Scientific Committee on Consumer Safety

SCCS

Addendum to the scientific Opinion on the safety of oxidative hair dye substances and hydrogen peroxide in products to colour eyelashes

The SCCS adopted this Opinion at its 9th plenary meeting

on 25 March 2015

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

Ulrike Bernauer, Qasim Chaudhry, Pieter Coenraads, Gisela Degen, Maria Dusinska, Werner Lilienblum, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Christophe Rousselle, Jan van Benthem

Contact
European Commission
Health and Food Safety
Directorate C: Public Health
Unit C2 – Health Information and Scientific Committees
Office: HTC 03/073
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

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SCCS Members

Prof. P.J. Coenraads
Dr. M. Dusinska
Dr. W. Lilienblum
Dr. E. Nielsen
Prof. T. Platzek (chairman)
Dr. S.C. Rastogi
Dr. C. Rousselle
Dr. J. van Benthem

External experts

Prof. A. Bernard
Prof. A.M. Giménez-Arnau
Prof. T. Vanhaecke (rapporteur)

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1. BACKGROUND

The definition of the term "hair product" was introduced in the preamble to Annexes II to VI of the EC Cosmetics Regulation n. 1223/2009[1]. A "hair product" is defined as a cosmetic product which is intended to be applied on the hair of head or face, except eyelashes.
The application of the newly defined term "hair product" in conjunction with provisions relating to specific substances in the new Annex III to the EU Cosmetics Regulation will restrict the use of relevant substances. Moreover, it is no longer legally possible to market oxidative colouring agents for application to eyelashes in the EU from July 2013.
The safety data on each hair dye substance submitted for risk assessment by the SCCS in the framework of the assessment strategy contain mandatory data on eye irritation. Therefore, the safety of these substances with regard to the application in the eye area was or will be assessed in the overall evaluation of each individual hair dye substance.
The Scientific Committee on Consumer Safety (SCCS) adopted the scientific Opinion by written procedure on 12 October 2012 (SCCS/1475/12) related to the use of oxidative hair dye substances and hydrogen peroxide for use in products to colour eyelashes with the following conclusion:

Information on eye irritation of oxidative dyes at or near anticipated use concentrations intended for eyelashes is available for only some of the dyes. For those tested at such concentrations there was either no or slight irritant potential and their use is considered safe in permanent eyelash dye formulations intended for the consumer. Dyes tested at higher concentration which was found to be not or slightly irritant can be considered safe. It is unknown what the irritant potential is of those dyes which have not been tested at use concentrations but which are irritant at the concentrations tested. For these substances, appropriate information is required before they can be assessed. For those hair dyes substances for which there is information on eye irritation at anticipated dilutions and which were found to have no or slight effects, there is no concern about eye irritation in the consumer.
For those hair dyes substances for which there is no information on eye irritant potential at anticipated dilutions, it is not possible to draw a conclusion without additional information on eye irritant potential at anticipated use concentrations. For those dyes which are skin sensitisers, there is a risk of allergic contact dermatitis developing in previously sensitised individuals. No detailed information is available on the ocular irritant properties of permanent eyelash dyes formulations intended for the consumer and these formulations need to be assessed on a case by case basis by the supplier.
In December 2013, the Commission received the attached submission by Cosmetics Europe[2]. According to the applicant the document contains the requested information for six out of the ten dyes and the selection reflects the commercial interest of companies who funded the studies.

2. TERMS OF REFERENCE

1. Does SCCS consider the submitted safety data, in particular the data provided on eye irritation, sufficient to conclude that oxidative hair dyes which were found safe for use in hair dye products can be safely used in products to colour eyelashes?

2. And/or does the SCCS have any further scientific concerns with regard to the use of the oxidative hair dyes (the ones related to the submitted data) intended to colour eyelashes (e.g. max conc. in the finish cosmetic product, warning)?

[1] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

[2] Cosmetics Europe - European Cosmetics Toiletry and Perfumery Association

3. OPINION

In its Opinion on the safety of oxidative hair dye substances and hydrogen peroxide in products to colour eyelashes (SCCS/1475/12), revised form of 11 December 2012, the SCCS concluded that "*For those hair dyes substances for which there is no information on eye irritant potential at anticipated dilutions, it is not possible to draw a conclusion without additional information on eye irritant potential at anticipated use concentrations."*
In response to these concerns, additional data at concentrations used in dyes for eyelashes were received for the Bovine Corneal Opacity and Permeability (BCOP) test and the EpiOcular TM assay for a selection of 6 out of 10 hair dye substances, namely for 2,5-Diamino Toluene Sulfate, p-Aminophenol, 2-Methylresorcinol, Tetraaminopyrimidinsulfate, Hydroxyethyl-p-phenylenediaminsulfate and 2-Amino-3-Hydroxypyridin. In addition to the selected 6 hair dyes for which data gaps had been identified, one hair dye, already accepted for use on eyelashes (Resorcinol), was tested using both *in vitro* methods. This reference substance was tested on top of the positive and negative reference chemicals that were included in all studies as an integral part of the study design.

