



Scientific Committee on Consumer Safety

SCCS

OPINION ON

**Hair dye Methylimidazoliumpropyl p-phenylenediamine
HCl (A166) (CAS 220158-86-1)
Submission I**



The SCCS adopted this Opinion by written procedure on 29 July 2019

1
2 **ACKNOWLEDGMENTS**
3
4 SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this
5 Opinion. The members of the Working Group are:
6

7 SCCS members:

8 Dr U. Bernauer
9 Dr L. Bodin
10 Prof. Q. Chaudhry (SCCS Chair)
11 Prof. P.J. Coenraads (SCCS Vice-Chair and Chairperson of the WG)
12 Prof. M. Dusinska
13 Dr J. Ezendam
14 Dr E. Gaffet (Rapporteur)
15 Prof. C. L. Galli
16 Dr B. Granum
17 Prof. E. Panteri
18 Prof. V. Rogiers (SCCS Vice-Chair)
19 Dr Ch. Rousselle
20 Dr M. Stepnik
21 Prof. T. Vanhaecke
22 Dr S. Wijnhoven

23
24
25 SCCS external experts:

26 Dr A. Simonnard
27 Dr A. Koutsodimou
28 Prof. W. Uter
29
30
31

32

33

34 All Declarations of Working Group members are available on the following webpage:
35 http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

36

37

38

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

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided, does the SCCS consider Methylimidazoliumpropyl p-phenylenediamine HCl (A166), safe when used in oxidative hair colouring products up to a maximum on-head concentration of 2 %?*

Although evaluation of the data provided for Methylimidazoliumpropyl p-phenylenediamine HCl (A166) has shown it to be safe, no safety data have been provided for the impurities and the residual solvents that are also present. Analysis of the potential risk carried out by the SCCS according to TTC principles has indicated that the levels of one of the impurities (3-aminopropyl imidazole) and a residual solvent (chloroethane) are above the TTC levels that can be considered safe. Therefore, the SCCS cannot conclude on the safety of Methylimidazoliumpropyl p-phenylenediamine HCl (A166) unless its purity is improved to bring the levels of the impurity and the residual solvent down to below the acceptable TTC levels.

2. *Does the SCCS have any further scientific concerns with regard to the use of Methylimidazoliumpropyl p-phenylenediamine HCl (A166) in cosmetic products?*

The SCCS has noted that Methylimidazoliumpropyl p-phenylenediamine HCl (A166) is a strong skin sensitiser.

Keywords: SCCS, scientific opinion, hair dye, Methylimidazoliumpropyl p-phenylenediamine HCl (A166), Regulation 1223/2009, CAS 220158-86-1

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166), SCCS/1609/19, preliminary version adopted on 29 July 2019

1

2 **About the Scientific Committees**

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

7 They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific
8 Committee on Health, Environmental and Emerging Risks (SCHEER) and are made up of
9 scientists appointed in their personal capacity.

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

13
14 **SCCS**

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

20
21 **Scientific Committee members**

22 Bernauer Ulrike, Bodin Laurent, Chaudhry Qasim, Coenraads Pieter-Jan, Dusinska Maria,
23 Ezendam Janine, Gaffet Eric, Galli Corrado Lodovico, Granum Berit, Panteri Eirini, Rogiers
24 Vera, Rousselle Christophe, Stępnik Maciej, Vanhaecke Tamara, Wijnhoven Susan

25
26 **Contact**

27 European Commission
28 Health and Food Safety
29 Directorate C: Public Health, Country Knowledge, Crisis Management
30 Unit C2 – Country Knowledge and Scientific Committees
31 L-2920 Luxembourg
32 SANTE-C2-SCCS@ec.europa.eu

33
34 © European Union, 2019

35
36 ISSN ISBN

37
38 Doi: ND-

39
40 The Opinions of the Scientific Committees present the views of the independent scientists
41 who are members of the committees. They do not necessarily reflect the views of the
42 European Commission. The Opinions are published by the European Commission in their
43 original language only.

44
45 http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

46

| | | |
|----|--|----|
| 1 | | |
| 2 | TABLE OF CONTENTS | |
| 3 | | |
| 4 | | |
| 5 | 1. ABSTRACT..... | 3 |
| 6 | 2. MANDATE FROM THE EUROPEAN COMMISSION..... | 6 |
| 7 | 3. OPINION | 7 |
| 8 | 3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS..... | 7 |
| 9 | 3.1.1 Chemical identity..... | 7 |
| 10 | 3.1.2 Physical form | 8 |
| 11 | 3.1.3 Molecular weight | 8 |
| 12 | 3.1.4 Purity, composition and substance codes..... | 8 |
| 13 | 3.1.5 Impurities / accompanying contaminants | 11 |
| 14 | 3.1.6 Solubility | 14 |
| 15 | 3.1.7 Partition coefficient (Log P _{ow}) | 14 |
| 16 | 3.1.8 Additional physical and chemical specifications..... | 15 |
| 17 | 3.1.9 Homogeneity and Stability..... | 15 |
| 18 | 3.2 FUNCTION AND USES | 16 |
| 19 | 3.3 TOXICOLOGICAL EVALUATION..... | 16 |
| 20 | 3.3.1 Acute Toxicity | 17 |
| 21 | 3.3.2 Irritation and corrosivity..... | 18 |
| 22 | 3.3.3 Skin sensitisation | 20 |
| 23 | 3.3.4 Toxicokinetics | 21 |
| 24 | 3.3.5 Repeated dose toxicity | 24 |
| 25 | 3.3.6 Reproductive toxicity | 27 |
| 26 | 3.3.7 Mutagenicity / genotoxicity..... | 29 |
| 27 | 3.3.8 Carcinogenicity | 34 |
| 28 | 3.3.9 Photo-induced toxicity..... | 34 |
| 29 | 3.3.10 Human data | 34 |
| 30 | 3.3.11 Special investigations..... | 34 |
| 31 | 3.3.12 Safety evaluation (including calculation of the MoS)..... | 35 |
| 32 | 3.3.13 Discussion | 35 |
| 33 | 4. CONCLUSION..... | 37 |
| 34 | 5. MINORITY OPINION | 37 |
| 35 | 6. REFERENCES | 38 |
| 36 | 7. GLOSSARY OF TERMS | 39 |
| 37 | 8. LIST OF ABBREVIATIONS | 39 |
| 38 | | |
| 39 | | |
| 40 | | |

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
45
46
47
48
49
50
51
52
53
54
55

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Submission I on the hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166) (CAS 220158-86-1) with the chemical name 1H-Imidazolium,3-[3-[(4-aminophenyl)amino]propyl]-1-methyl-,chloride, hydrochloride was transmitted by Cosmetics Europe in 2017.

The ingredient Methylimidazoliumpropyl p-phenylenediamine HCl (A166) is intended to be used in oxidative hair colouring products up to a maximum on-head concentration of 2 %.

Terms of reference

1. *In light of the data provided, does the SCCS consider Methylimidazoliumpropyl p-phenylenediamine HCl (A166), safe when used in oxidative hair colouring products up to a maximum on-head concentration of 2 %?*
2. *Does the SCCS have any further scientific concerns with regard to the use of Methylimidazoliumpropyl p-phenylenediamine HCl (A166) in cosmetic products?*

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
45
46
47
48
49
50

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Methylimidazoliumpropyl p-phenylenediamine HCl (INCI)

SCCS comment

The salt used is chloride, dihydrochloride therefore the following name will be used throughout the whole Opinion:

Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl

3.1.1.2 Chemical names

1H-Imidazolium, 3-[3-[(4-aminophenyl)amino]propyl]-1-methyl-,chloride, hydrochloride (1:1:2)

1H-Imidazolium, 1-[3-[(4-aminophenyl)amino]propyl]-3-methyl-, chloride, dihydrochloride

1-(3-((4-Aminophenyl)amino)propyl)-3-methyl-1H-imidazol-3-ium chloride hydrochloride

Ref: Wohr 2019

3.1.1.3 Trade names and abbreviations

IMEXINE® OBL

Cosmetics Europe code A166

Other codes: R0027494A

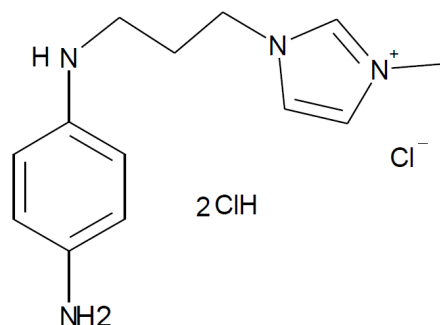
CI6778

3.1.1.4 CAS / EC number

CAS: 220158-86-1

EC: /

1
2 **3.1.1.5 Structural formula**



12 IR spectra, ^1H and ^{13}C NMR spectra of the studied batches were in accordance with the proposed structure.

13 Mass spectra of the studied batches were compatible with the proposed structure.

14 UV-Visible spectra of the studied batches were compatible with the proposed structure.

15
16
17
18 **3.1.1.6 Empirical formula**

19 $\text{C}_{13}\text{H}_{19}\text{N}_4, \text{Cl}, 2\text{HCl}$
20 or $\text{C}_{13}\text{H}_{21}\text{N}_4\text{Cl}_3$

21 Ref: Wöhr 2019

22
23 **3.1.2 Physical form**

24 Very light beige to beige powder.

25
26
27 **3.1.3 Molecular weight**

28 Molecular weight: 339.70 g/mol.

29 Exact mass: 338.09

30 Note:

31 Cl: Molecular weight: 35.453

32 Cl: Exact mass: 34.97

33
34 **3.1.4 Purity, composition and substance codes**

35 The HPLC titre was carried out on all batches against batch R0027494A 019 L 001,
36 reference standard considered as pure (99% w/w).

37 All batches have a titre $\geq 99\%$ w/w.

38 The separation was achieved by reversed phase LC and performed on a HPLC system
39 equipped with a photodiode array detector. Detection wavelength: $\lambda = 245\text{nm}$

40 All samples and eluents were filtered through a $0.2\mu\text{m}$ membrane filter (Pall Acrodisc GHP)
41 prior to use.

42 The dilution solvent is a mixture of water + 0.01% of dithionite of sodium/acetonitrile
43 95/5[v/v].
44

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) is the main component detected on the HPLC chromatograms: its HPLC purity is above 95% in all batches (Relative purity, UV- area %).

Some impurities are detected in the tested batches in particular in: Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - batch 002 L 001.

This batch was one of the first synthesized batches.

According to the Applicant 'The impurities content in the other tested batches is very weak. The impurity content the future productions is now under control'.

One impurity was checked in all batches and detected:

(R0011102A: 4-Aminophenylamine dihydrochloride).

Its content was determined against reference standard R00011102A L 139, considered as pure (99.8% w/w):

R001102A content: < 0.2% w/w in all batches.

