9 ± 2.5

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	155	B	-161
3	A	259	B	-261

Paraph	
Date	01-Mar-05

Filter	
0.1	1
0.3	16
0.6	51
0.8	91
1	143

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	polyethylene glycol 400 [25322-68-3]		
Batch No.	S23152-394		
Concentration	100%	Treatment time	10 min
Code	C1 (3)		
Sequence	2005/ intern 1 kalveren		**OP-KIT**

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.023		0.3
2	NaCl 0.9%	0	0	0		0.069		1.0
3	100%	0	0	0		0.044		0.7
	Mean ± S.D.			0.0 ± 0.0		0.045 ± 0.023		0.7 ± 0.4
4	Test article	0	0	0	0.0	0.102	0.057	0.9
5		0	0	0	0.0	0.178	0.133	2.0
6	100%	0	0	0	0.0	0.080	0.035	0.5
	Mean ± S.D.				0.0 ± 0.0		0.075 ± 0.051	1.1 ± 0.8

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	157	B	-161
	3	A	260	B	-259

Paraph	
Date	28-Feb-05

Filter	
0.1	1
0.3	16
0.6	50
0.8	88
1	140

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	glycerol [56-81-5]
Batch No.	13574HC
Concentration	100%
Code	B2
Sequence	2005/ interni2 kalveren

Treatment time	10 min

OP-KIT

No. Comea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.084		1.3
2	NaCl 0.9%	0	2	2		0.085		3.3
3	100%	1	1	0		0.036		0.5
	Mean ± S.D.			0.7 ± 1.2		0.068 ± 0.028		1.7 ± 1.4
7	Test article	0	0	0	-0.7	0.008	-0.060	-1.6
8		0	0	0	-0.7	0.009	-0.059	-1.6
9	100%	2	2	0	-0.7	0.161	0.093	0.7
	Mean ± S.D.				-0.7 ± 0.0		-0.009 ± 0.088	-0.8 ± 1.3

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	155	B	-161
3	A	259	B	-261

Paraph	
Date	01-Mar-05

Filter	
0.1	1
0.3	16
0.6	51
0.8	91
1	143

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl cyclopentane [96-37-7]
Batch No.	1097605
Concentration	95%
Code	D5 (5)
Sequence	2005/ Intern3 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.008		0.1
2	NaCl 0.9%	0	0	0		0.026		0.4
3	100%	0	0	0		0.006		0.1
	Mean ± S.D.			0.0 ± 0.0		0.013 ± 0.011		0.2 ± 0.2
16	Test article	0	2	2	2.0	0.444	0.431	8.5
17		1	2	1	1.0	0.181	0.168	3.5
18	100%	0	1	1	1.0	0.193	0.180	3.7
	Mean ± S.D.				1.3 ± 0.6		0.260 ± 0.149	5.2 ± 2.8

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	158	B	-160
3	A	256	B	-258

Paraph	
Date	07-Mar-05

Filter	
0.1	1
0.3	15
0.6	50
0.8	90
1	145

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Tween 20 {9005-64-5}
Batch No.	094K01761
Concentration	100%
Code	C2 (6)
Sequence	2005/Intern4 kalverogen

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.045		0.7
2	NaCl 0.9%	0	0	0		0.022		0.3
3	100%	0	0	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.026 ± 0.017		0.4 ± 0.3
19	Test article	0	0	0	0.0	0.028	0.002	0.0
20		0	0	0	0.0	0.021	-0.005	-0.1
21	100%	0	0	0	0.0	0.013	-0.013	-0.2
	Mean ± S.D.				0.0 ± 0.0		-0.005 ± 0.008	-0.1 ± 0.1

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	156	B	-158
	3	A	263	B	-258

Paraph	
Date	14-Mar-05

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Methyl iso-butyl ketone (4 methyl-2-pentanone) [108-10-1]
Batch No.	1127250
Concentration	100%
Code	A2 (7)
Sequence	2005/ intern1 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.023		0.3
2	NaCl 0.9%	0	0	0		0.069		1.0
3	100%	0	0	0		0.044		0.7
	Mean ± S.D.			0.0 ± 0.0		0.045 ± 0.023		0.7 ± 0.4
7	Test article	0	3	3	3.0	0.714	0.669	13.0
8		0	8	8	8.0	0.861	0.816	20.2
9	100%	0	6	6	6.0	1.059	1.014	21.2
	Mean ± S.D.				5.7 ± 2.5		0.833 ± 0.173	18.1 ± 4.5