3.1 Toxicological Evaluation

3.1.1 Irritation and corrosivity

3.1.1.1 Eye irritation

Bovine Corneal Opacity and Permeability (BCOP) test method

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**2,5 Diamino Toluene Sulfate (A005)**
Test batch:	3153-M63-0000
Purity:	99.8%
Test item:	2% (v/v) free base in saline
Test volume:	750µl
Treatment period:	10 minutes
Post-treatment incubation time:	120 minutes
Positive control:	2-Ethoxyethanol
Negative control:	Saline
GLP:	In compliance
Study period:	June 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneae were rinsed with saline, incubated for a further 2 hours at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean *In vitro* Irritancy Score (IVIS)=1.65). The positive control showed opacity and a distinctive permeability of the corneas (mean IVIS=66.06) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause an increase of the corneal opacity or permeability (mean IVIS=1.37).

Conclusion

Under the experimental conditions of this study, the 2% (v/v) dilution of the free base of 2,5 Diamino Toluene Sulfate in saline is not corrosive/not a severe irritant to the eye.

Ref: 1

SCCS comment

On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 2% free base of 2,5 Diamino Toluene Sulfate does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of 2,5 Diamino Toluene Sulfate at 2% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**p-Aminophenol (A016)**
Test batch:	3002-M51
Purity:	99.9%
Test item:	0.9% (w/v) suspension in saline
Test volume:	750µl
Treatment period:	240 minutes
Post-treatment incubation time:	90 minutes
Positive control:	10% (w/v) Benzalkonium chloride in saline
Negative control:	Saline
GLP:	In compliance
Study period:	May 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 90 minutes at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=3.0). The positive control caused opacity of the corneas (mean IVIS=216.1), corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause any increase of the corneal opacity or permeability (mean IVIS=16.4).

Conclusion

Under the experimental conditions of this study, the 0.9% (w/v) suspension of p-Aminophenol in saline is not corrosive/not a severe irritant to the eye.

Ref: 2

SCCS comment

No rationale is given in the study report for the used (post-)treatment periods.
On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 0.9% of p-Aminophenol does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of p-Aminophenol at 0.9% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**2-Methylresorcinol (A044)**
Test batch:	0210123453/201107058
Purity:	99.5%
Test item:	1.25% (w/v) in saline
Test volume:	750µl
Treatment period:	240 minutes
Post-treatment incubation time:	90 minutes
Positive control:	10% (w/v) Benzalkonium chloride in saline
Negative control:	Saline
GLP:	In compliance
Study period:	May 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 90 minutes at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=3.0). The positive control caused opacity of the corneas (mean IVIS=216.1) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause any increase of the corneal opacity or permeability (mean IVIS=1.4).

Conclusion

Under the experimental conditions of this study, the 1.25% (w/v) 2-Methylresorcinol in saline is not corrosive/not a severe irritant to the eye.

Ref: 3

SCCS comment

No rationale is given in the study report for the used (post-)treatment periods.
On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 1.25% 2-Methylresorcinol does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of 2-Methylresorcinol at 1.25% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**Tetraaminopyrimidinesulfate (A053)**
Test batch:	3911-M02
Purity:	98.3%
Test item:	2% (v/v) free base in saline
Test volume:	750µl
Treatment period:	10 minutes
Post-treatment incubation time:	120 minutes
Positive control:	2-Ethoxyethanol
Negative control:	Saline
GLP:	In compliance
Study period:	May 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 2 hours at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=1.01). The positive control caused opacity and distinctive permeability of the corneas (mean IVIS=69.01) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause an increase of the corneal opacity or permeability (mean IVIS=1.42).

Conclusion

Under the experimental conditions of this study, the 2% (v/v) dilution of the free base of 2,5 Tetraaminopyrimidinsulfate in saline is not corrosive/not a severe irritant to the eye.

