EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

# MEMORANDUM on

# the *in vitro* test EPISKIN™ for skin irritation testing

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 14th plenary on 18 December 2007

About the Scientific Committees

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

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

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

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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## 1. ESAC* STATEMENT ON THE VALIDITY OF EPISKIN™ FOR SKIN IRRITATION

At its 26th meeting, held on 26-27 April 2007, the ESAC made the following statement on the validity of *in vitro* tests for skin irritation:
"The EPISKIN™ method is considered to be a reliable and relevant stand-alone test for predicting rabbit skin irritation, when the endpoint is evaluated by MTT† reduction, and for being used as a replacement for the Draize skin irritation test (OECD TG 404 & Method B.4 of Annex V to Directive 67/548/EEC) for the purposes of distinguishing between R38 skin irritating and non-skin irritating substances."

A second endpoint, IL-1α‡, was found to increase the sensitivity of the test, without reducing its specificity. This endpoint is advised to be used to confirm negatives obtained with the MTT endpoint (*http://ecvam.jrc.it/index.htm*, consulted November 2007).

## 2. DISCUSSION ON THE VALIDITY OF EPISKIN™ FOR COSMETIC INGREDIENTS

The release of the *in vitro* EPISKIN™ method is highly welcomed since it is urgently needed to limit the number of experimental animals used not only in assessing the safety of chemicals in general, but more specifically, for the safety assessment of cosmetics and their ingredients. In its recent memorandum on the actual status of alternative methods on the use of experimental animals in the safety assessment of cosmetic ingredients in the European Union (SCCP/1111/07§), the SCCP pointed to the importance and urgency of validated replacement alternative methods for all toxicological endpoints needed to guarantee the safety of cosmetic ingredients and finished cosmetic products for the consumer (SCCP/1005/06).

Indeed, the availability of validated replacement alternatives becomes key because of the testing and marketing ban taken up in the EU cosmetic legislation (76/768/EEC and 2003/15/EC). The testing ban on ingredients or combination of ingredients will apply step by step as soon as alternative methods are validated and adopted, but with a maximum cut-off date of 6 years after entry into force of the Directive, i.e., 11 March 2009, irrespective of the availability of alternative non-animal tests. The marketing ban will apply step by step as soon as alternative methods are validated and adopted in EU legislation with due regard to the OECD validation process. This marketing ban will be introduced at the latest 6 years after entry into force of the Directive, i.e., 11 March 2009, for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity and toxicokinetics.

For these specific health effects, a deadline of 10 years after entry into force of the Directive is foreseen, i.e., 11 March 2013, irrespective of the availability of alternative non-animal tests.

As one of the tasks of the SCCP is giving advice to the Commission about the applicability of validated alternative methods to the field of cosmetic products (2003/15/EC), a review took place of the background information leading to the positive ESAC opinion on EPISKIN™. The inter- and intra-laboratory relevance and reproducibility were assessed by making use of a set of 60 coded test chemicals for which high quality animal data were available. These

* ESAC = ECVAM Scientific Advisory Committee
† 3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide
‡ Interleukin 1-α
§ Available through *http://ec.europa.eu/health/ph_risk/committees/04_sccp/sccp_statements_en.htm* (consulted November 2007)

chemicals were selected from the ECETOC[**] and TSCA[††] (USA) databases and are listed in Table 1.

**Table 1:** Selected test chemicals for the EPISKIN™ validation (substances found in the coming update of the cosmetic ingredients list are indicated with *)