Table 1: Comparative table of the main results (Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - batches: 009 D 003, 008 L 002, 002 L 001, 000 L 006)

| Analytical test | 009 D 003 | 008 L 002 | 002 L 001 | 000 L 006 |
|--|--|-------------------------|---|--------------|
| Identification | | | | |
| Appearance | Light beige powder | Very light beige powder | Light beige powder | Beige powder |
| Infrared spectrometry | In accordance with the proposed structure | | | |
| UV spectrometry | Compatible with the proposed structure | | | |
| NMR spectrometry | In accordance with the proposed structure | | | |
| Mass spectrometry | Compatible with the proposed structure | | Compatible with the proposed structure | |
| Assays | | | | |
| HPLC Titre ⁽¹⁾ (% w/w) | 99.3 ± 0.3 | 99.0 ± 0.35 | 99.3 ± 0.6 | 99.5 ± 0.1 |
| HPLC Profile ⁽²⁾ (UV purity- Area %) | Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) > 95 % Some impurities are detected | | | |
| R0011102A ⁽³⁾ (impurity content by HPLC): (µg/g) | 328 | 633 | 638 | 1595 |
| Water content (for information) | 1.1% w/w (KF method) | | 0.8% w/w (calculated from elemental analysis) | |
| Chloride content Elemental analysis (Theoretical value: 31.3% w/w) | 31.0 | 31.5 | 30.6 | 31.1 |

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1
2 *Note: in Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) batch 002 L 001, 4*
3 *% of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) is under sulphate form*
4 *(calculated from corresponding elemental analysis).*

5
6 *(1) Titre against batch R0027494A 019 L 001 reference standard considered as pure (99.0%*
7 *w/w)*

8 *(2) UV detection: UV purity - Area %, without response factor.*

9 *Irrespective of residual solvents, salts and other non-detectable products*

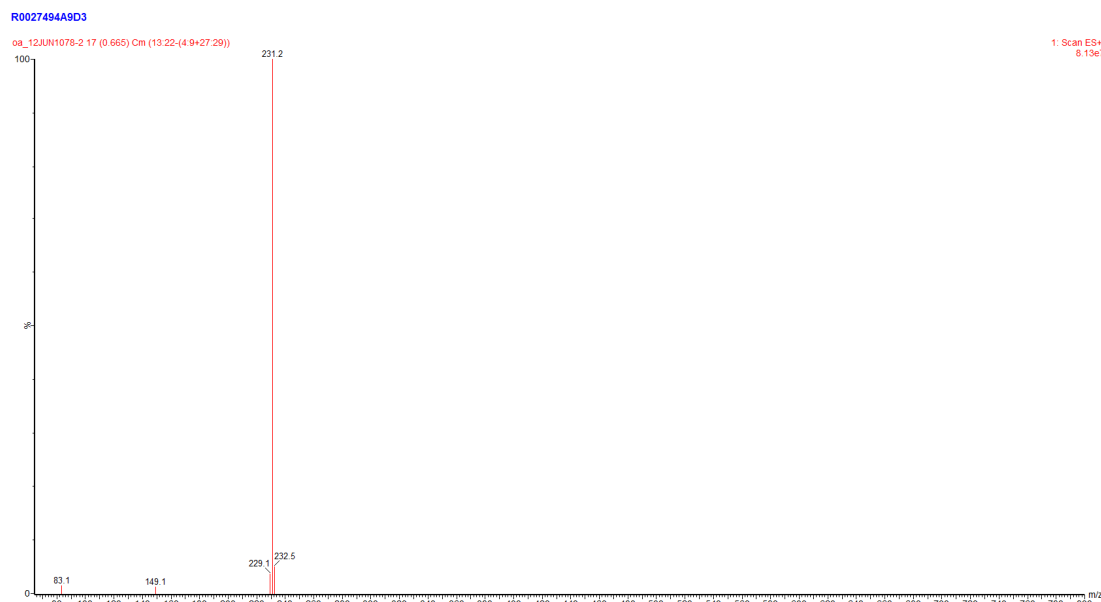
10 *(3) Determination of 4-aminophenylamine dihydrochloride against batch R0011102A 000 L*
11 *139 reference standard considered as pure (99.8% w/w)*

12
13 The main results of the four batches are comparable.

14
15 **Mass spectra**

16 According to the Applicant, an Analysis FIA / MS on Methylimidazoliumpropyl p-

17 phenylenediamine, Cl, 2HCl (A166) batch 009 D 003 was realized via OpenLynx.
18 The expected cation was mainly detected. Presence of secondary ions not characterised by
19 low intensity.



21
22
23 Ref: Analytical file – Imexine OBL – June 2017

24
25
26 **SSCS comment**

27 Filtration of the analysed samples through a 0.2µm filter prior to the analysis has to be
28 clearly justified. All samples should be fully dissolved in the dilution solvent prior to the
29 HPLC-PDA analysis.

30
31 According to the Applicant, peak purity of the test substance based on the use of a
32 reference standard is greater than 99% w/w, while peak purity based on % area calculation
33 is > 95%. Data regarding peak purity of the test substance based on the use of a reference
34 standard method are different from peak purity data presented in the Certificate of Analysis
35 for the batch 009 D003.

36
37 The purity of the test substance has been quantified by HPLC against batch R0027494A 019
38 L 001, without the use of a proper reference standard of the compound. A proper
39 quantification of the test substance and impurities is therefore needed.

1
2 **3.1.5 Impurities / accompanying contaminants**
3

4 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) is the main component
5 detected on the HPLC chromatograms: its HPLC purity is above 95% in all batches (Relative
6 purity, UV- area %).
7

8 Some impurities are detected in the tested batches in particular in:
9 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - batch 002 L 001. This
10 batch was one of the first synthesized batches. According to the Applicant, the impurity
11 content in future productions is now under control.
12

13 The determination of R0011102A was carried out by HPLC (external calibration) in the four
14 batches against a primary reference standard:
15

16 R0011102A batch 000 L 139 (purity considered at 99.8%).
17 R0011102A 000 L 139 is a dichlorhydrated form.
18

19 R0011102A content in Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166):
20 - Batch 009 D 003: 328 µg/g
21 - Batch 008 L 002: 633 µg/g
22 - Batch 002 L 001: 638 µg/g
23 - Batch 000 L 006: 1595 µg/g
24

25 R001102A content: < 0.2% w/w in all batches.
26

27 For Batches 009 D 003 and 008 L 002, no impurity has been detected.

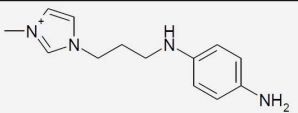
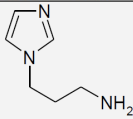
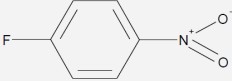
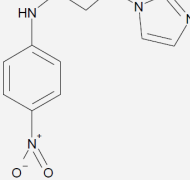
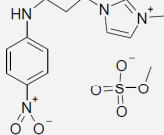
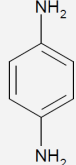
28 For Batch 002 L001, impurities eluting at 2.4, 7.8, and 8.8 min. have been detected,
29 identified and quantified (area %) as 3-aminopropyl imidazole (0.1), R0027060A (0.2) and
30 R0026760A (0.3) respectively. Impurities eluting at 6.4 min (< 0.1) and traces at 3.7min
31 and 5.3 min (without identification) are detected.

32 For batch 000 L 006, only impurities eluting at 2.4 and 3.7 min have have been detected,
33 identified and quantified (area %) as 3-aminopropyl imidazole (0.1) and R0011102A (0.2)
34 respectively.
35

36 Impurities, which have a content $\geq 0.1\%$ (area% without response factor – UV detection),
37 were identified using a standard mixture.
38

39 Potential impurities in Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) can
40 originate from starting material, synthesis intermediates, or by-products.

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

| | |
|--|---|
| <p>Imexine OBL (Main product)</p> |  <p>Molecular weight= 339.696</p> |
| <p>3-aminopropyl imidazole (Synthesis reagent)</p> |  <p>Molecular Weight =125.17</p> |
| <p>R001872A (Starting material)</p> |  <p>Molecular Weight =141.10</p> |
| <p>R0026760A (N-2)</p> |  <p>Molecular Weight =246.27</p> |
| <p>R0027060A (N-1)</p> |  <p>Molecular weight = 372.41</p> |
| <p>R0011102A 4-aminophenylamine dihydrochloride (Potential impurity)</p> |  <p>Molecular weight :181.065</p> |

1
2
3
4
5
6

Residual solvents

Determination of residual solvents in Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) by Gas Chromatography (GC/HS - Standard addition method) (Results in µg/g).

| Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) | batch 009 D 003 | batch 008 L 002 | batch 002 L 001 | batch 000 L 006 |
|---|-----------------|--------------------|-----------------|-----------------|
| Chloroethane | < 500 | < 25 | < 500 | # 560 |
| Diethylether | < 100 | < 100 | < 100 | < 200 |
| Methylethylether | < 100 | Not detected < 100 | < 100 | < 200 |
| Ethanol | < 1000 | < 500 | < 1500 | < 500 |
| Chloropropane | | | | < 500 |

7
8

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 *Heavy metals content*

2 Determination of metal content ($\mu\text{g/g}$ unless otherwise specified: % w/w):

3 Dosage of 24 elements using ICP/AES, ICP-MS or atomic fluorescence (for Hg) after
4 mineralization in microwave bomb according to method: CONS 285 LA2012

5 - Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - Batch 009 D003 ($\mu\text{g/g}$)
6 except Na: % w/w):

7 As, Cd, Hg, Pb, Pd, Sb: Each < 1

8 Al, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: Each <5

9 Ca, K, P: <50

10 Na: 0.66 % (w/w)

11 - Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - Batch 008 L 002 ($\mu\text{g/g}$):

12 As, Cd, Hg, Pb, Pd, Sb: Each < 1

13 Al, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: Each <5

14 Ca, K, P, Na: <50

15 - Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - Batch 002 L 001 ($\mu\text{g/g}$)
16 except Na: % w/w):

17 As, Cd, Hg, Sb: Each < 1

18 Ba, Co, Cr, Cu, Mn, Mo, Ni, Se, Sn, Ti, V: Each <5

19 Ca, K, P: <50

20 Al, Pb: 17

21 Fe: 11

22 Pd: 8

23 Zn: 5

24 Na: 1.03 % (w/w)

25 - Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) - Batch 000 L 006 ($\mu\text{g/g}$):

26 As, Cd, Hg, Pb, Pd, Sb: Each < 1

27 Al, Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: Each <5

28 Ca, K, P: <50

29 Na: 501

30

31 *Elemental analysis*

32 Determination of carbon, hydrogen and nitrogen contents were carried out by measure of
33 thermic conductivity (method MO 240 LA 2008).

34 Determination of oxygen content was carried out by infrared (method MO 238 LA 2008).

35 Determination of Chloride content was carried out by potentiometry (method MO108 LA
36 2005)

37

38 For Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) batches 008 L 002 and
39 000 L 006, the experimental elemental analysis values were in accordance with theoretical
40 values.

41 For batches 002 L 001 and 009 D 003, the experimental elemental analysis values were in
42 accordance with theoretical values, after recalculation.

43

44 Ref: Analytical file – Imexine OBL – June 2017

45

46 **SCCS comments**

47 Impurities eluting at 5.3 and 6.4 min have not been identified. Chemical characterisation of
48 all impurities should be provided.

49 Percentage content of all of the impurities should be calculated at $\lambda_{\text{max}} = 246 \text{ nm}$ of the
50 test substance. Quantitative determination of R0011102A (p-phenylenediamine) content by
51 HPLC was carried out at 240 nm.

52

53 Filtration of the analysed samples through a $0.2\mu\text{m}$ filter prior to the analysis has to be
54 clearly justified because this would prevent detection of any impurities that are not soluble
55 in the dilution solvent. All of the analysed samples should be fully solubilised/diluted prior to
56 the HPLC-PDA analysis.

57

1 In the absence of experimental data, the SCCS has calculated the potential risk of the
2 presence of impurities and residual solvents according to the TTC principles. This has
3 indicated that the levels of one of the impurities (3-aminopropyl imidazole) and a residual
4 solvent (chloroethane) are above the TTC levels that can be considered safe.