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY A	OPACITY B	OPACITY B
	1	A	75	B	-75
	2	A	157	B	-161
	3	A	260	B	-259

Paraph	
Date	28-Feb-05

Filter	
0.1	1
0.3	16
0.6	50
0.8	88
1	140

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Toluene [108-88-3]		
Batch No.	A0204558001		
Concentration	100%	Treatment time	10 min
Code	D4 (8)		
Sequence	2005/ Interm3 kalveren		**OP-KIT**

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.008		0.1
2	NaCl 0.9%	0	0	0		0.026		0.4
3	100%	0	0	0		0.006		0.1
	Mean ± S.D.			0.0 ± 0.0		0.013 ± 0.011		0.2 ± 0.2
13	Test article	0	2	2	2.0	1.550	1.537	25.1
14		0	9	9	9.0	1.852	1.839	36.6
15	100%	0	7	7	7.0	1.030	1.017	22.3
	Mean ± S.D.				6.0 ± 3.6		1.464 ± 0.416	28.0 ± 7.6

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	158	B	-160
	3	A	256	B	-258

Paraph	
Date	07-Mar-05

Filter	
0.1	1
0.3	15
0.6	50
0.8	90
1	145

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	methyl amyl ketone (2 heptanone) [110-43-0]		
Batch No.	13622JC		
Concentration	100%	Treatment time	10 min
Code	A3 (9)		
Sequence	2005/ intern 1 kalveren	**OP-KIT**	

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.023		0.3
2	NaCl 0.9%	0	0	0		0.069		1.0
3	100%	0	0	0		0.044		0.7
	Mean ± S.D.			0.0 ± 0.0		0.045 ± 0.023		0.7 ± 0.4
10	Test article	0	5	5	5.0	1.065	1.020	20.3
11		0	5	5	5.0	1.030	0.985	19.8
12	100%	2	4	2	2.0	0.995	0.950	16.3
	Mean ± S.D.				4.0 ± 1.7		0.985 ± 0.035	18.8 ± 2.2

NC: Negative Control

REMARKS	Filter		OPACITY			
	1	A	75	B	-75
	2	A	157	B	-161
	3	A	260	B	-259

Paraph	
Date	28-Feb-05

Filter	
0.1	1
0.3	16
0.6	50
0.8	88
1	140

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	2-methyl-1-pentanol [105-30-6]
Batch No.	451942/1
Concentration	100%
Code	B3 (10)
Sequence	2005/ intern2 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.084		1.3
2	NaCl 0.9%	0	2	2		0.085		3.3
3	100%	1	1	0		0.036		0.5
	Mean ± S.D.			0.7 ± 1.2		0.068 ± 0.028		1.7 ± 1.4
10	Test article	0	11	11	10.3	1.801	1.733	36.3
11		0	8	8	7.3	2.436	2.368	42.8
12	100%	0	9	9	8.3	1.773	1.705	33.9
	Mean ± S.D.				8.6 ± 1.5		1.935 ± 0.375	37.7 ± 4.6

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	155	B	-161
3	A	259	B	-261

Paraph	
Date	01-Mar-05

Filter	
0.1	1
0.3	16
0.6	51
0.8	91
1	143

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Ethanol [64-17-5]
Batch No.	K33957583 448
Concentration	100%
Code	D1 (11)
Sequence	2005/ Intern3 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.008		0.1
2	NaCl0.9%	0	0	0		0.026		0.4
3	100%	0	0	0		0.006		0.1
	Mean ± S.D.			0.0 ± 0.0		0.013 ± 0.011		0.2 ± 0.2
7	Test article	0	18	18	18.0	2.308	2.295	52.4
8		0	16	16	16.0	1.702	1.689	41.3
9	100%	0	15	15	15.0	1.531	1.518	37.8
	Mean ± S.D.				16.3 ± 1.5		1.834 ± 0.408	43.8 ± 7.6

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	158	B	-160
3	A	256	B	-258

Paraph	
Date	07-Mar-05

Filter	
0.1	1
0.3	15
0.6	50
0.8	90
1	145

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Sodium hydroxide 1% [1310-73-2]
Batch No.	014K0006
Concentration	1%
Code	D3 (12)
Sequence	2005/Intern4 kalverogen