Ref: 4

SCCS comment

On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 2% 2,5 Tetraaminopyrimidinsulfate does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of 2,5 Tetraaminopyrimidinsulfate at 2% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**Hydroxyethyl-p-phenylenediaminesulfate (A080)**
Test batch:	3355-M04
Purity:	98.50%
Test item:	1.75% (v/v) free base in saline
Test volume:	750µl
Treatment period:	10 minutes
Post-treatment incubation time:	120 minutes
Positive control:	2-Ethoxyethanol
Negative control:	Saline
GLP:	In compliance
Study period:	May 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 2 hours at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=1.01). The positive control caused opacity and distinctive

permeability of the corneas (mean IVIS=69.01) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause an increase of the corneal opacity or permeability (mean IVIS=0.05).

Conclusion

Under the experimental conditions of this study, the 1.75% (v/v) dilution of the free base of Hydroxyethyl-p-phenylenediaminesulfate in saline is not corrosive/not a severe irritant to the eye.

Ref: 5

SCCS comment

On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 1.75% free base of Hydroxyethyl-p-phenylenediaminesulfate does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of Hydroxyethyl-p-phenylenediaminesulfate at 1.75% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**2-Amino-3-Hydroxypyridin (A132)**
Test batch:	3006-M24
Purity:	99.96%
Test item:	0.5% (w/v) in saline
Test volume:	750µl
Treatment period:	240 minutes
Post-treatment incubation time:	90 minutes
Positive control:	10% (w/v) Benzalkonium chloride in saline
Negative control:	Saline
GLP:	In compliance
Study period:	May 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 90 minutes at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=3.0). The positive control caused opacity of the corneas (mean

IVIS=216.1) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause any increase of the corneal opacity or permeability (mean IVIS=1.8).

Conclusion

Under the experimental conditions of this study, the 0.5% (w/v) 2-Amino-3-Hydroxypyridin in saline is not corrosive/not a severe irritant to the eye.

Ref: 6

SCCS comment

No rationale is given in the study report for the used (post-)treatment periods.
On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 0.5% 2-Amino-3-Hydroxypyridin does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of 2-Amino-3-Hydroxypyridin at 0.5% cannot be excluded.

Guideline:	OECD 437 (September 2009)
Test system:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	**Resorcinol (A011)**
Test batch:	0209123173 (81/11-12)
Purity:	99.95%
Test item:	1.25% (w/v) in saline
Test volume:	750µl
Treatment period:	240 minutes
Post-treatment incubation time:	90 minutes
Positive control:	10% (w/v) Benzalkonium chloride in saline
Negative control:	Saline
GLP:	In compliance
Study period:	July 2013

Bovine eyes (from cattle aged at least 9 months) were collected from the slaughterhouse. On the same day, the corneas were isolated and used in the BCOP test. Corneas showing defects such as vascularisation, pigmentation, scratches and an opacity value > 7 were discarded. After a first basal opacity measurement of the fresh corneas, 750µl of the test item, and of the positive and the negative control, respectively, were applied to the corneas (triplicates) and incubated for 10 minutes at 32±1 °C. At the end of the contact period, the corneas were rinsed with saline, incubated for a further 90 minutes at 32±1 °C and measured again for their opacity. Following the opacity readings, the permeability endpoint was measured as an indication of the integrity of the epithelial cell layers. Hereto, the corneas were brought into contact via the anterior chamber of the corneal holder with 1ml of a 0.5% (w/v) sodium fluorescein solution in HBSS and incubated for 90 minutes at 32±1 °C. Complete medium from the posterior compartment of the corneal holder was removed, well mixed and the optical density was determined at 490nm.

Results

With the negative control, neither an increase of opacity nor permeability of the corneas could be observed (mean IVIS=0.98). The positive control caused opacity of the corneas (mean IVIS=66.06) corresponding to a classification as corrosive/severe irritant to the eye. Relative to the negative control, the test item did not cause any increase of the corneal opacity or permeability (mean IVIS=0.00).

Conclusion

Under the experimental conditions of this study, the 1.25% (w/v) Resorcinol in saline is not corrosive/not a severe irritant to the eye.

Ref: 7

SCCS comment

No rationale is given in the study report for the used (post-)treatment periods.
On the basis of the results obtained in the BCOP study, it can be considered that a maximum use concentration of 1.25% Resorcinol does not cause severe occular irritation. This does, however, not exclude a mild eye irritancy potential. Under the conditions of this study, an eye irritation potential of Resorcinol at 1.25% cannot be excluded.