| Chemical name | CAS number [‡‡] | EU class. | GHS[§§] class. |
|---|---|---|---|
| 1-bromo-4-chlorobutane | 6940-78-9 | none | none |
| 3-diethylaminopropionitrile | 1260545 | none | none |
| diethyl phthalate * | 84-66-2 | none | none |
| di-propylene glycol * | 25265-71-8 | none | none |
| dipropylene glycol monobutyl ether | 29911-28-2 | none | none |
| naphthalene acetic acid | 86-87-3 | none | none |
| silane A-1430 | 2530-87-2 | none | none |
| triethylene glycol * | 112-27-6 | none | none |
| allyl phenoxyacetate * | 7493-74-5 | none | none |
| isopropanol * | 67-63-0 | none | none |
| 4-methylthio-benzaldehyde | 3446-89-7 | none | none |
| methyl stearate * | 112-61-8 | none | none |
| Phenylethylalcohol * | 60-12-8 | none | none |
| 3-chloro-4-fluoronitrobenzene | 350-30-1 | none | none |
| allyl heptanoate * | 142-19-8 | none | Cat. 3 |
| heptyl butyrate * | 5870-93-9 | none | Cat. 3 |
| hexyl salicylate * | 6259-76-3 | R38 | Cat. 3 |
| terpinyl acetate * | 80-26-2 | R38 | Cat. 3 |
| tri-isobutyl phosphate | 126-71-6 | R38 | Cat. 3 |
| 1-decanol * | 112-30-1 | R38 | Cat. 2 |
| cyclamen aldehyde * | 103-95-7 | R38 | Cat. 2 |
| 1-bromohexane | 111-25-1 | R38 | Cat. 2 |
| a-terpineol * | 98-55-5 | R38 | Cat. 2 |
| butyl methacrylate * | 97-88-1 | R38 | Cat. 2 |
| di-n-propyl disulphide | 629-19-6 | R38 | Cat. 2 |
| 2,6-dimethyl-4-nitrobenzeneamine | 16947-63-0 | none | none |
| 2S-(2-furyl)-5R-hydroxy-4R-(1R,2-dihydroxy)ethyl-6S-hydroxymethyl-1,3-dioxane | 7089-59-0 | none | none |
| Mixture of isomers:<br>1-(spiro[4.5]dec-7-en-7-yl)pent-4-en-1-one (CAS# 224031-70-3)<br>1-(spiro[4.5]dec-6-en-7-yl)pent-4-en-1-one (CAS# 224031-71-4) | 224031-70-3 | none | none |
| propyl (2S)-2-(1,1-dimethylpropoxy)-propanoate | 0319002-92-1 | none | none |
| ethyl cis-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine-1-carboxylate | 67914-69-6 | none | none |
| disodium 2,2'-(1,4-phenylene)bis-(1H-benzimidazole-4,6-disulfonic acid or monosulfonic acid, monosulfonate or disulfonate * | 180898-37-7 | none | none |
| cyclohexadecanone | 2550-52-9 | none | none |
| Mixture of:<br>diethyl cis-1,4-cyclohexanedicarboxylate;<br>diethyl trans-1,4-cyclohexanedicarboxylate | 0072903-27-6 | none | none |
| 2-ethylhexyl 4-aminobenzoate | 26218-04-2 | none | none |
| capryl-isostearate | 209802-43-7 | none | none |
| 2,5-dimethyl-4-oxo-4,5-dihydrofuran-3-yl acetate | 4166-20-5 | none | none |
| 3-mercaptohexanol | 51755-83-0 | none | none |

[**] European Centre for Ecotoxicology and Toxicology of Chemicals
[††] Toxic Substances Control Act
[‡‡] Chemical Abstracts Service registry number
[§§] Globally Harmonized System