5 One of the impurities, 4-aminophenylamine dihydrochloride (synonym p-phenylenediamine
6 dihydrochloride), has already been the subject of an SCCS Opinion (SCCS/1443/11 Revision
7 of 18 September 2012), which raised concerns over its strong skin sensitising potency.

3.1.6 Solubility

11 Following request from SCCS, data on solubility of Methylimidazoliumpropyl-p-
12 Phenylenediamine, Cl, 2HCl (A166) in water media and in the solvent systems used in
13 various studies have been provided.

14 According to the Applicant, water was the solvent used in all safety studies performed on
15 Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166). Accordingly, the solubility
16 was measured in water only.

17 This study was conducted in compliance with:

- 18 - OECD guideline for the testing of chemicals: N° 105 - Water solubility adopted July 27,
19 1995.
- 20 - OECD principles of Good Laboratory Practice.

21 *Test material*

22 Batch R0027494A 008 L 002 (99.9% pure) was used in this study.

23 A preliminary test for the water solubility was carried out. The water solubility of the test
24 item was estimated to be greater than 1 kg/L in pure water at room temperature (20°C ±
25 5°C). At this high level of solubility, no pH-dependency could be observed. Based on the
26 guideline the shake flask method was used for the test performance for substances with
27 solubility > 10 mg/L.

28 *Test Principle*

29 *Shake flask method*

30 The closed vessels containing (5 g test item, 1 ml solvent) were incubated in a shaking
31 water bath at 30 °C for 24 h, 48 h and 72 h, respectively. Soon after the start of the pre-
32 incubation time, the samples appeared as deep dark brown solution with colourless crystals
33 of 2-5 mm size inside.

34 At the end of each incubation period the vessels were removed from the water bath and
35 equilibrated for another 24 hours at 20 °C under shaking. The remaining test item was
36 separated by filtration through a 0.45 µm syringe tip filter and analysed by HPLC / UV.

37 *Results*

38 The results showed no tendency to change over time and met all validity criteria, therefore
39 the study is considered to be valid. Based on the analyses, the solubility of the test item in
40 water was determined to be 931 g/L at 20 °C.

41 The determination of the concentration in the saturated solutions was done under usage of
42 a quadratic regression. The arrangements made to prevent a possible oxidation of the test
43 item (overlay with argon, addition of ascorbic acid in the solvent) seem to be sufficient.

44 Ref : Wöhr T. (2019)

3.1.7 Partition coefficient (Log P_{ow})

45 n-Octanol/Water partition coefficient: experimental Log P_{ow} is not determined.

1 LogP_{ow} (calculated value): -3.3 (ClogP_{ow}, v.5.2)
2

3 **3.1.8 Additional physical and chemical specifications**

- 4
5 - organoleptic properties (colour, odour, taste if relevant): Very light beige to beige
6 powder
7
8 - melting point: /
9
10 - boiling point: /
11
12 - flash point: /
13
14 - vapour pressure: /
15
16 - density: /
17
18 - viscosity: /
19
20 - pKa: 2.09 and 5.9 (25°C, ionic strength 0.15 M) for basic equilibria. (Potentiometry,
21 GLpKa Sirius)
22 - pH: The pH of A166 (Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166))
23 diluted at 2% and 10% in water is 1.5 and 1.27/1.24, respectively, as reported in
24 Cannamela (2011) and Cannamela (2015).
25
26 - refractive index: /

27
28
29 The following analyses have been performed: UV/visible light absorption, Infra-red
30 spectrometry, ¹H NMR and ¹³C NMR spectrometry, Mass spectrometry. Their results are
31 compatible with the proposed structure of Methylimidazoliumpropyl-p-Phenylenediamine, Cl,
32 2HCl (A166).

33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

3.1.9 Homogeneity and Stability

The compound is considered to be stable when stored at 4 ± 4 °C in the dark under argon shielded from air and humidity.

Ref: Certificates of Analysis (version 2), Analytical file – Imexine OBL – June 2017; Wöhr T. 2019

Following the request of SCCS, the Applicant has provided information on the stability of Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) for dosing formulations used in systemic toxicity studies at 10, 30 and 100 mg/ml in water.

Stability of Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) in dose formulation was determined by measuring Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) contents using HPLC at different time intervals up to 4 h. The hair dye ingredient Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) was found stable in dose formulation for 4h (Raithatha, 2013).

In addition, in the skin penetration study on Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) contained in the Applicant's initial safety dossier and performed under typical consumer use conditions (Toner, 2014), an HPLC analysis of the oxidative hair coloring formulation containing Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) at 2% (final, on-head concentration) was performed immediately after the application period and 24 hours afterwards. The radiochemical purity and test item concentration was determined by HPLC in the Hair Dye Formulation 24 h post dose to determine the stability of the test item over the live phase of the study.

These analyses yielded Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) concentrations of 103.73% and 100.96% of the nominal concentration (2%), respectively, which confirms that Methylimidazoliumpropyl-p-Phenylenediamine, Cl, 2HCl (A166) is stable over a 24-hour period under typical consumer use conditions.

Ref: Raithatha A. (2013), Toner F. (2014)

SCCS comment

The information provided on stability of A166 is not convincing. The use of ascorbic acid and argon for the shaking flask method, and the storage conditions (4 ± 4 °C, in the dark under argon), indicates that the test substance is easily oxidised. The Applicant has provided further information on the stability studies performed for the dose formulation used for toxicity studies, where the dissolution media is distilled water. However, details on the solvent media and the procedure used for preparation of solutions are not fully described in the report.

It is necessary to provide data on the stability of A166 under conditions of use with a selective analytical method. Liquid scintillation counting is not a selective method for this purpose. The stability study was performed before mixing the test substance with the developer, whereas it should be performed after the mixing with the developer. Any degradation products should also be chemically characterised.

The procedure described under 7.13.4 Formulation Stability is different than the procedure described in Appendix 1 - 13.6 Formulation Stability (Toner, 2014),. Any degradation products should be chemically characterised.

3.2 FUNCTION AND USES

The ingredient Methylimidazoliumpropyl-p-Phenylenediamine Cl, 2HCl (A166) is intended to be used in oxidative hair colouring products at on-head concentration of up to 2%.

3.3 TOXICOLOGICAL EVALUATION

The following table provides an overview of the guidelines under which each study submitted in the present dossier was conducted.

| STUDY | TEST GUIDELINE | GLP | REFERENCES |
|---|----------------|-----|-------------------|
| <i>In vitro</i> primary cutaneous tolerance test using human reconstructed epidermis (Episkin SM) | OECD 439 | Yes | Brémond, 2012 |
| <i>In vitro</i> ocular primary irritation test using Bovine Cornea Opacity and Permeability method | OECD 437 | Yes | Cannamela, 2015 |
| Local lymph node assay in mice | OECD 429 | Yes | Verma, 2012 |
| Bacterial reverse mutation test | OECD 471 | Yes | Hobson, 2013 |
| Mammalian cell gene mutation test (<i>hprt</i> locus) in mouse lymphoma cells | OECD 476 | Yes | Lloyd, 2013 |
| Micronucleus test in cultured human lymphocytes | OECD 487 | Yes | Watters, 2012 |
| Acute oral toxicity study in rats | OECD 401 | Yes | De Jouffrey, 1997 |
| 13-week oral toxicity study in rats | OECD 408 | Yes | Gohel, 2014 |
| Prenatal developmental toxicity study by | OECD 414 | Yes | Patel, 2014 |

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

the oral route in rats

| | | | |
|---|-----------------------------|-----|---------------|
| <i>In vitro</i> percutaneous absorption study using human dermatomed skin | SCCS 1358/10 OECD 428 | Yes | Toner, 2014 |
| Bioavailability across intestinal barrier in cultured human intestinal epithelial cells | - | No | Lee, 2015 |
| 14-day toxicity study by the oral route in rats | - | Yes | Ujawane, 2013 |

3.3.1 Acute Toxicity

3.3.1.1 Acute oral toxicity

Guideline: OECD 401
Species/strain: Sprague-Dawley rats
Group size: One group of 3 males and 3 females
Test substance: C16778
Batch: RF006
Vehicle: Purified water
Dose levels: 500 mg/kg (volume of 10 ml/kg)
Administration: oral (gavage), single
GLP: In compliance
Study period: 2 June 1997 - 4 August 1997

The acute oral toxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was evaluated following single administration to fasted rats at 500 mg/kg.

Three Sprague-Dawley (SD) rats per sex were used in this study to assess the acute oral toxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166).

On a day designated as day 1, 3 overnight fasted SD rats per sex received a single oral (gavage) dose of 500 mg/kg Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) in purified water (10 mL/kg). Animals were observed at least daily for clinical signs and twice daily for mortality. Body weights were recorded on day 1 just before treatment and on days 5 and 8. Animals found dead or killed prematurely and all surviving animals killed at the end of the study were subjected to macroscopic examination. Organs showing gross anomalies were sampled and preserved, but no histopathological examination was performed. Neither mortality nor clinical signs were observed during the study. Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

Conclusion

Under the conditions of this study, no clinical signs and no deaths were observed after a single oral administration of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) at the dose level of 500 mg/kg in rats. The oral LD50 was higher than 500 mg/kg.

Ref: De Jouffrey S (1997)

SCCS comments

Animals were observed until day 8 instead of for 14 days, but since no mortality was registered, it can be deduced that the oral LD50 is higher than 500 mg/kg b.w.

1
2 3.3.1.2 Acute dermal toxicity

3
4 /

5
6 3.3.1.3 Acute inhalation toxicity

7
8 /

9 **3.3.2 Irritation and corrosivity**

10
11 3.3.2.1 Skin irritation - *In vitro* Primary Cutaneous Tolerance

12
13 Guideline: ECVAM validated protocol (ESAC Statement 2007)
14 Test system: 96 reconstructed human epidermis EpiskinSM samples (0.38 cm²)
15 Number of replicates: Triplicate tissues for each item
16 Test substance: R0027494A
17 Test item: 10% (w/w) R0027494A in water
18 Batch: R0027494A 000 L 006
19 Purity: >95%
20 Dose: 10 µl
21 Treatment period: 15± 1 minutes
22 Post-treatment incubation time: 42 h± 1h
23 Positive control: 5% aqueous solution of SDS
24 Negative control: PBS
25 Direct interaction with MTT: Positive
26 Colouring potential test item: Positive
27 GLP: in compliance
28 Study period: November 2011 – May 2012

29
30 *Test Procedure*

31 The test item diluted at 10% (w/w) in water was evaluated in 3 different batches of a
32 reconstructed human epidermis model. After preliminary tests (dilution and staining
33 tests), the coloured test item was tested according to the specific colouring protocol.

34 The test item, negative control (PBS+), positive control (5% aqueous solution of Sodium
35 Dodecyl Sulfate), solvent control (water), and a dead epidermis negative control (PBS+)
36 were tested in triplicate. Two additional negative controls (one dead and one living
37 tissue) were used which followed the same treatment as the negative control (PBS+)
38 but underwent an additional MTT incubation period.

39 Six additional tissues (three living and three dead tissues) were used and followed the
40 same treatment as the other tissues (except for the MTT incubation period).

41 These tissues as well as the additional negative control (PBS+) were used as specific
42 controls in order to quantify the extent of Non-Specific Colour due to the colouring
43 chemical interactions with the tissue.