Summary of BCOP results for the hair dyes substances of Submission 2:

Substance name	Colipa No.	Conc.	Mean IVIS	Appli-cants con-clusion	IVIS Negative Control (saline)	IVIS Positive Control	Ref.
Toluene-2.5-diamine sulfate (free base)	A005	2.00 %	1.37	non corrosive/ non severe irritant	1.65	66.06 (2-ethoxyethanol)	1
p-Aminophenol	A016	0.90 %	16.40	non corrosive/ non severe irritant	3.00	216.1 (10 % benzalkonium chloride in saline)	2
2-Methylresorcinol	A044	1.25 %	1.40	non corrosive/ non severe irritant	3.00	216.1 (10 % benzalkonium chloride in saline)	3
Tetraamino-pyrimidine sulfate (free base)	A053	2.00 %	1.42	non corrosive/ non severe irritant	1.01	69.01 (2-ethoxyethanol)	4
Hydroxethyl-p-phenylene-diaminesulfate (free base)	A080	1.75 %	0.05	non corrosive/ non severe irritant	1.01	69.01 (2-ethoxyethanol)	5
2-Amino-3-hydroxypyridine	A132	0.50 %	1.80	non corrosive/ non severe irritant	3.00	216.1 (10 % benzalkonium chloride in saline)	6
Resorcinol	A011	1.25 %	0.00	non corrosive/ non severe irritant	0.98	155.83 (10 % benzalkonium chloride in saline)	7

EpiOcular™ Eye Irritation Test (EIT)

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**2,5 Diamino Toluene Sulfate (A005)**
Test batch:	3153-M63-0000
Purity:	99.8%
Test item:	2% (v/v) free base in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	June 2013

2,5 Diamino Toluene Sulfate (A005) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3-hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 20 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60 minutes treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 48,2% (after 15 minutes treatment) or 21,4% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The ET_{50}-value was < 15 minutes.
The relative absorbance values of the free base dilution, corresponding to the cell viability, did not decrease or were reduced irrelevantly compared with the result of the negative control. They spaced within a range of 89,1% to 106,8%, consequently the free base dilution was classified as non-irritant.

Conclusion

Under the experimental conditions of this study, the 2% (v/v) dilution of the free base of 2,5 Diamino Toluene Sulfate in deionised water does not possess any eye-irritating potential.

Ref: 8

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of 2% (v/v) dilution of the free base of 2,5 Diamino Toluene Sulfate in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**p-Aminophenol (A016)**
Test batch:	3002-M51
Purity:	99.9%
Test item:	0.9% (w/v) suspension in saline
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	May 2013

p-Aminophenol (A016) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3-hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 2-3 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60 minutes treatment interval thus showing the quality of the tissues.

Treatment with the positive control induced a sufficient decrease in the relative absorbance compared with the negative control to 57,2% (after 15 minutes treatment) or 13,3% (after 45 minutes treatment), thus ensuring the validity of the test system. The calculated ET_{50}-value was 19,9 minutes.
The relative absorbance values of the test item suspension, corresponding to the cell viability, did not decrease or were reduced irrelevantly compared with the result of the negative control. They spaced within a range of 89,3% to 101,4%, consequently the test item suspension was classified as non-irritant.

Conclusion

Under the experimental conditions of this study, the 0,9% (w/v) suspension of p-Aminophenol in deionised water does not possess any eye-irritating potential.

Ref: 9

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of a 0,9% (w/v) suspension of p-Aminophenol in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**2-Methylresorcinol (A044)**
Test batch:	0210123453/201107058
Purity:	99.5%
Test item:	1.25% (w/v) in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	June 2013

2-Methylresorcinol (A044) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3 hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for

about 20 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60 minutes treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 48,2% (after 15 minutes treatment) or 21,4% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The calculated ET_{50}-value was <15 minutes.
The relative absorbance values of the test item solution, corresponding to the cell viability, did not decrease compared with the result of the negative control. They spaced within a range of 103,8% to 111,7%, consequently the test item solution was classified as non irritant.

Conclusion

Under the experimental conditions of this study, the 1,25% (w/v) solution of 2-Methylresorcinol in deionised water does not possess any eye-irritating potential.

Ref: 10

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of a 1,25% (w/v) solution of 2-Methylresorcinol in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**Tetraaminopyrimidinesulfate (A053)**
Test batch:	3911-M02
Purity:	98.3%
Test item:	2% (v/v) free base in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	May 2013

Tetraaminopyrimidinesulfate (A053) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from

the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3-hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 2-3 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60-minute treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 57,2% (after 15 minutes treatment) or 13,3% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The calculated ET_{50}-value was 19,9 minutes.
The relative absorbance values of the free base dilution, corresponding to the cell viability, were reduced irrelevantly compared with the result of the negative control. They spaced within a range of 83,3% to 101,3%, consequently the free base dilution was classified as non-irritant.