| Chemical name | CAS number ‡‡ | EU class. | GHS§§ class. |
|---|---|---|---|
| 3,4-dimethyl-1H-pyrazole | 2820-37-3 | none | none |
| 2-(formylamino)-3-thiophenecarboxylic acid | 43028-69-9 | none | none |
| A mixture of:<br>(E)-oxacyclohexadec-12-en-2-one; (E)-oxacyclohexadec-13-en-2-one;<br>a) (Z)-oxacyclohexadec-12-en-2-one and b) (Z)-oxacyclohexadec-13-en-2-one [a] | not allocated | none | none |
| 2-phenylhexanenitrile | 3508-98-3 | none | Cat. 3 |
| 2-methyl-3-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]-1-propanol, bornyl isomer * | 128119-70-0 | none | Cat. 3 |
| A mixture of:<br>5-exo-decylbicyclo[2.2.1]hept-2-ene;<br>5-endo-decylbicyclo[2.2.1]hept-2-ene | 22094-85-5 | none | Cat. 3 |
| 1-[4-(2-dimethylaminoethoxy)phenyl]-2-phenylbutan-1-one | 68047-07-4 | R38 | Cat. 3 |
| Mixture of isomers:<br>1-(2-isopropylphenyl)-1-phenylethane (CAS# 191044-60-7)<br>1-(3-isopropylphenyl)-1-phenylethane (CAS# 191044-59-4)<br>1-(4-isopropylphenyl)-1-phenylethane (CAS# 2320-06-1) | 52783-21-8 | R38 | Cat. 3 |
| Mixture of:<br>2-methyl-4-(2',2',3'-trimethyl-3'-cyclopenten-1'-yl)-4-penten-1-ol<br>56% (1'R,2R) & 40%(1'R,2S) isomer | 014864-90-6 | R38 | Cat. 3 |
| isostearic acid monoisopropanolamide * | 152848-22-1 | R38 | Cat. 3 |
| A mixture of isomers:<br>ethyl exo-tricyclo[5.2.1.0(2,6)]decane-endo-2-carboxylate;<br>ethyl endo-tricyclo[5.2.1.0(2,6)]decane-exo-2-carboxylate * | 80657-64-3 | R38 | Cat. 3 |
| 4-methyl-8-methylenetricyclo[3.3.1.1(3,7)]decan-2-ol * | 122760-84-3 | R38 | Cat. 3 |
| 4-methyl-8-methylenetricyclo[3.3.1.1(3,7)]dec-2-yl acetate * | 122760-85-4 | R38 | Cat. 3 |
| bis[(1-methylimidazol)-(2-ethyl-hexanoate)], zinc complex | not allocated | R38 | Cat. 3 |
| A mixture of isomers:<br>1-(1,1-dimethylpropyl)-4-ethoxy-cis-cyclohexane;<br>1-(1,1-dimethylpropyl)-4-ethoxy-trans-cyclohexane | 181258-87-7 (cis)<br>181258-89-9 (trans) | R38 | Cat. 3 |
| diisononyl cyclohexane-1,2-dicarboxylate [a] | 166412-78-8 | R38 | Cat. 3 |
| (E,E)-3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol | 125474-34-2 | R38 | Cat. 2 |
| [2-(cyclopentyloxy)ethyl]benzene(cyclopentyl 2-phenylethyl ether) | not allocated | R38 | Cat. 2 |
| benzenethiol, 5-(1,1-dimethylethyl)-2-methyl | 7340-90-1 | R38 | Cat. 2 |
| 1-methyl-3-phenyl-1-piperazine | 5271-27-2 | R38 | Cat. 2 |
| 2-chloromethyl-3,5-dimethyl-4-methoxypyridine hydrochloride | 86604-75-3 | R38 | Cat. 2 |
| (+/-) trans-3,3-dimethyl-5-(2,2,3-trimethyl-cyclopent-3-en-1-yl)-pent-4-en-2-ol * | 107898-54-4 | R38 | Cat. 2 |
| 2-isopropyl-2-isobutyl-1,3-dimethoxypropane | 129228-21-3 | R38 | Cat. 2 |

Careful examination of this list reveals that only two of the chemicals have been part of a former SCC(NF)P dossier. The first one is the UV-filter 'Monosodium salt of 2-2'-bis-(1,4-phenylene) 1H-benzimidazole-4,6-disulphonic acid', present on Annex VII, Reference nr.24 and discussed by the SCCNFP in 1999 (SCCNFP 1999). The other compound is diethyl phthalate, discussed in 2002 and 2003, but for other toxicological endpoints than skin irritation (SCCNFP/0411/01, SCCNFP/0767/03). As the SCCP (as was the former SCCNFP) is responsible for the safety assessment of the cosmetic ingredients listed in the Annexes of Directive 76/768/EEC, being Annex II (forbidden substances), Annex III (restricted substances), Annex IV (colorants), Annex VI (preservatives) and Annex VII (UV filters), the availability of more data on substances present on these Annexes and tested with EPISKIN™, seems necessary to fully support the implementation of this *in vitro* methodology in the safety assessment process carried out by the SCCP.

The request for additional data to support the applicability domain of a replacement test is not new. An extra study was also requested in the past by the SCCNFP on the occasion of the establishment of the *in vitro* phototoxicity test (SCCNFP 1998). Consequently, a study was run on the UV filters present in Annex VII, applying the *in vitro* phototoxicity test to all these UV filters for which a favourable safety opinion was already expressed earlier by the SCCNFP. The study was successful (Spielmann et al. 1998) and the methodology was endorsed by the SCCNFP and subsequently taken up in the Notes of Guidance.