44
45 10 µl of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) diluted at 10%
46 and 10 µL of different controls were applied onto the epidermis using a positive
47 displacement pipette. After 15 minutes' treatment period at room temperature, tissues
48 were rinsed with PBS+, and then epidermis were transferred in 2 ml/well of fresh
49 maintenance medium and incubated for 42 hours +/- 1 hour at 37°C.

50
51 *MTT test:*

52 At the end of the 42 hours +/- 1-hour treatment period, each epidermis unit was
53 transferred to the wells of a 12 well plate containing a dye solution (MTT) except for the
54 negative control and the test item-treated epidermis without MTT which were
55 transferred into a 12 well plate containing fresh medium. Plates were incubated for 3

1 hours to 3 hours 15 minutes at 37°C. At the end of the incubation period, a biopsy of
2 the entire epidermis was taken. For all tissues with the test item, the superficial
3 epidermis layer (containing most of the remaining color) was removed and discarded.
4 The epidermis was separated from the collagen matrix and both were transferred into a
5 tube containing 500 µL of acidified isopropanol. Formazan crystals were extracted and
6 stirred to homogenize the solution. 2 x 200 µL of each extract were transferred onto a
7 96 well plate and the optical density (OD) was measured at 570 nm versus acidified
8 isopropanol.

9
10 Determination of IL-1α concentrations in the culture medium:

11 IL-1α released in the culture medium was determined by a classic quantitative sandwich
12 enzyme immunoassay technique. The optical density (OD values related to the IL-1α
13 amount) was measured at 450 nm.

14
15 The test item is predicted to be non-irritant when mean viability value is above 50% and
16 final IL-1α release is below 50 pg/mL. The test item is predicted to be irritant when mean
17 viability value is lower than (\leq) 50% or final IL-1α release is above (\geq) 50 pg/mL.

18
19 *Results*

20 The mean viability value for the test item diluted at 10% was 76.2±12.8% and the final
21 IL-1α release was 1.6±2.9 pg/mL.

22
23 *Conclusion*

24 Under the conditions of this study, Methylimidazoliumpropyl p-phenylenediamine, Cl,
25 2HCl (A166) at the concentration of 10%(w/w) in water is considered to be non-irritant
26 to the skin.

27 Ref: Brémond C. (2012)

28
29 **SCCS comment**

30 IL-release was not taken up in the Guideline. It is not clear whether the positive control
31 yielded the expected results to indicate that the test had worked.

32
33
34 **3.3.2.2 Eye irritation**

35
36 **Bovine corneal opacity and permeability method (BCOP)**

37
38 Guideline: Not stated
39 Test system: Bovine eyes (from cattle aged less than 12 months)
40 Group size: 3 corneas
41 Test substance: R0027494A
42 Test item: 10% (w/w) R0027494A in distilled water
43 Batch: R0027494A 009 L 006
44 Purity: >95% (UV detection)
45 Dose applied: 750 ± 8µl
46 Treatment period: 30 ± 10 minutes, 4 hours ± 10 minutes
47 Post-treatment
48 Incubation time: 2 hours for 30 minutes incubation time, none for 4 hours
49 Negative control: Nutritive medium (MEM, sodium bicarbonate, distilled water)
50 Positive control: 0,5% (w/w) Cetyl Trimethylammonium Bromide (CTAB)
51 GLP: In compliance
52 Study period: June- November 2011

53
54 *Test Procedure*

55 Bovine eyes (from cattle less than 12 months old) were collected at slaughterhouses and
56 prepared within 4 hours of collection. Eyes that were too big or were presenting defects
57 were rejected. After the pre-incubation and equilibration period of the corneas at 32 ± 1 C

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 for 1h ± 10 minutes, 750 ± 8 µL of the test item and controls were applied onto the
2 corneas. The treatment period of 30 ± 5 minutes and 4 hours ± 10 minutes was followed by
3 three rinsing steps and visual examination of rinsing efficiency. The corneal opacity and
4 permeability were measured. Corneas were incubated with 0.5% fluorescein solution for 90
5 ± 5 minutes at 32 ± 1 C; corneal permeability was performed by measuring optical density
6 at 490 nm.

7 The following validity criteria were applied in the study:

- 8 - opacity of the negative control (nutritive medium) corneas: between 0 and 10
- 9 - O.D. of the negative control (nutritive medium) corneas: lower than 0.100
- 10 - O.D. of the fluorescein solution to 5 µg/mL: between 0.850 and 0.940
- 11 - score CTAB to 0.5%:
 - 12 • 30 minutes: value between 85.0 and 105.0
 - 13 • 10 minutes: value between 25.0 and 40.0.

15 Results

16 The study fulfilled the validity criteria stated in the study report.

17 The corneal scores (a combination of opacification and permeability measurements)
18 obtained after a 30-minute and 4-hour contact time were 5.5±0.9 and 96.7±0.7,
19 respectively.

20 Under the conditions of this study, the test item at the concentration of 10% is predicted to
21 be moderately irritant when administered to the isolated bovine cornea.

22
23 Ref: Cannamela N. (2011), Cannamela N (2015)

25 SCCS comment

26 The BCOP assay is an *in vitro* method that can be used to identify ocular corrosive and
27 severe irritants, but not moderate irritants. Considering that the test item is brown coloured
28 and that colouration of epithelial tissue by the test item has been reported in the *in vitro*
29 skin irritation test, it is expected that colouration of the cornea might have occurred.

30 Even though R0027494A diluted to 10% w/w in water has a very low pH (reported to be
31 1.24 and 1.27), which may lead to corrosive effects, the BCOP assay did not indicate severe
32 eye irritation. This, however, does not exclude a mild or moderate eye irritancy potential.
33 Under the conditions of this study, a mild to moderate eye irritation potential of the test
34 item cannot be excluded.

37 3.3.3 Skin sensitisation

39 Local Lymph Node Assay (LLNA)

| | | |
|----|-------------------|---|
| 41 | Guideline: | OECD 429, EC B.42 |
| 42 | Species/strain: | Female CBA/J mice |
| 43 | Group size: | 4 mice per group (main study), 2 mice per group (preliminary assay) |
| 44 | Test substance: | Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) |
| 45 | Batch: | R00227494A 002 L 001 |
| 46 | Purity: | >95% |
| 47 | Vehicle: | Pluronic® L92 1% in water |
| 48 | Concentration: | 1, 5, 25 and 50% (w/v) |
| 49 | Positive control: | alpha-hexylcinnamaldehyde (HCA) 25%(v/v) in water with 1% 50 Pluronic® L92 |
| 51 | Vehicle control: | Pluronic® L92 1% in water |
| 52 | GLP: | In compliance |
| 53 | Study period: | Febr – Sept 2012 |

55 The skin sensitising potential of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
56 (A166) was evaluated in a Local Lymph Node Assay (LLNA) in mice.

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

- 1 Animals (28 mice) were separated in 7 groups (4 mice/group) consisting of:
 2 • 5 treated groups receiving Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
 3 (A166) at 1, 5, 10, 25 or 50% (w/v) in 1% Pluronic L-92 (vehicle). Due to unsatisfactory
 4 solubility tests with several recommended vehicles, Pluronic L-92 was selected on the
 5 basis of a solubility study revealing 50% (w/v) Methylimidazoliumpropyl p-
 6 phenylenediamine, Cl, 2HCl (A166) as the maximal technically feasible concentration.
 7 • A negative control group receiving the vehicle (1% Pluronic L-92) alone
 8 • A positive control group receiving alpha-hexylcinnamaldehyde (HCA) at 25% (v/v) in 1%
 9 Pluronic L-92

10
 11 The test substance Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) and 1%
 12 Pluronic L-92 or HCA were applied on the ears (25 µL per ear) of mice for three consecutive
 13 days designated as days 0, 1 and 2. After 2 days of resting (day 5), mice received a single
 14 intravenous injection of tritiated methyl thymidine (³H-TdR). Lymph nodes draining the
 15 application sites (auricular nodes) were sampled, pooled per group, and the proliferation of
 16 lymphocytes was evaluated by measuring the incorporation of ³H-TdR. The values obtained
 17 were used to calculate stimulation indices (SI). The irritant potential of the test item was
 18 assessed by measuring ear thickness on days 0, 2 and 5.

19
 20 *Results*

21 All validity criteria were fulfilled and the study was therefore considered to be valid. In
 22 particular, a SI value of 8.04 was obtained with the positive control HCA.
 23 The threshold value of 3 for positive results was exceeded at 5% with a dose-response
 24 effect (SI values of 2.69, 7.99, 10.77, 12 and 15.91 at 1%, 5%, 10%, 25% and 50%
 25 respectively) and the EC3 value was calculated to be 1.23%.

26
 27
 28 *Conclusion*

29 Under the conditions of this study, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
 30 (A166) induced delayed contact hypersensitivity. According to the EC3 value calculated
 31 (1.23%), Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was considered to
 32 have a moderate sensitising potential.

33 Ref: Verma, 2012

34
 35 **SCCS comment**

36 SCCS considers Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) as a strong
 37 skin sensitiser, based on the EC3 value of 1.23% observed in the LLNA (see SCCP, 2005).
 38
 39

40 **3.3.4 Toxicokinetics**

41
 42 **3.3.4.1 Dermal / percutaneous absorption**

43
 44 ***In Vitro* Percutaneous Absorption of Radiolabelled R0027494A Through Human Skin.**

45
 46
 47 Guideline: OECD 428 (2004); OECD 28 (2004), SCCS 1358/10 (2010),
 48 COLIPA Cosmetic Ingredients: Guidelines for Percutaneous
 49 Absorption/Penetration (1997)
 50 Test system: Split-thickness human skin 350-400 µm, from 5 donors aged 40
 51 – 56 years (1 breast, 4 abdomen)
 52 Membrane integrity: Checked by a tritiated water method
 53 Replicates: 12 skin samples from 4 different donors
 54 Test substance: R0027494A (non-labelled); [¹⁴C]-R0027494A (labelled)
 55 Batch: R0027494A 009 D 003 (non-labelled); CFQ41883 (labelled)

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

| | | |
|----|-------------------------------|---|
| 1 | Purity: | 98% (non-labelled material); 96.8% (radiochemical purity by |
| 2 | | HPLC) |
| 3 | Test item: | Hair dye formulation containing 4% (w/w) [¹⁴ C]-R0027494A |
| 4 | Dose applied: | 20 mg/cm ² of the test formulation |
| 5 | Exposure area: | 0.64 cm ² |
| 6 | Exposure period: | 30 minutes |
| 7 | Sampling period: | 24 hours (every hour between 0-6h, then every 2 hour) |
| 8 | Receptor fluid: | Phosphate buffered saline (PBS) |
| 9 | Solubility in receptor fluid: | 100g/l in water |
| 10 | Mass balance analysis: | Provided |
| 11 | Tape stripping: | Yes (20) |
| 12 | Method of Analysis: | Liquid Scintillation Counting (LSC) |
| 13 | GLP: | In compliance |
| 14 | Study period: | March– April 2014 |

15
16 The percutaneous absorption of 2% Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
17 (A166) was investigated *in vitro* in a oxidative hair dye formulation, in the presence of
18 hydrogen peroxide by using human skin preparations. A total of 12 samples of human skin
19 obtained from 4 different donors were used for each test group. Mean values were
20 calculated from all valid skin samples (n=12, four donors). The integrity of the skin was
21 demonstrated prior to application by a tritiated water method. Only skin samples within the
22 acceptable range of <0.6% were used.