Conclusion

Under the experimental conditions of this study, the 2,0% (v/v) dilution of the free base of Tetraaminopyrimidinesulfate in deionised water does not possess any eye-irritating potential.

Ref: 11

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of a 2,0% (v/v) dilution of the free base of Tetraaminopyrimidinesulfate in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**Hydroxyethyl-p-phenylenediaminesulfate (A080)**
Test batch:	3355-M04
Purity:	98.50%
Test item:	1.75% (v/v) free base in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	May 2013

Hydroxyethyl-p-phenylenediaminesulfate (A080) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3-hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 2-3 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60 minutes treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 57,2% (after 15 minutes treatment) or 13,3% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The calculated ET_{50}-value was 19,9 minutes.
The relative absorbance values of the free base dilution, corresponding to the cell viability, were reduced irrelevantly compared with the result of the negative control. They spaced within a range of 85,1% to 94,1%, consequently the free base dilution was classified as non-irritant.

Conclusion

Under the experimental conditions of this study, the 1,75% (v/v) dilution of the free base of Hydroxyethyl-p-phenylenediaminesulfate in deionised water does not possess any eye-irritating potential.

Ref: 12

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of a 1,75% (v/v) dilution of the free base of Hydroxyethyl-p-phenylenediaminesulfate in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**2-Amino-3-Hydroxypyridin (A132)**
Test batch:	3006-M24

Purity:	99.96%
Test item:	0.5% (w/v) in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	May 2013

2-Amino-3-Hydroxypyridin (A132) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3 hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 2-3 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60-minute treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 57,2% (after 15 minutes treatment) or 13,3% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The calculated ET_{50}-value was 19,9 minutes.
The relative absorbance values of the test item solution, corresponding to the cell viability, were reduced irrelevantly compared with the result of the negative control. They spaced within a range of 89,3% to 99,6%, consequently the test item solution was classified as non irritant.

Conclusion

Under the experimental conditions of this study, the 0,5% (w/v) solution of 2-Amino-3-Hydroxypyridin in deionised water does not possess any eye-irritating potential.

Ref: 13

SCCS comment

Only 2 tissue replicates have been used per condition.

Under the conditions of this study, a mild eye irritation potential of a 0,5% (w/v) solution of 2-Amino-3-Hydroxypyridin in deionised water cannot be excluded.

Protocol:	Test kit manual
Test system:	Human EpiOcular™ tissue model
Replicates:	2 tissues per condition
Test substance:	**Resorcinol (A011)**
Test batch:	0209123173 (81/11-12)
Purity:	99.95%
Test item:	1.25% (w/v) in deionised water
Test volume:	100µl
Treatment periods:	3, 30 and 60 minutes
Positive control:	0.3% Triton X-100 in deionised water
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	No evidence present in the study report
GLP:	In compliance
Study period:	June 2013

Resorcinol (A011) was tested for its eye-irritating potential using the human EpiOcular™ tissue model according to the procedures described by MatTek Corporation (Ashland, MA 01721, USA). After pre-incubation of the EpiOcular™ tissues between 60-90 minutes at 37.0±1.5 °C and 5.0±0.5% CO_2 in pre-warmed assay medium, the medium was replaced by fresh assay medium. 100µl of the negative control, positive control and the test item were added in duplicate into the inserts onto the tissues. The cell plates were placed in the incubator at 37.0±1.5 °C and 5.0±0.5% CO_2. The negative control was tested for 60 minutes, the positive control was tested for 15 and 45 minutes and the test item was tested for 3, 30 and 60 minutes. At the end of the treatment intervals, the inserts were removed from the cell culture plates. The tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in cell culture plates containing adequate volumes of medium and remained there for at least 10 minutes but not longer than 20 minutes. Next, the cell culture inserts were transferred to new plates containing 300µl MTT assay medium. After a 3-hour incubation period at 37.0±1.5 °C and 5.0±0.5% CO_2, the MTT solution was aspirated from the wells and the tissues were rinsed three times with PBS. Inserts were transferred into new 24-well plates and 2ml isopropanol was added to each insert. The 24-well plate was sealed to inhibit isopropanol evaporation. The formazan was extracted in each test group for about 19 hours at room temperature. The optical density was determined at 570nm in a microplate reader and the time of the test item to reduce absorbance to 50% (ET_{50}) was calculated. Mean values were calculated from the 3 wells per tissue.