With regard to the EPISKIN™ method currently under study, the SCCP acknowledges that the list of substances displayed in Table 1 contains several cosmetic ingredients. Unfortunately, the vast majority of them are, as mentioned before, not present on the Annexes to Dir. 76/768/EEC. The SCCP considers this as a missed opportunity. Although cosmetic ingredients are expected to be mostly non-irritating, it can be extracted from the publicly available SCC(NF)P reports[***] issued between 2000 and 2006 (in total 182 opinions), that *in vivo* irritation data exist for 112 substances, either tested in their undiluted form or at foreseen in-use concentrations or other dilutions. The majority of the test substances (75) was indeed found non-irritating, whereas 23 compounds were called "slightly" or "mildly" irritating, and 11 were considered "irritating" or "moderately irritating". One was "severely irritating", one was indecisive and one was even corrosive (Pauwels et Rogiers 2007). Since many of the test compounds belonged to the category of hair dyes, colouration of the animals' skin frequently interfered with the visual scoring.

For one particular substance, the skin colouration caused by the tested substance even rendered the scoring impossible.
As colouration of the skin seems to be a commonly occurring problem during the *in vivo* skin irritation testing of hair dyes and colorants (occurring in 17 of the 112 cases examined), the question could be raised whether this problem can be adequately addressed in the EPISKIN™ protocol. Considering that the endpoint of the test is MTT reduction, in which colour formation is essential, it seems necessary to collect some data on the proposed *in vitro* skin irritation testing of hair dyes and colorants. Moreover, in a scientific publication on the use of the EPISKIN™ model for the assessment of *in vitro* phototoxicity, the authors describe that dyes can hardly be evaluated with the MTT colorimetric method (Lelièvre et al. 2007).

Finally, the fact that the barrier of *in vitro* skin models is less developed than the *in vivo* barrier (Poumay et Coquette 2007) could have potential consequences for the dermal absorption of colorants/hair dyes and thus also interfere with colouration of the skin model.
These aspects require special attention in any further studies.

## 3. STATEMENT OF THE SCCP

The SCCP welcomes the availability of the validated EPISKIN™ method, which is a replacement alternative highly needed for the animal-free assessment of skin irritation of cosmetic ingredients. As only one substance of the test set used for validation, however, is present on the Annexes to Dir. 76/768/EEC and as no information is available to the SCCP with respect to colouration effects, potentially important in the case of colorants and hair dyes, the SCCP is of the opinion that additional data are necessary to fully support the EPISKIN™ method for the safety assessment of cosmetic ingredients present on the Annexes of Directive 76/768/EEC. The problem of dyes interfering with the MTT colorimetric method was already mentioned in a study assessing the applicability of the EPISKIN™ model for phototoxicity testing (Lelièvre et al. 2007).

[***] http://ec.europa.eu/health/ph_risk/committees/sccp/sccp_opinions_en.htm (consulted November 2007)
http://ec.europa.eu/health/ph_risk/committees/04_sccp/sccp_opinions_en.htm (consulted November 2007)

Therefore additional work specifically addressing substances present on the Annexes would be highly welcomed.
For a number of cosmetic ingredients present in the annexes, data have recently[†††] been received and are presently under review, while further information on coloured substances has been announced. Should review of these data reveal remaining knowledge gaps, an additional set of compounds could be determined based on the *in vivo* skin irritation data described in the publicly available SCC(NF)P opinions and stored in a databank accessible to the SCCP, and included in an additional validation step of the EPISKIN™ method for cosmetic ingredients present on the Annexes, should this be necessary. The SCCP offers its help for such an additional study and would welcome any information that sectors of industry may already have and be useful as part of the evaluation.

## 4. REFERENCES

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SCCNFP/0411/01 - Opinion of the Scientific Committee on Cosmetic products and Non-Food Products intended for consumers on Diethyl Phthalate, adopted during the 20th plenary meeting of 04 June 2002.

SCCNFP/0767/03 - Opinion of the Scientific Committee on Cosmetic products and Non-Food Products intended for consumers concerning Diethyl phthalate, adopted during the 26th plenary meeting of 9 December 2003.

SCCP/1005/06: The SCCP's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, adopted by the SCCP during the 10th plenary meeting of 19 December 2006

SCCP/1111/07: Memorandum on the Actual Status of Alternative Methods on the use of Experimental Animals in the Safety Assessment of Cosmetic Ingredients in the European Union, adopted by the SCCP during the 12th plenary on 19 June 2007

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[†††] Received on 17 December 2007; data included results on 5 in house-synthetized colourants Received on 18 December 2007;data included results on 15 regulated cosmetic ingredients (UV-filters, preservatives and "other purpose " ingredients; no colourants)