23
24 Split-thickness human skin membranes were mounted into flow-through diffusion cells,
25 receptor fluid was pumped underneath the skin at a flow rate of 1.5 mL/h ± 0.15 mL/h. The
26 skin surface temperature was maintained at 32°C ± 1°C throughout the experiment. Test
27 item at 2% (w/w) was applied at an application rate of ca 20 mg/cm² for 30 minutes.

28
29 Absorption of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was evaluated
30 by collecting receptor fluid in 60 min fractions from 0 to 6 h post dose, then in 2-hourly
31 fractions from 6 to 24h post dose. The stratum corneum was removed by tape stripping and
32 the remaining skin was divided into exposed and unexposed skin. The exposed epidermis
33 was then heat separated from the dermis. The radioactivity of radiolabelled
34 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was quantified by liquid
35 scintillation counting.

36
37 *Results*

38 The majority of the test substance was removed in the skin wash after the 30-minute
39 exposure (97.52%). A further 0.07% of the applied dose was removed at 24 h (donor
40 wash). The total dislodgeable dose was 97.59% of the applied dose. The mean total
41 unabsorbed dose was 99.35% of the applied dose. This consisted of the dislodgeable dose,
42 unexposed skin (0.01%) and the radioactivity associated with the stratum corneum
43 (1.74%). The first five tape strips contained 1.12% of the applied dose. There was a steady
44 decrease in the recovery of radioactivity associated with the stratum corneum. Tapes 6-10,
45 11-15 and 16-20 contained 0.40%, 0.16% and 0.07%, respectively. Those are not
46 considered to be dermally absorbed and thus do not contribute to the systemic dose. Where
47 any epidermis was removed during tape stripping this tape strip value was added to the
48 epidermis and not to the stratum corneum value.

49 The absorbed dose (0.06%) was the sum of the receptor fluid (0.05%), the receptor rinse
50 (<0.01%) and the receptor wash (0.01%). Dermal delivery: 0.29% (1.15 ± 0.77 µg
51 equiv./cm²) was the sum of the absorbed dose and the epidermis (0.19%) and dermis
52 (0.04%). The mean mass balance was 99.63% of the applied dose after 24 h.

53
54 The table below shows a summary of the mean test results:

55

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

| Test Preparation | Test Preparation |
|--|------------------|
| Target [¹⁴ C]-R0027494A Concentration in Formulation (% w/w) | 4 |
| Actual [¹⁴ C]- R0027494A Concentration in Formulation (% w/w) | 3.98 |
| Target [¹⁴ C]- R0027494A Concentration in Test Preparation (% w/w) | 2 |
| Actual [¹⁴ C]- R0027494A Concentration in Test Preparation (% w/w) | 1.95 |
| Target Application Rate of Test Preparation (mg/cm ²) | 20 |
| Actual Application Rate of Test Preparation (mg/cm ²) | 20.4 |
| Total Number of Donors | 4 |
| Total Number of Replicates Dosed | 3 |
| Total Number of Replicates Contributing to Mean ± SD Data | 12 |
| (% Applied Dose) | (Mean ± SD) |
| Dislodgeable Dose | 97.59 ± 1.47 |
| Unabsorbed Dose * | 99.35 ± 1.33 |
| Absorbed Dose ** | 0.06 ± 0.02 |
| Dermal Delivery *** | 0.29 ± 0.19 |
| Mass Balance | 99.63 ± 1.17 |
| (µg equiv./cm ²) | (Mean ± SD) |
| Dislodgeable Dose | 388.63 ± 5.79 |
| Unabsorbed Dose * | 395.60 ± 4.87 |
| Absorbed Dose ** | 0.23 ± 0.07 |
| Dermal Delivery *** | 1.15 ± 0.77 |
| Mass Balance | 396.75 ± 4.35 |

* Unabsorbed dose = dislodgeable dose + stratum corneum + unexposed skin

** Absorbed dose = receptor fluid + receptor rinse + receptor wash

*** Dermal delivery = epidermis + dermis + absorbed dose

Conclusion

Under the experimental conditions of the study, the mean dose of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) considered to be penetrated following application of a typical oxidative hair colouring formulation containing this ingredient at 2% was estimated to be $1.15 \pm 0.77 \mu\text{g equiv./cm}^2$ ($0.29 \pm 0.19\%$ of the applied dose).

Ref: Toner F. (2014)

SCCS comment

Correct calculation of the standard deviation is $0.74 \mu\text{g/cm}^2$.

In accordance with the SCCS Notes of Guidance, the mean + 1 SD of dermal absorption value, i.e. $1.89 \mu\text{g/cm}^2$ (0.48%), will be used for MoS calculation.

3.3.4.2 Other studies on toxicokinetics

Study to Investigate the Permeability of the Test Compound, R0027494A, in Caco-2 Cells

Guideline: Study performed according to the recommendations by ECVAM and is generally recognized as scientifically relevant (ECVAM, DB-ALM Protocol n° 142)

Species/strain: Human intestinal epithelial cells line Caco-2

Test substance: Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)

Batch: R0027494A 009 D 003

Purity: 98% pure

Concentration: 10 µM in HBSS (pH 7.4) with a final DMSO concentration of 1%

GLP: non-GLP conditions

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1
2 *Test Procedure*
3 The test item was prepared at 10 µM in HBSS (pH 7.4) with a final DMSO concentration of
4 1%. The permeability from the apical (A, pH 7.4) to the basolateral (B, pH 7.4) side was
5 investigated at 37°C in 96-well Multiscreen plates with shaking for a 60 min contact period.
6 Analysis of the donor (apical) and receiver (basolateral) samples was done by means of the
7 LC-MS/MS and the apparent permeability coefficient (P_{app}) of the test item and its recovery
8 were calculated for two independent experiments. Lucifer yellow was used to demonstrate
9 the integrity of the cell monolayer. Only monolayers revealing a permeability of less than
10 1.5×10^{-6} cm/sec were used. Propranolol (highly permeable~90%), atenolol (moderately
11 permeable~50%), and talinolol (P-glycoprotein substrate) were analyzed concurrently to
12 demonstrate the validity of the assay.

13 According to the laboratory's classification system, a low permeability is considered for test
14 items revealing a $P_{app} = 0.2 - 2 \times 10^{-6}$ cm/sec. A P_{app} of $2 - 20 \times 10^{-6}$ cm/sec and a $P_{app} = 20$
15 $- 45 \times 10^{-6}$ cm/sec classify a substance to have a moderate and a high permeability,
16 respectively. As recommended by FDA, atenolol (50% absorption in humans) was used as
17 the low permeability reference compound and propranolol (90% absorption in humans) was
18 used as the high permeability reference compound.

19
20 *Results*

21 The total recovery for the reference substances ranged from 76.5 to 94.4% and was 93.4%
22 for Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166).

23 The figures for the reference substances propranolol ($P_{app} = 34.0 \times 10^{-6}$ cm/sec) and
24 atenolol ($P_{app} = 0.39 \times 10^{-6}$ cm/sec) were well within the acceptance range for these
25 compounds of $20 - 45 \times 10^{-6}$ cm/sec and $P_{app} 0.2 - 2 \times 10^{-6}$ cm/sec, respectively, and
26 demonstrated the validity of the assay.

27 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) revealed a P_{app} of 5.73×10^{-6}
28 cm/sec and thus, was classified to be of moderate permeability, indicating a moderate
29 absorption from the gastro-intestinal tract.

30
31 *Conclusion*

32 A mean permeability in human intestinal epithelial cells (Caco-2) of 5.73×10^{-6} cm/sec was
33 obtained with Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) which
34 indicates a moderate permeability. As the absorption from the gastro-intestinal epithelium is
35 considered to be the limiting factor of the uptake through the gastro-intestinal tract, the
36 moderate permeability observed in this assay points to a moderate absorption of
37 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) after oral administration.

38
39 Ref: Lee S. (2015)
40

41 **SCCS comment**

42 A moderate permeability observed in this study supports the default value of 50% for oral
43 bioavailability, which will be used for the calculation of the MoS.
44

45 **3.3.5 Repeated dose toxicity**46
47 **3.3.5.1 Repeated dose 14 Days oral toxicity**

48
49 Species/strain: Wistar rats
50 Group size: 5 per dose per sex
51 Test substance: R0027494A, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
52 (A166)
53 Batch: 008 L 002
54 Purity: 99.9%
55 Vehicle: distilled water

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 Dose levels: 0 (G1), 30 (G2), 100 (G3), 300 (G4) and 1000 (G5) mg/kg b.w.
2 Dose volume: 10 mL/kg
3 Route: oral
4 Administration: oral gavage of 14 consecutive days
5 GLP: yes
6 Study period: May 11, 2012 to October 10, 2103
7
8

9 *Method*

10 Total 25 male and 25 female Wistar were randomly divided into five groups. Four dose
11 levels of R0027494A were tested: 30 (G2-Low dose), 100 (G3-Mid dose-I), 300 (G4-Mid
12 dose-II) and 1000 (G5 High dose) mg/kg b. wt./day. A concurrent control group of rats was
13 given distilled water only. The fix dose volume of 10 mL/kg body weight was used. The dose
14 formulations were prepared by dissolving the test item in distilled water.
15

16 All rats were observed once a day for clinical signs and twice a day for mortality and
17 morbidity throughout the study period. Body weight and food consumption of individual
18 animals were determined twice weekly throughout the study. Haematological and
19 biochemical analyses were performed on blood samples of all rats at the end of the
20 treatment period.
21

22 All the surviving rats were sacrificed by carbon dioxide asphyxiation and subjected to gross
23 pathological examination at the end of the treatment period. Absolute organ weights were
24 recorded and relative organ weights were calculated for the organs defined in study plan.
25

26 *Results*

27 No mortality was observed from any of the groups (G1 to G5) during the study period.
28 All animals belonging to groups G1 and G5 were found normal throughout the study period
29 Some changes were observed in mean body weight gain of rats belonging to group G5 and
30 in food consumption of female rats from groups G2, G3, G4 and G5 when compared to the
31 G1 rats. These changes were limited, not dose related in female and therefore of uncertain
32 biological significance.
33 No treatment-related alterations were observed in haematology and clinical chemistry
34 parameters of R0027494A treated groups when compared with the control group.
35 No treatment related change in polychromatic to total erythrocytes ratio (P/E) was observed
36 in all the R0027494A treated groups as compared to control group.
37 No external and internal gross lesions were observed across all the groups.
38 No treatment related microscopic changes were observed in rats treated with R0027494A at
39 1000 mg/kg b.wt./day.
40

41 *Conclusion*

42 Based on the results of the study, it is concluded that, with the exception of a slight
43 decrease in body weight gain at the highest dose in males and a non dose-related decrease
44 in body weight gain at all doses in females with a decreased food consumption in the two
45 highest dosed groups, R0027494A did not produce any adverse effect up to the dose level
46 of 1000 mg/kg b.wt./day when administrated through oral gavage for 14 consecutive days
47 in Wistar rats under the conditions and the procedures followed in the present study.
48

49 Consequently, the dose level of 1000 mg/kg b.wt./day can be selected as the highest dose
50 to be tested for further repeated toxicity study.