Results

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD≥0,8 for the 60 minutes treatment interval thus showing the quality of the tissues.
Treatment with the positive control induced a decrease in the relative absorbance compared with the negative control to 38,7% (after 15 minutes treatment) or 18,3% (after 45 minutes treatment), respectively, thus ensuring the validity of the test system. The calculated ET_{50}-value was <15 minutes.
The relative absorbance values of the test item solution, corresponding to the cell viability, did not decrease compared with the result of the negative control. They spaced within a range of 105,9% to 109,0%, consequently the test item solution was classified as non irritant.

Conclusion

Under the experimental conditions of this study, the 1,25% (w/v) solution of Resorcinol in deionised water does not possess any eye-irritating potential.

Ref: 14

SCCS comment

Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of a 1,25% (w/v) solution of Resorcinol in deionised water cannot be excluded.

Summary of EpiOcular™ results for the hair dye substances of Submission 2:

Substance name	Colipa No.	Conc.	Rel. Absorbance (% of Negative Control)	Applicants conclusion	Negative Control (deionised water)	Positive Control (0.3 % triton X-100 in deionised water)	Ref.
Toluene-2.5-diamine sulfate (free base)	A005	2.00 %	89.1 (3 min) 99.6 (30 min) 106.8 (60 min)	does not possess any eye-irritating potential	100 (60 min)	48.2 (15 min) 21.4 (45 min)	8
p-Aminophenol	A016	0.90 %	99.8 (3 min) 89.3 (30 min) 101.4 (60 min)	does not possess any eye-irritating potential	100 (60 min)	57.2 (15 min) 13.3 (45 min)	9
2-Methylresorcinol	A044	1.25 %	103.8 (3 min) 111.7 (30 min) 105.1 (60 min)	does not possess any eye-irritating potential	100 (60 min)	48.2 (15 min) 21.4 (45 min)	10
Tetraamino-pyrimidine sulfate (free base)	A053	2.00 %	101.3(3 min) 84.0 (30 min) 88.3 (60 min)	does not possess any eye-irritating potential	100 (60 min)	57.2 (15 min) 13.3 (45 min)	11
Hydroxethyl-p-phenylene-diaminesulfate (free base)	A080	1.75 %	93 (3 min) 94.1 (30 min) 85.1 (60 min)	does not possess any eye-irritating potential	100 (60 min)	57.2 (15 min) 13.3 (45 min)	12
2-Amino-3-hydroxypyridine	A132	0.50 %	99.6 (3 min) 89.3 (30 min) 99.3 (60 min)	does not possess any eye-irritating potential	100 (60 min)	57.2 (15 min) 13.3 (45 min)	13
Resorcinol	A011	1.25 %	106.1 (3 min) 109.0 (30 min) 105.9 (45 min)	does not possess any eye-irritating potential	100 (60 min)	38.7 (15 min) 18.3 (45 min)	14

Overall SCCS comment

The SCCS notes that different extraction periods were used for the different test items.
Only 2 tissue replicates have been used per condition.
Under the conditions of this study, a mild eye irritation potential of the different test items cannot be excluded.

Summary of the eye irritation and skin sensitisation results for the dyes related to the current submission.

Colipa N°	INCI name (CAS N°)	Eye irritation potential	Sensitising potency category, based on results from LLNA or guinea pig assays	LLNA EC3 value (%)	Guinea pig assay	References
A005	Toluene-2,5-diamine (95-70-5) Use concentration in hair dye formulation: 2.0% (free base), 3.6% (sulfate) Use concentration in eyelash dye formulation: 4.0% (free base)	Eye irritation studies have demonstrated that 50.6% toluene-2,5-diamine is irritant to the rabbit eye. Some irritant effects were also seen with 2.5% toluene-2,5-diamine. A 2% (v/v) dilution of the free base of 2,5 diamino toluene sulfate is not a strong eye irritant.	Extreme	0.31	GPMT 0.1/100 (modified method)	SCCS/1479/12 new *in vitro* data
A011	Resorcinol (108-46-3) Use concentration in hair dye formulation: 1.25%. Use concentration in eyelash dye formulation: 0.6%	A 2.5% concentration of resorcinol caused mild conjunctival irritation to the rabbit eye. 1.25% (w/v) resorcinol is not a strong eye irritant.	Strong	1.4	-	SCCS/1270/09 new *in vitro* data
A016	p-Aminophenol (123-30-8, free base) (51-78-5, HCl) Use concentration in hair dye formulation: 0.9%.	p-Aminophenol was irritant on mucous membranes at 2.5% aqueous solution and when applied neat to rabbit eye.	Strong	-	Non-guideline tests indicating strong sensitising potency	SCCS/1409/11