Treatment time: 10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.045		0.7
2	NaCl 0.9%	0	0	0		0.022		0.3
3	100%	0	0	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.026 ± 0.017		0.4 ± 0.3
16	Test article	0	139	139	139.0	4.540	4.514	206.7
17		0	145	145	145.0	2.600	2.574	183.6
18	100%	0	123	123	123.0	4.164	4.138	185.1
	Mean ± S.D.				135.7 ± 11.4		3.742 ± 1.029	191.8 ± 12.9

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	75	-75
	2	156	-158
	3	263	-258

Paraph	
Date	14-Mar-05

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Triton X-100 (5%) [9002-93-1]		
Batch No.	A019437801		
Concentration	5%	Treatment time	10 min
Code	C4 (13)		
Sequence	2005/Interim4 kalverogen		**OP-KIT**

No. Cornea	Treatment	Opacity at t0	t120	t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.045		0.7
2	NaCl 0.9%	0	0	0		0.022		0.3
3	100%	0	0	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.026 ± 0.017		0.4 ± 0.3
10	Test article	0	5	5	5.0	4.300	4.274	69.1
11		0	5	5	5.0	3.004	2.978	49.7
12	100%	0	4	4	4.0	3.860	3.834	61.5
	Mean ± S.D.				4.7 ± 0.6		3.695 ± 0.659	60.1 ± 9.8

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	156	B	-158
	3	A	263	B	-258

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141

Paraph

Date 14-Mar-05

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	1-octanol [111-87-5]
Batch No.	S02961-454
Concentration	100%
Code	B4 (14)
Sequence	2005/ interm2 kalveren

Treatment time	10 min
OP-KIT	

No. Comea	Treatment	Opacity at t0	t120	t120 - t0		Permeability		In vitro score	
1	NC	0	0	0		0.084		1.3	
2	NaCl 0.9%	0	2	2		0.085		3.3	
3	100%	1	1	0		0.036		0.5	
	Mean ± S.D.			0.7	± 1.2	0.068	± 0.028	1.7	± 1.4
					Corrected value		Corrected value		
13	Test article	1	7	6	5.3	1.263	1.195	23.2	
14		0	18	18	17.3	1.904	1.836	44.8	
15	100%	0	9	9	8.3	1.637	1.569	31.8	
	Mean ± S.D.				10.3 ± 6.2		1.533 ± 0.322	33.3	± 10.9

NC: Negative Control

REMARKS	Filter	OPACITY			
	1	A	75	B	-75
	2	A	155	B	-161
	3	A	259	B	-261

Paraph	
Date	01-Mar-05

Filter	
0.1	1
0.3	16
0.6	51
0.8	91
1	143

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	2-ethyl-1-hexanol [107-76-7]
Batch No.	S01263-011
Concentration	100%
Code	A4 (15)
Sequence	2005/ intern 1 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.023		0.3
2	NaCl 0.9%	0	0	0		0.069		1.0
3	100%	0	0	0		0.044		0.7
	Mean ± S.D.			0.0 ± 0.0		0.045 ± 0.023		0.7 ± 0.4
13	Test article	0	4	4	4.0	0.523	0.478	11.2
14		0	0	0	0.0	0.401	0.356	5.3
15	100%	0	3	3	3.0	1.803	1.758	29.4
	Mean ± S.D.				2.3 ± 2.1		0.864 ± 0.777	15.3 ± 12.6

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	157	B	-161
3	A	260	B	-259

Paraph	
Date	28-Feb-05

Filter	
0.1	1
0.3	16
0.6	50
0.8	88
1	140

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	n-Hexanol [111-27-3]
Batch No.	A020123401
Concentration	98%
Code	D2 (16)
Sequence	2005/ Intern3 kalveren

Treatment time	10 min

OP-KIT

No. Comea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.008		0.1
2	NaCl 0.9%	0	0	0		0.026		0.4
3	100%	0	0	0		0.006		0.1
	Mean ± S.D.			0.0 ± 0.0		0.013 ± 0.011		0.2 ± 0.2
10	Test article	0	16	16	16.0	3.624	3.611	70.2
11		0	13	13	13.0	3.232	3.219	61.3
12	100%	0	13	13	13.0	4.028	4.015	73.2
	Mean ± S.D.				14.0 ± 1.7		3.615 ± 0.398	68.2 ± 6.2