Ref: Jawane D (2013)

51
52
53 **SCCS comment**

54 The study was performed as a range-finder for an oral 90 d repeat-dose toxicity study. The
55 results of this study support a low acute toxicity of Methylimidazoliumpropyl p-
56 phenylenediamine, Cl, 2HCl (A166).
57

3.3.5.2 Sub-chronic (90 days) oral toxicity

Guideline: OECD 408 (1998)
Species/strain: Wistar rats
Group size: 10 males and 10 females

Test substance: R0027494A, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
Batch: 009 D 003
Purity: 99.9 %
Vehicle: distilled water
Dose levels: 0 (G1), 100 (G2), 300(G3), 1000 (G4) mg/kg b.w.
Dose volume: 10 mL/kg b.w.
Route: oral
Administration: gavage – 13 weeks
GLP: yes
Study period: December 11, 2012 to November 14, 2014
The experimental phase of the 13-week oral toxicity in rats was conducted from December 2012 to March 2013, and the corresponding study report was completed in 2014

Test Procedure

The subchronic toxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was investigated in Wistar rats (10/sex/group) after daily oral gavage at 0, 100, 300 or 1000 mg/kg/day in distilled water (10 mL/kg) for 13 weeks. These dose levels were selected on the basis of the results of a preliminary 14-day study (see section 3.3.5.1). Evaluations and measurements included twice daily mortality checks and clinical observations, weekly body weight, food intake and neurobehavioral observation, ophthalmoscopy prior to dosing and at the end of the treatment period, neurotoxicological evaluation during week 12, haematology, blood clinical chemistry and urinalysis (week 13). Neurobehavioral observations were conducted on each rat once prior to treatment initiation and at weekly intervals thereafter. At the end of the treatment period, surviving animals were sacrificed and subjected to macroscopic examination; selected organs were weighed, and a wide range of organs/tissues were preserved. Microscopic examination was performed for specified tissues/organs from control and high dose rats killed at the end of the dosing period, as well as for any gross anomaly.

Results

The analysis of the dose formulations administered during the study showed that the concentrations given were within the appropriate range.

At 100 and 300 mg/kg bw, the test item Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) did not induce any relevant treatment-related changes.

At 1000 mg/kg/day in males, statistically significant decreases in mean body weights were observed during week 9, 10, 11 and 13 and in mean body weight changes (throughout treatment period). There were no apparent abnormalities in ophthalmology, neurobehaviour and functional observational battery.

In males of the highest dose, relative weights of liver, spleen, brain, kidneys and adrenals were statistically significantly higher when compared to controls and absolute weights of thymus and epididymides were statistically significantly lower when compared to controls. In females at the highest dose a significant increase was observed in absolute and relative weights of livers and ovaries and in relative weights of adrenals.

The weight changes were not accompanied by histopathological findings but considered test-item related by the study authors. Treatment-related changes were observed in clinical chemistry in males and females at the highest dose (significantly lower blood glucose, alkaline phosphatase and albumin concentrations levels; significantly higher blood phosphorus concentrations). Females at the highest dose also had significantly higher blood

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 AST concentrations and significantly lower blood sodium and chloride concentrations.
2 Furthermore, haematology revealed significantly higher Hb, MCH and MCHA values and
3 significantly lower APTT (Activated Partial Thromboplasin Time) and PT (prothrombin time)
4 values in males at the highest dose and significantly lower PT values in females at 300 and
5 1000 mg/kg bw/d. These effects were not considered treatment-related by the study
6 authors.

7
8 *Conclusion*

9 Thus, under the conditions of the study, the NOEL (No Observed Effect Level) of this
10 90-day repeated oral toxicity study with Methylimidazoliumpropyl p-phenylenediamine, Cl,
11 2HCl (A166) in Wistar rats was defined at 300 mg/kg /day.

12
13 Ref: Gohel D (2014), Ujawane D (2013)

14
15 **SCCS comment**

16 Based on limited adverse effects (changes in clinical chemistry and haematology at the
17 highest dose), the SCCS derives a NOAEL of 300 mg/kg bw/d from this study.

18
19
20 **3.3.5.3 Chronic (> 12 months) toxicity**

21 /

22
23
24 **3.3.6 Reproductive toxicity**

25
26 **3.3.6.1 Fertility and reproduction toxicity**

27 /

28
29
30
31 **3.3.6.2 Developmental Toxicity**

32
33 Guideline: OECD 414 (2001)
34 Species/strain: Wistar rats
35 Group size: 25 mated females per group
36 Test substance: R0027494A, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
37 (A166)
38 Batch: 009 D 003
39 Purity: 99.9%
40 Vehicle: distilled water
41 Dose levels: 0 (G1), 100 (G2), 300 (G3), 1000 (G4) mg/kg b.w. from gestational days
42 (GD) 6 to 19
43 Dose volume: 10 mL/kg b.w.
44 Route: oral
45 Administration: gavage
46 GLP: Yes
47 Study period: December 11, 2012 to October 30, 2014
48 The experimental phase of the Prenatal development toxicity by the oral
49 route in rats was conducted from December 2012 to January 2013, and
50 the corresponding study report was completed in 2014

51
52 *Test Procedure*

53 The potential effects of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) on
54 pregnant rats and embryo-foetal development were evaluated through daily oral gavage in
55 which mated Wistar female rats (25/group) were dosed at 0, 100, 300 or 1000 mg/kg/day

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 during the sensitive period of organogenesis from gestation day 6 to day 19 [the day of
2 mating was designated as Gestation Day 0 (GD 0)]. The test item was dissolved in water
3 and given at 10 mL/kg. These dose levels were selected on the basis of the results of a
4 preliminary 14-day repeated toxicity study performed at 100, 300 and 1000 mg/kg/day
5 where no adverse effects were observed [Ujawane 2013]. Maternal evaluations and
6 measurements included daily observation of clinical signs and body weight/food intake at
7 designated intervals. The dams were killed on GD 20 and subjected to macroscopic
8 examination. Usual litter parameters were recorded and foetuses were sexed, weighed and
9 submitted to external examination. About one half of the foetuses were also examined for
10 soft tissue anomalies, and remaining foetuses were examined for skeletal anomalies.

Results

11
12 The analysis of the dose formulations administered during the study showed that the
13 concentrations given were within the appropriate range.

14 No mortality was observed in dams up to the dose level of 1000 mg/kg/day. Lethargy was
15 observed in animals given 300 mg/kg bw/d and above, associated with weakness,
16 hypoesthesia and somnolence at 1000 mg/kg bw/d. Decreases in mean body weight, mean
17 body weight changes, corrected body weight gain and food consumption were observed at
18 the same dose level when compared to the control group. At 100 and 300 mg/kg/day, no
19 clinical signs of toxicological relevance were observed in dams. External and visceral
20 examination of dams did not reveal any lesion of pathological significance. Mean numbers of
21 corpora lutea, implantations, live foetuses, dead foetuses and mean rates of pre-
22 implantation losses, post-implantation losses, live foetuses and dead foetuses in treated
23 animals were not different from control. Slight increase in resorptions was also observed in
24 treated dams at 300 and 1000 mg/kg/day but not statistically significant.

25 Mean number of litters and fetal body weights in treated animals were not different from
26 controls. There were no gross findings, no external malformations and no skeletal findings in
27 foetuses of treated animals. Visceral examination indicated isolated gross lesions and were
28 considered incidental by the study authors. Thus, there were no indications for a teratogenic
29 potential.

30
31 No significant changes in foetal parameters were observed at any dose level of
32 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) when compared to the
33 control group.

Conclusion

34
35 On the basis of the results obtained in the present study, the No Observed Adverse Effect
36 Levels (NOAEL) for maternal and developmental toxicity of Methylimidazoliumpropyl p-
37 phenylenediamine, Cl, 2HCl (A166) were set at 100 mg/kg/day and 1000 mg/kg/day,
38 respectively. Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was considered
39 to have no teratogenic potential.

40
41
42 Ref: Patel MV (2014)

SCCS comment

43
44 Methylimidazoliumpropyl phenylenediamine, Cl, 2HCl (A166) is not teratogenic in rats.

45
46 A NOAEL of 1000 mg/kg bw/d is derived for developmental toxicity.

47
48 A NOAEL of 100 mg/kg bw/d is derived for maternal toxicity due to changes in lethargy at
49 300 and 1000 mg/kg bw/d and weakness, hypoaesthesia and somnolence and decreased
50 mean body weight gain and mean percent body weight and of absolute uterine weight and
51 uterine weight corrected to body weight at 1000 mg/kg bw/d.

3.3.7 Mutagenicity / genotoxicity**3.3.7.1 Mutagenicity / genotoxicity *in vitro*****Bacterial Reverse Mutation Assay**

| | |
|-----------------|--|
| Guideline: | OECD 471 (1997) |
| Species/Strain: | <i>Salmonella typhimurium</i> TA1535, TA1537, TA98, TA100 and TA102 |
| Replicates: | two independent experiments in the absence and presence of metabolic activation (S9 mix prepared from the livers of rats given Aroclor 1254) |
| Test substance: | Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) |
| Batch: | R0027494A 008 L 002 |
| Purity: | 99.9% |
| Solvent: | deionised water |
| Concentrations: | 5, 15.81, 50, 158.1, 500, 1581 and 5000 µg/plate in experiment 1, both in the absence and presence of S9 mix 20.48, 51.2, 128, 320, 800, 2000 and 5000 µg/plate in experiment 2, both in the absence and presence of S9 mix |
| Treatment: | Experiment I direct plating incorporation method, Experiment II without S9 MIX direct incorporation method with S9 mix the pre-incubation method (20 min). |
| GLP: | in compliance |
| Study period: | June 2012 – January 2013 |

Test Procedure

The test item Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was evaluated in two independent experiments in the absence and presence of metabolic activation (S9 mix prepared from the livers of rats given Aroclor 1254). The experiments were conducted according to the direct plating incorporation method, apart from the second test with S9 mix which was performed according to the pre-incubation method. Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was freely soluble at 50 mg/mL in water.

Toxicity was evaluated for range of concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Negative and positive controls were in accordance with the OECD guideline. Known mutagens were used as positive controls, and cultures treated with purified water (solvent) were used as negative controls. Three plates per treatment condition were used except for negative control (five plates).

Results

All solvent and positive controls gave counts of revertants within expected ranges, and the experiments were therefore considered to be valid.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in both experiments.

Experiment 1: Evidence of toxicity in the form of a slight thinning of the background bacterial lawn and/or a marked reduction in revertant numbers was observed following treatments at 1581 and/or 5000 µg/plate in strains TA98, TA1535, TA1537 and TA102 in the absence and presence of S-9 and strain TA100 in the absence of S-9 only.

Experiment 2: Evidence of toxicity in the form of a slight thinning of the background bacterial lawn and/or a reduction in revertant numbers was observed following treatments at 2000 and/or 5000 µg/plate in strains TA98, TA1537 and TA102 in the absence and presence of S-9 and strain TA1535 in the absence of S-9 only.

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 When compared to controls, no increases in the number of revertants were observed after
2 treatment with Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166), either in the
3 absence or presence of S9 mix.

4
5 *Conclusion*

6 Under the conditions of this study, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
7 (A166) was not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA98,
8 TA100 and TA102 neither in the presence nor absence of metabolic activation.

9
10 Ref: Hobson S (2013)

11
12 **SCCS comment**

13 In experiment 1, R0027494A was clearly bacteriotoxic in low concentrations in the strain
14 TA1537 and in the absence of S9-mix.