	Use concentration in eyelash dye formulation: 0.5%	A 0.9% (w/v) suspension of p-aminophenol is not a strong eye irritant.				new *in vitro* data
A044	Methylresorcinol (608-25-3) Use concentration in hair dye formulation: 1.8% Use concentration in eyelash dye formulation: 0.3%	Undiluted 2-methylresorcinol was severely irritant to the rabbit eye. 1.25% (w/v) 2-methylresorcinol is not a strong eye irritant.	Moderate	50	A 5% (w/v) aqueous solution did not produce dermal sensi-tisation on Guinea pigs.	SCCP/1206/08 new *in vitro* data
A053	Tetraaminopyrimidine (5392-28-9, sulfate) Use concentration in hair dye formulation: 2.0% (free base); 3.4% (sulfate) Use concentration in eyelash dye formulation: 2.0% (free base); 3.4% (sulfate)	Undiluted tetraaminopyrimidine was irritant to the eye. A 2% (v/v) dilution of free base of tetraaminopyrimidinesulfate is not a strong eye irritant.	Insufficient testing	No value. Should have been tested at higher conc. or in other vehicle.	A test formulation containing 4.2% A53 did not cause sensi-tisation.	SCCP/1118/07 new *in vitro* data
A080	Hydroxyethyl-p-phenylenediamine sulphate (93841-25-9) Use concentration in hair dye formulation: 2.0% Use concentration in eyelash dye formulation: 1.75%	Under the conditions of the test, undiluted hydroxyethyl-p-phenylenediamine sulfate was irritant to rabbit eyes. A 1.75% (v/v) dilution of the free base of hydroxyethyl-p-phenylenediamine sulfate is not a strong eye irritant.	Strong	0.57	-	SCCS/1310/10 new *in vitro* data

A132	2-Amino-3-hydroxypyridine (16867-03-1) Use concentration in hair dye formulation: 1.0% Use concentration in eyelash dye formulation: 0.5%	Undiluted 2-Amino-3-hydroxypyridine was irritating to the rabbit eye. 0.5% (w/v) 2-Amino-3-hydroxypyridine is not a strong eye irritant.	Not classifiable	No value.	-	SCCS/1126/07 new *in vitro* data

SCCS comment

Information on eye irritation at anticipated use concentrations in products intended for eyelashes is available for Tetraaminopyrimidine (A053), Hydroxyethyl-p-phenylenediamine sulphate (A080) and 2-Amino-3-hydroxypyridine (A132). Resorcinol (A011), p-Aminophenol (A016) and Methylresorcinol (A044) were tested at concentrations higher than the anticipated use concentrations in eyelash formulations. The eye irritant potential of Toluene-2,5-diamine (A005) has been tested at a lower concentration than the anticipated use concentration for eyelash colouring products.

3.1.2 Skin sensitisation

No new data with respect to skin sensitisation have been submitted.

3.1.3 Discussion

The critical toxicological endpoints considered of relevance to the use of oxidative dyes on eyelashes are skin and eye irritation and skin sensitisation, either by the dye substance or hydrogen peroxide used as oxidant.

In response to concerns stated in the Opinion on the safety of oxidative hair dye substances and hydrogen peroxide in products to colour eyelashes (SCCS/1475/12), with respect to the lack of information on eye irritation of some oxidative hair dyes at or near anticipated use concentrations intended for eyelashes, new *in vitro* eye irritation data for 2,5-Diamino Toluene Sulfate, p-Aminophenol, 2-Methylresorcinol, Tetraaminopyrimidinsulfate, Hydroxyethyl-p-phenylenediaminsulfate, 2-Amino-3-Hydroxypyridin have been submitted.

Resorcinol (A011) that is already accepted for use on eyelashes (SCCS/1475/12) at a maximum use concentration of 1.25%, was also included in the study design as reference substance. From the BCOP and EpiOcular tests, it appears that no severe eye irritant potential is to be expected at the intended use concentration. However, a mild eye irritation potential cannot be excluded.

For Tetraaminopyrimidine (A053), Hydroxyethyl-p-phenylenediamine sulphate (A080) and 2-Amino-3-hydroxypyridine (A132), the test concentrations were identical to the anticipated use concentrations of the dyes in products to colour eyelashes, namely 2.0% (free base), 1.75% (free base) and 0.5%, respectively.

Based upon the new results obtained in the BCOP and EpiOcular tests, no severe eye irritant potential is to be expected for these substances at the intended use/test concentrations. However, a mild eye irritation potential cannot be excluded.

For p-Aminophenol (A016) and Methylresorcinol (A044), the eye irritant potential has been tested at higher concentrations (0.9% and 1.25% respectively) than the anticipated use concentrations in eyelash formulations (0.5% and 0.3% respectively). Based on the submitted studies, no severe eye irritant potential is to be expected for these substances at the tested concentrations. However, a mild eye irritation potential cannot be excluded.