NC: Negative Control

REMARKS

Filter	OPACITY A	OPACITY	B	
1	A	75	B	-75
2	A	158	B	-160
3	A	256	B	-258

Paraph	
Date	07-Mar-05

Filter	
0.1	1
0.3	15
0.6	50
0.8	90
1	145

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Acetone [67-64-1]
Batch No.	442942/1
Concentration	100%
Code	A5 (17)
Sequence	2005/ intern 1 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.023		0.3
2		0	0	0		0.069		1.0
3	100%	0	0	0		0.044		0.7
	Mean ± S.D.			0.0 ± 0.0		0.045 ± 0.023		0.7 ± 0.4
16	Test article	0	101	101	101.0	2.824	2.779	142.7
17		0	92	92	92.0	2.452	2.407	128.1
18	100%	0	81	81	81.0	3.428	3.383	131.7
	Mean ± S.D.				91.3 ± 10.0		2.856 ± 0.493	134.2 ± 7.6

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	157	B	-161
3	A	260	B	-259

Paraph	
Date	28-Feb-05

Filter	
0.1	1
0.3	16
0.6	50
0.8	88
1	140

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	cyclohexanol
Batch No.	S05238-044
Concentration	100%
Code	B5 (18)
Sequence	2005/ interm2 kalveren

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	t120	t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.084		1.3
2	NaCl 0.9%	0	2	2		0.085		3.3
3	100%	1	1	0		0.036		0.5
	Mean ± S.D.			0.7 ± 1.2		0.068 ± 0.028		1.7 ± 1.4
16	Test article	1	13	12	11.3	1.768	1.700	36.8
17		0	12	12	11.3	1.892	1.824	38.7
18	100%	0	13	13	12.3	2.940	2.872	55.4
	Mean ± S.D.				11.6 ± 0.6		2.132 ± 0.644	43.6 ± 10.2

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	155	B	-161
3	A	259	B	-261

Paraph	
Date	01-Mar-05

Filter	
0.1	1
0.3	16
0.6	51
0.8	91
1	143

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Cetylpyridinium bromide (6%) [140-72-7]
Batch No.	038H2509
Concentration	6%
Code	C5 (19)
Sequence	2005/Intern4 kalverogen

Treatment time: 10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.045		0.7
2	NaCl 0.9%	0	0	0		0.022		0.3
3	100%	0	0	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.026 ± 0.017		0.4 ± 0.3
13	Test article	0	20	20	20.0	2.252	2.226	53.4
14		0	13	13	13.0	1.879	1.853	40.8
15	100%	0	12	12	12.0	0.919	0.893	25.4
	Mean ± S.D.				15.0 ± 4.4		1.657 ± 0.688	39.9 ± 14.0

NC: Negative Control

REMARKS	Filter	OPACITY A	OPACITY B
	1	75	-75
	2	156	-158
	3	263	-258

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141

Paraph

Date 14-Mar-05

VALIDATION

Calculation of the in vitro eye irritation score for liquids

Test article	Benzalkoniumchloride [8001-54-5]
Batch No.	033K2544
Concentration	10g/g%
Code	C3 **20**
Sequence	2005/Intern4 kalverogen

Treatment time	10 min

OP-KIT

No. Cornea	Treatment	Opacity at t0	Opacity at t120	Opacity at t120 - t0	Corrected value	Permeability	Corrected value	In vitro score
1	NC	0	0	0		0.045		0.7
2	NaCl 0.9%	0	0	0		0.022		0.3
3	100%	0	0	0		0.012		0.2
	Mean ± S.D.			0.0 ± 0.0		0.026 ± 0.017		0.4 ± 0.3
7	Test article	0	115	115	115.0	4.016	3.990	174.9
8		0	95	95	95.0	3.856	3.830	152.5
9	100%	0	107	107	107.0	4.356	4.330	172.0
	Mean ± S.D.				105.7 ± 10.1		4.050 ± 0.255	166.5 ± 12.2

NC: Negative Control

REMARKS

Filter	OPACITY			
1	A	75	B	-75
2	A	156	B	-158
3	A	263	B	-258

Paraph	
Date	14-Mar-05

Filter	
0.1	0
0.3	15
0.6	50
0.8	89
1	141