15
16
17
18 **Bacterial Reverse Mutation assay on strain TA1537 in the absence of metabolic
19 activation**

20
21 Guideline: OECD 471 (1997)
22 Species/Strain: *Salmonella typhimurium* TA1537
23 Replicates: two independent experiments in the absence of metabolic activation (S9
24 mix prepared from the livers of rats given Aroclor 1254)
25 Test substance: Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
26 Batch: R0027494A 008 L 002
27 Purity: 99.9%
28 Solvent: water for irrigation (purified water),
29 Concentrations: stock concentration at 50 mg/mL
30 Experiment 1: 5, 16, 50, 160, 500, 1600 and 5000 µg/plate in the
31 absence of S9 mix
32 Experiment 2: 160, 300, 625, 1250, 2500, and 5000 µg/plate in the
33 absence of S9 mix
34 Treatment: Experiment I direct plating incorporation method,
35 Experiment II with the pre-incubation method (20 min).
36 Positive control: 9-aminoacridine (AAC) at 50 µg/plate
37 GLP: in compliance
38 Study period: December 4, 2018 – February 22, 2019
39
40

41 *Material and methods*

42 R0027494A was tested for mutation (and toxicity) in one strain of *Salmonella typhimurium*
43 (TA1537), in two separate experiments, at the concentrations detailed above, using
44 triplicate plates without S-9 for test article, vehicle and positive controls.

45 As the results of Experiment 1 were negative, treatments in Experiment 2 included a pre-
46 incubation step. Quantities of test article, vehicle or positive control, bacteria and buffer
47 solution were mixed together and incubated for 20 minutes at 37±1°C, with shaking, before
48 the addition of 2 mL molten agar at 45±1°C. Plating of these treatments then proceeded as
49 for the normal plate-incorporation procedure.

50
51 *Results*

52 Experiment 1 treatments of the tester strain were performed in the absence of S-9, using
53 final concentrations of R0027494A at 5, 16, 50, 160, 500, 1600 and 5000 µg/plate, plus
54 vehicle and positive controls. Following these treatments, evidence of toxicity in the form of
55 a slight thinning of the background bacterial lawn was observed at 5000 µg/plate.

56 Experiment 2 treatments of the tester strain were performed in the absence of S-9. The
57 maximum test concentration of 5000 µg/plate was retained. Narrowed concentration

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 intervals were employed covering the range 160 to 5000 µg/plate, in order to examine
2 more closely those concentrations of R0027494A approaching the maximum test
3 concentration and considered therefore most likely to provide evidence of any mutagenic
4 activity. In addition, all treatments were further modified by the inclusion of a pre-
5 incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that
6 could be detected using this assay system. Following these treatments, evidence of toxicity
7 in the form of a slight thinning of the background bacterial lawn was observed at 5000
8 µg/plate.

9 The test article was completely soluble in the aqueous assay system at all concentrations
10 treated, in each of the experiments performed. Intense colouration caused by the test
11 article was observed on the test plates at 5000 µg/plate in Experiments 1 and 2.

12 Following R0027494A treatments of the test strain in the absence of S-9, no increases in
13 revertant numbers were observed that were ≥3-fold the concurrent vehicle control in strain
14 TA1537. This study was considered therefore to have provided no evidence of any
15 R0027494A mutagenic activity in this assay system.

16 17 *Conclusion*

18 It was concluded that R0027494A did not induce mutation in a histidine-requiring strain
19 (TA1537) of *Salmonella typhimurium* when tested under the conditions of this study. These
20 conditions included treatments at concentrations up to 5000 µg/plate (the maximum
21 recommended concentration according to current regulatory test guidelines, which was a
22 toxic concentration) in the absence of an endogenous metabolic activation system (S-9).

23
24 Ref: Lloyd (2019)

25 26 **SCCS comment**

27 The bacteriotoxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166), batch
28 R0027494A occurred at the highest tested concentration of 5000 µg/plate in the *S.*
29 *typhimurium* TA1537 strain.

30 31 32 ***In vitro* Mammalian Cell Gene Mutation Test in Mouse Lymphoma cells (*Hprt*-locus)**

33
34 Guideline: OECD 476 (1997)
35 Cells: L5178Y cells (MLA)
36 Replicates: duplicate cultures in three independent experiments
37 Test substance: Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
38 Batch: R0027494A 008 L 002
39 Purity: ~99.9% pure
40 Solvent: deionised water
41 Concentrations: Experiment 1 In the absence of S9 mix: 2.5, 5, 10, 20, 40, 60, 80, 100,
42 150 and 200 µg/mL (cytotoxicity: 18% relative survival at 200 µg/mL)
43 In the presence of S9 mix: 100, 200, 400, 800, 1200, 1600, 2000,
44 2400, 2800 and 3397 µg/mL (10% relative survival at 3397 µg/mL)
45 Experiment 2 In the absence of S9 mix: 10, 20, 40, 60, 80, 120, 160,
46 200 and 250 µg/mL (cytotoxicity: 17% relative survival at 250 µg/mL)
47 In the presence of S9 mix: 150, 300, 600, 1600, 2000, 2400, 2700,
48 3000 and 3397 µg/mL (16% relative survival at 3397 µg/mL)
49 Experiment 3 In the absence of S9 mix: 15, 30, 90 and 120 µg/mL
50 (cytotoxicity: 11% relative survival at 120 µg/mL)
51 Treatment: Experiments I and II: 3 h treatment both without and with S9-mix;
52 Expression period 7 days and a selection period of
53 11 days.
54 Experiment III: 3 h treatment without S9-mix;
55 Expression period 7 days and a selection period of
56 11 days.
57 GLP: in compliance

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 Study period: July 2012 – April 2013
2
3
4

5 *Test Procedure*

6 The test item Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was evaluated
7 in three independent experiments using duplicate cultures each (single cultures for positive
8 controls). The three experiments used a pulse (3-hour), two conducted in the absence and
9 presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial
10 fraction (S-9) and a third, confirmatory experiment (conducted in the absence of S-9 only)
11 to clarify the data obtained in Experiments 1 and 2. The test material was formulated in
12 purified water.
13

14 The ingredient Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was tested at
15 a broad range of concentrations. The concentrations were selected on the basis of
16 cytotoxicity criteria for final test statistics as follows:

17 Known mutagens in the presence (Benzo(a)pyrene, BP) or absence of S9 mix (4-
18 nitroquinoline 1-oxide, NQO) were tested at two different concentrations and served as
19 positive controls. Negative controls consisted of cultures treated with the solvent alone
20 (purified water).
21

22 *Results*

23 Mutation frequencies in solvent negative controls fell within normal ranges, and treatment
24 with positive controls NQO and BP yielded distinct increases in mutant frequency.
25 Accordingly, the study was considered to be valid.
26

27 In the absence of S-9 in Experiment 1, no significant increases in mutant frequency (MF)
28 were observed at any concentration analysed (up to a maximum of 200 µg/mL, limited by
29 toxicity) with no linear trend of MF. In Experiment 2, a statistically significant increase in MF
30 was observed only in the absence of S-9 at the highest cytotoxic concentration (250 µg/mL)
31 with a significant linear trend. This isolated increase in Experiment 2 in the absence of S-9
32 was not reproduced in Experiments 1 and 3 and is therefore considered of no biological
33 relevance.
34

35 Also in the presence of metabolic activation when tested up to the limit of cytotoxicity or
36 solubility, there were no relevant statistically significant increases in mutant frequency and
37 no significant linear trends following treatment with Methylimidazoliumpropyl p-
38 phenylenediamine, Cl, 2HCl (A166).
39

40 *Conclusion*

41 Under the conditions of this study, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
42 (A166) was considered not to be mutagenic in the mouse lymphoma assay (*hprt* locus),
43 neither in the absence nor presence of metabolic activation.
44

Ref: Loyd M (2013)

45
46 **SCCS comments**

47 In experiment 2, there was a statistically significant increase in mutant frequency with a
48 linear trend up to a concentration of 250 µg/mL without S9 mix, and a statistically
49 significant increase in mutant frequency without linear trend with S9 mix. In the
50 confirmation experiment 3, there was no treatment in the presence of S9-mix. Results from
51 the treatment in the absence of S9-mix do not meet the acceptance criteria, since only 3
52 concentrations were selected for the assessment of the study (up to 120 µg/mL). The
53 Applicant has claimed that this is due to high toxicity. However, the concentration of 175
54 µg/mL was still within the acceptable limits of cytotoxicity (around 10%) and the SCCS is of
55 opinion that it should have been included into the evaluation.
56
57

1
2 **In vitro Micronucleus Test in human lymphocytes**
3

4 Guideline: In compliance with the OECD draft guideline OECD 487 (2009)
5 Test system: human peripheral blood lymphocytes from two female volunteers.
6 Replicates: duplicate cultures in a single experiments with 2 exposure times
7 Test substance: Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
8 Batch: R0027494A 008 L 002
9 Purity: ~99.9% pure
10 Solvent: water
11 Concentrations: 500, 1500, 2500 and 3397 µg/mL (equivalent to 10 mM) in 3h-
12 treatment in the absence of S9-mix
13 500, 1500, 2500 and 3397 µg/mL (equivalent to 10 mM) in 3h-
14 treatment in the presence of S9-mix
15 3, 9, 18, and 30 µg/mL in 24h-treatment in the absence of S9 (limited
16 by cytotoxicity)
17 Treatment 48 h PHA (phytohaemagglutinin), 3 h treatment and 21 h recovery
18 without or with S9-mix
19 24 h PHA, 24 h treatment without S9-mix
20 GLP: in compliance
21 Study period: August 2012 – November 2012
22

23 *Test Procedure*

24 The test item Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) was evaluated
25 in the absence and presence of metabolic activation (S9 mix prepared from the livers of
26 Aroclor 1254-treated rats).
27

28 Duplicate cultures were treated with each concentration of Methylimidazoliumpropyl p-
29 phenylenediamine, Cl, 2HCl (A166) or with known clastogens in the presence
30 (cyclophosphamide, CPA) or absence of S9 (mitomycin C, MMC and vinblastine, VIN).
31 Solvent-treated cultures (purified water, four replicates) were used as negative controls.
32 Cytochalasin B was added after the 3-hour treatments or before the 24-hour treatments
33 The test article was formulated in water for irrigation (purified water). Cytotoxicity was
34 determined in a preliminary cytotoxicity Range-Finder Experiment. The highest
35 concentrations tested was 3397 µg/mL for the 3+21 hour treatments in the absence and
36 presence of S-9 (equivalent to 10 mM) and 80 µg/mL for the 24+0 hour treatment in the
37 absence of S-9 (limited by toxicity)
38

39 *Results*

40 When compared to concurrent solvent controls, treatment of cultures with positive controls
41 CPA, MMC and VIN resulted in consistent significant increases in MNBN frequencies, thus
42 validating the sensitivity of the test system and procedure used.
43 Treatment of cells Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) in the
44 absence and presence of S9-mix resulted in frequencies of MNBN cells, which were similar
45 to those observed in concurrent vehicle controls for all concentrations analyzed under all
46 treatment conditions.
47

48 *Conclusion*

49 Under the conditions of the study, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
50 (A166) did not produce micronuclei in cultured human peripheral blood lymphocytes neither
51 in the absence nor presence of metabolic activation and was therefore considered to have
52 no clastogenic or aneugenic potential.
53

Ref: Watters G (2012)

54
55 **SCCS overall comments**

56 The genotoxic potential of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
57 was evaluated in the required battery of *in vitro* studies (Submission I, July 2017) including:

Opinion on hair dye Methylimidazoliumpropyl p-phenylenediamine HCl (A166)