For Toluene-2,5-diamine (A005), the eye irritant potential has been tested at a lower concentration (2.0% (free base)) than the anticipated use concentration in eyelash formulations (4.0% (free base)). Based on the submitted studies, no severe eye irritant potential is to be expected at the 2.0% test concentration. However, a mild eye irritation potential cannot be excluded.

SCCS recommends testing different concentrations of the dyes in order to allow concentration-response and threshold setting.

No information is given on potential ocular irritant properties of mixtures of the dyes with hydrogen peroxide.

Based on a previous SCCP Opinion (SCCP/1129/07), it could be extrapolated that transient exposure to 2% hydrogen peroxide may be slightly irritant to the eye (SCCS/1475/12).

Accidental introduction of an oxidative dye or hydrogen peroxide or a mixture into the eye would cause rapid elimination by lachrymation. Only mild and transient irritation is expected from those substances for which information is available (SCCS/1475/12).

No new information is given on the skin sensitising potential of the 6 dyes. Therefore, the potential exists for an allergic contact reaction to occur in case the eyelid is exposed to dyes which are sensitisers or when the exposure to the eyelid skin is higher than under normal use conditions (accidental). Individuals with a history of an allergic reaction to hair dyes are at particular risk (SCCS/1475/12).

4. CONCLUSION

1. Does SCCS consider the submitted safety data, in particular the data provided on eye irritation, sufficient to conclude that oxidative hair dyes which were found safe for use in hair dye products can be safely used in products to colour eyelashes?

In the previous Opinion on Oxidative hair dye substances and hydrogen peroxide used in products to colour eyelashes, the SCCS was of the Opinion that the information submitted was inadequate to assess the safe use of those hair dyes for which there was no information on eye irritation potential at anticipated dilutions (SCCS/1475/12).

Based on the newly submitted *in vitro* studies, the SCCS considers that there is no concern for severe eye irritation in the consumer for the use of Toluene-2,5-diamine (A005), p-Aminophenol (A016), Methylresorcinol (A044), Tetraaminopyrimidine (A053), Hydroxyethyl-p-phenylenediamine sulphate (A080) and 2-Amino-3-hydroxypyridine (A132) in products to colour eyelashes at the tested maximum concentrations for eyelashes and their use is considered safe in eyelashes dye formulations.

No information is available on the ocular irritant properties of permanent eyelash dyes formulations intended for the consumer and these formulations need to be assessed on a case-by-case basis by the supplier.

The SCCS considers that the potential risk to the consumer from the use of these products is greater from non-professional use compared with professional use of the same products as there may be increased eyelid contamination.

2. And/or does the SCCS have any further scientific concerns with regard to the use of the oxidative hair dyes (the ones related to the submitted data) intended to colour eyelashes (e.g. max conc. in the finish cosmetic product, warning)?

As no new data has been submitted with respect to skin sensitisation, the risk of allergic contact dermatitis developing in previously sensitised individuals for those hair dyes which are skin sensitisers cannot be excluded (SCCS/1475/12).

Based on SCCP/1129/07 and other information, it could be extrapolated that transient exposure to 2% hydrogen peroxide may be slightly irritant to the eye. Therefore, up to 2% hydrogen peroxide could be considered safe for the consumer when applied on eyelashes and direct eye contact is avoided (SCCS/1475/12).

5. MINORITY OPINION

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6. REFERENCES

1. 2,5-Diamino Toluene Sulfate; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548201)
2. p-Aminophenol; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548202)
3. 2-Methylresorcinol; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548203)
4. Tetraaminopyrimidinsulfat; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548206)
5. Hydroxyethyl-p-phenylendiaminsulfat; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548204)
6. 2-Amino-3-hydroxypyridin; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548205)
7. Resorcinol; Bovine Corneal Opacity and Permeability Assay (BCOP); Harlan Report dated 18 September 2013 (Harlan Study Number 1548214)
8. 2,5-Diamino Toluene Sulfate; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548207)
9. p-Aminophenol; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548208)
10. 2-Methylresorcinol; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548209)
11. Tetraaminopyrimidinsulfat; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548212)
12. Hydroxyethyl-p-phenylendiaminsulfat; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548210)
13. 2-Amino-3-hydroxypyridin; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548211)
14. Resorcinol; *In Vitro* Eye Irritation Test: Human Cornea Model Test; Harlan Report dated 18 September 2013 (Harlan Study Number 1548216)