1 the bacterial reverse mutation test, a mammalian cell gene mutation test in mouse
2 lymphoma cells (*Hprt* locus) and a micronucleus assay in cultured human lymphocytes.
3 The bacterial gene mutation test was negative. However, bacteriotoxicity was noted mainly
4 in the strain TA1537 in the absence of S9-mix even in the lowest concentrations.
5 Although the study on the gene mutation in mammalian cells (mouse lymphoma cells, *Hprt*
6 locus) raised some concern, the SCCS considered it negative. In the micronucleus test *in*
7 *vitro*, Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) did not produce
8 micronuclei in cultured human peripheral blood lymphocytes either in the absence or in the
9 presence of metabolic activation and it was therefore considered to have no clastogenic or
10 aneugenic potential.
11 Following the Applicant's submission of a safety dossier on the new hair dye ingredient
12 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) in July 2017, several
13 information exchanges occurred between SCCS and the Applicant, mainly pertaining to the
14 genetic toxicity profile of this new hair dye ingredient.
15 Following a request from the SCCS, the Applicant conducted a complementary bacterial
16 reverse mutation assay (Ames test) on strain TA 1537. The results of this new study
17 confirmed bacteriotoxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
18 in *S. typhimurium* TA1537 strain but only at the highest tested concentration of 5000
19 µg/plate.
20 Based on the analysis of all the studies submitted, the SCCS considered
21 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) as not inducing gene
22 mutations in bacteria and mammalian cells or clastogenic/aneugenic effects in mammalian
23 cells in tested conditions.
24

25 **3.3.1.2 Mutagenicity / genotoxicity *in vivo***

26 /
27 /
28

29 **3.3.8 Carcinogenicity**

30 /
31 /
32

33 **3.3.9 Photo-induced toxicity**

34 /
35 /
36

37 **3.3.10 Human data**

38 /
39 /
40

41 **3.3.11 Special investigations**

42 /
43 /
44 /
45 /
46 /
47 /
48 /
49 /
50

3.3.12 Safety evaluation (including calculation of the MoS)**CALCULATION OF THE MARGIN OF SAFETY**

(on-head concentrations of up to 2%)

| | | | |
|---|----------------------|---|--------------------------------|
| Absorption through the skin | A | = | 1.89 $\mu\text{g}/\text{cm}^2$ |
| Skin Area surface | SAS | = | 580 cm^2 |
| Dermal absorption per treatment | SAS x A x 0.001 | = | 1.10 mg |
| Typical body weight of human | bw | = | 60 kg |
| Systemic exposure dose (SED) | SAS x A x 0.001/bw | = | 0.0183mg/kg |
| No observed adverse effect level (developmental toxicity, oral, rat) | NOAEL _{sys} | = | 100 mg/kg bw/d |
| Bioavailability 50%* | | = | 50 mg/kg bw/d |

Margin of Safety **NOAEL_{sys}/SED** = **2732**

* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

3.3.13 Discussion***Physicochemical properties*****Impurities**

Physicochemical data indicate the presence of six impurities and five residual solvents.

In the absence of experimental data, the SCCS has calculated the potential risk of the presence of impurities and residual solvents according to the TTC principles. This has indicated that the levels of one of the impurities (3-aminopropyl imidazole) and a residual solvent (chloroethane) are above the TTC levels that can be considered safe.

The impurity, 4-aminophenylamine dihydrochloride (synonym p-phenylenediamine dihydrochloride), has already been the subject of an SCCS Opinion (SCCS/1443/11 Revision of 18 September 2012), which raised concerns over its strong skin sensitising potency.

Stability

The information provided on stability of A166 is not convincing. The use of ascorbic acid and argon for the shaking flask method, and the storage conditions (4 ± 4 °C, in the dark under argon), indicates that the test substance is easily oxidised. The Applicant has provided further information on the stability studies performed for the dose formulation used for toxicity studies, where the dissolution media is distilled water. However, details on the solvent media and the procedure used for preparation of solutions are not fully described in the report.

It is necessary to provide data on the stability of A166 under conditions of use with a selective analytical method. Liquid scintillation counting is not a selective method for this purpose. The stability study was performed before mixing the test substance with the developer, whereas it should be performed after the mixing with the developer. Any degradation products should also be chemically characterised.

1
2 **Toxicological Evaluation**
3
4

5 **Acute toxicity**

6 The acute oral toxicity of Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166)
7 was evaluated following single administration to fasted rats at 500 mg/kg. Animals were
8 observed until day 8 instead of for 14 days, but since no mortality was registered, it can be
9 deduced that the oral LD50 is higher than 500 mg/kg b.w.

10
11
12 **Irritation and corrosivity**

13 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) at the concentration of
14 10%(w/w) in water applied on reconstructed human epidermis model is considered to be
15 non-irritant to the skin.

16 A mild to moderate eye irritation potential of the test item cannot be excluded.
17

18
19 **Skin sensitisation**

20 Based on the EC3 value of 1.23% observed in Local Lymph Node Assay (LLNA) in mice,
21 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) is considered as a strong
22 skin sensitiser.
23

24
25 **Toxicokinetics**

26 The percutaneous absorption of 2% Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl
27 (A166) was investigated *in vitro* in a oxidative hair dye formulation, in the presence of
28 hydrogen peroxide by using human skin preparations. The mean + 1 SD of dermal
29 absorption value, i.e. 1.89 µg/cm² (0.48%), will be used for MoS calculation.
30

31 A moderate permeability observed in this study supports the default value of 50% for oral
32 bioavailability, which will be used for the calculation of the MoS.
33

34
35 **Repeated dose toxicity**

36 Based on limited adverse effects (changes in clinical chemistry and haematology at the
37 highest dose) observed after Repeated dose 90-day oral toxicity study of R0027494 in
38 wistar rats, the SCCS derives a NOAEL of 300 mg/kg bw/d.
39

40
41 **Reproductive toxicity**

42 Methylimidazoliumpropyl phenylenediamine, Cl, 2HCl (A166) is not teratogenic in rats.
43 NOAEL for developmental toxicity is 1000 mg/kg b.w.

44 NOAEL for maternal toxicity is 100 mg/kg b.w. due to changes in lethargy at 300 and 1000
45 mg/kg b.w. and weakness, hypoaesthesia and somnolence and decreased mean body weight
46 gain and mean percent body weight and of absolute uterine weight and uterine weight
47 corrected to body weight at 1000 mg/kg b.w.
48

49
50 **Mutagenicity / genotoxicity**

51 Based on the analysis of all the *in vitro* studies submitted, the SCCS considered
52 Methylimidazoliumpropyl p-phenylenediamine, Cl, 2HCl (A166) as not inducing gene
53 mutations in bacteria and mammalian cells or clastogenic/aneugenic effects in mammalian
54 cells in tested conditions.
55
56
57
58

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

4. CONCLUSION

- 1) *In light of the data provided, does the SCCS consider Methylimidazoliumpropyl p-phenylenediamine HCl (A166), safe when used in oxidative hair colouring products up to a maximum on-head concentration of 2 %?*

Although evaluation of the data provided for Methylimidazoliumpropyl p-phenylenediamine HCl (A166) has shown it to be safe, no safety data have been provided for the impurities and the residual solvents that are also present. Analysis of the potential risk carried out by the SCCS according to TTC principles has indicated that the levels of one of the impurities (3-aminopropyl imidazole) and a residual solvent (chloroethane) are above the TTC levels that can be considered safe. Therefore, the SCCS cannot conclude on the safety of Methylimidazoliumpropyl p-phenylenediamine HCl (A166) unless its purity is improved to bring the levels of the impurity and the residual solvent down to below the acceptable TTC levels.

- 2) *Does the SCCS have any further scientific concerns with regard to the use of Methylimidazoliumpropyl p-phenylenediamine HCl (A166) in cosmetic products?*

The SCCS has noted that Methylimidazoliumpropyl p-phenylenediamine HCl (A166) is a strong skin sensitiser.

5. MINORITY OPINION

/

1
2 **6. REFERENCES**

3
4 Brémond C. (2011). Primary cutaneous tolerance – Prediction of the acute irritant potential
5 on human reconstructed epidermis EpiskinSM model. R0027494A. Episkin Study No 11-BPL-
6 1061

7
8 Brémond C. (2012). R0027494A: Primary Cutaneous Tolerance. Prediction of the acute
9 irritant potential on human reconstructed epidermis EpiskinSM model. Episkin Study No. 11-
10 BPL-1061.

11
12 Cannamela N. (2011). Ocular primary irritation B.C.O.P. – Study performed on the isolated
13 bovine cornea measurement of the bovine corneal opacity and permeability. R0027494A.
14 I.E.C. France Study No. 111555RD

15
16 Cannamela N (2015). Ocular primary irritation B.C.O.P. – Study Performed on the Isolated
17 Bovine Cornea. Measurement of the Bovine Corneal Opacity and Permeability. IEC Report
18 No 150044RD

19
20 De Jouffrey S. (1997). Acute Oral Toxicity Study in Rats. C16778. CIT Study No. 15726 TAR

21
22 Gohel D. (2014). Repeated dose 90-Day oral toxicity study of R0027494A in Wistar rats. Vol
23 I and II. JRF Study No. 443-01-03-5653

24
25 Hobson S. (2013). Reverse mutation in five histidine-requiring strains of *Salmonella*
26 *typhimurium*. R0027494A. Covance Study No. 8268088

27
28 Lloyd M. (2013). Mutation at the *hprt* locus of mouse lymphoma L5178Y cells (MLA) using
29 the Microtitre^R fluctuation technique. R0027494A. Covance Study No. 8268089

30
31 Lloyd M. (2019). R0027494A: Bacterial Reverse Mutation Assay. Covance Study No.
32 8401295.

33
34 Lee S. (2015). Study to investigate the permeability of the test compound, R0027494A, in
35 Caco-2 cells. Cyprotex study No CY0775-R7

36
37 Patel MV. (2014). Prenatal Developmental Oral Toxicity Study in Rats of R0027494A. JRF
38 Study No. 461-1-04-5654

39
40 Raithatha A. (2013). Validation of analytical method for determination of R0027494A active
41 ingredient concentration in dose formulation. JRF Study Number:228-2-13-5666. February
42 4, 2013.

43
44 Thybaud *et al.* (2007). Strategy for genotoxicity testing: Hazard identification and risk
45 assessment in relation to *in vitro* testing. Mutation Research 627:41-58

46
47 Toner F. (2014). R0027494A *In Vitro* Percutaneous Absorption of Radiolabelled R0027494A
48 Through Human Skin. Charles River Study No. 35246

49
50 Ujawane D. (2013). Repeated Dose 14-day Oral Toxicity Study of R0027494A in Wistar
51 Rats. JRF Study No. 410-1-03-4691

52
53 Verma R. (2012). Skin Sensitization study of R0027494A by Local Lymph Node Assay
54 (LLNA). JRF Study No. 409-1-01-4299

55

1 Watters G. (2012). Induction of micronuclei in cultured human peripheral blood
2 lymphocytes. R0027494A. Covance Study No. 8268090

3
4 Wöhr T. (2019). Imexine OBL: Determination of the Water Solubility. Ibacon Study
5 Number: 142281185. March, 15, 2019

6
7

8 **7. GLOSSARY OF TERMS**

9
10 See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of
11 Cosmetic Ingredients and their Safety Evaluation – from page 141

12
13

14 **8. LIST OF ABBREVIATIONS**

15
16 See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of
17 Cosmetic Ingredients and their Safety Evaluation – from page 141

18