



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (sensitisation only)

Adopted by the SCCP
during the 2nd plenary meeting of 7 December 2004

TABLE OF CONTENTS

1.	BACKGROUND	3
2.	TERMS OF REFERENCE	3
3.	OPINION	3
4.	CONCLUSION	20
5.	MINORITY OPINION	20
6.	REFERENCES	20
7.	ACKNOWLEDGEMENTS	23

1. BACKGROUND

4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-carboxaldehyde (HMPCC) is used as a fragrance ingredient in cosmetic products. It is not regulated in an annex to the Cosmetics Directive.

On 9 December, 2003 the SCCNFP¹ adopted opinion SCCNFP/0743/03 on HMPCC stating that the current use levels of HMPCC have both caused the induction and elicitation of contact allergy to it. Further it stated that based on the information presently available, a concentration of up to 0.02 % in a finished cosmetic product would have a low potential to *induce* sensitisation, or *elicit* allergic contact reactions in those consumers already sensitised to this fragrance chemical.

Since the opinion was adopted, the European Commission has received a submission on HMPCC from industry with regard to its sensitisation potential proposing a NOEL (no observed effect level) for an induction-based risk assessment. It has also received a submission from COLIPA (European Cosmetics Toiletry and Perfumery Association) concerning the data requested by the SCCNFP.

2. TERMS OF REFERENCE

On the basis of currently available information, the SCCP is asked to review and if necessary revise the opinion of the SCCNFP of 9 December 2003, as concerns:

- induction of sensitisation in consumers
- elicitation of contact allergic reactions in previously sensitised consumers.

Does the SCCP recommend any further restrictions with regard to the use of 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde as a fragrance in cosmetic products?

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name
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Hydroxyisohexyl 3-cyclohexene carboxaldehyde (INCI name)

¹ SCCNFP - Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers

3.1.1.2. Chemical names

Lyral® is a mixture of 2 isomers, namely:

4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde and 3-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde

The chemical composition of the 2 isomers is variable.

3.1.1.3. Trade names and abbreviations

Lyral®; Kovanol®; HMPCC

3.1.1.4. CAS / EINECS number

4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde

CAS : 31906-04-4

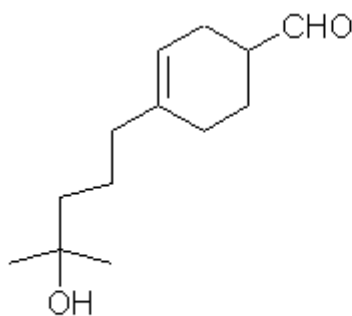
EINECS : 250-863-4

3-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde

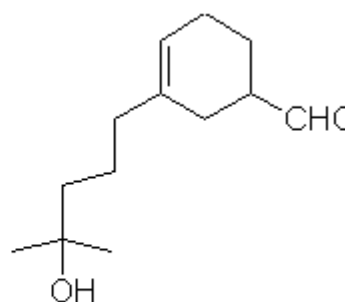
CAS : 51414-25-6

EINECS : 257-187-9

3.1.1.5. Structural formula



Major



Minor

HMPCC isomer ratio is approximately 2:1. The major isomer is the 1,4-disubstituted cyclohexenyl compound and the minor is the 1,3-disubstituted.

3.1.1.6. Empirical formula

Formula : C₁₃H₂₂O₂

3.1.2. Physical form

A colourless viscous liquid

3.1.3. Molecular weight

Molecular weight : 210.32

3.1.4. Purity, composition and substance codes

Minimum 98%

3.1.5. Impurities / accompanying contaminants

No data submitted

3.1.6. Solubility

184.6 mg/l at 25 °C

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow} : /

3.1.8. Additional physical and chemical specifications

Organoleptic properties	:	colourless liquid with a sweet, light and floral odour
Melting point	:	/
Boiling point	:	280 °C
Flash point	:	> 100 °C (closed cup)
Vapour pressure	:	/
Density	:	0.994
Viscosity	:	/
pKa	:	/
Refractive index	:	1.490

3.2. Function and uses

HMPCC is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its worldwide use is in the region of 1000 metric tonnes per annum.

The determinant factors for fragrance exposure are quantities of cosmetic used, frequency of use, and concentration of the fragrance material in these products (Ford *et al.*, 2000). Using these factors, the total maximum exposure to HMPCC has been calculated from ten types of cosmetic products (See Table 1). For consideration of potential sensitization the exposure is calculated as a percent concentration used on the skin. Thus the maximum fragrance level in formulae that go onto the skin has been reported to be 3.35% (IFRA, 1998), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 9.529% (IFRA, 1998), which would result in a

maximum daily exposure on the skin of 0.24 mg/kg for high end users of these products (See Table 1). Exposure data are provided by the fragrance industry. An explanation of how the data are obtained and how exposure was determined has been reported by Cadby *et al.* (2002) and Ford *et al.* (2000).

Table 1: Calculation of the total human skin exposure from the use of multiple cosmetic products containing HMPCC.

Type of Cosmetic Product	Grams Applied	Applications per day	Retention Factor	Mixture/Product %	Ingredient/Mixture ¹	Ingredient mg/kg/day ²
Body lotion	8.00	1.00	1.000	0.004	9.529	0.051
Face cream	0.80	2.00	1.000	0.003	9.529	0.0076
Eau de toilette	0.75	1.00	1.000	0.080	9.529	0.0953
Fragrance cream	5.00	0.29	1.000	0.040	9.529	0.0921
Antiperspirant	0.50	1.00	1.000	0.010	9.529	0.0079
Shampoo	8.00	1.00	0.10	0.005	9.529	0.0064
Bath products	17.00	0.29	0.01	0.020	9.529	0.0016
Shower gel	5.00	2.00	0.10	0.012	9.529	0.0191
Toilet soap	0.80	6.00	0.10	0.015	9.529	0.0114
Hair spray	5.00	2.00	0.10	0.005	9.529	0.0079
Total						0.2527

¹ Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products

² Based on a 60-kilogram adult

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

/

3.3.1.2. Acute dermal toxicity

/

3.3.1.3. Acute inhalation toxicity

/

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

/

3.3.2.2. Mucous membrane irritation

/

3.3.3. Skin sensitisation

Human Predictive (induction) Studies

Table 2: Summary of human skin sensitization studies with HMPCC

Test Method	Test Concentration	Dose/unit area ($\mu\text{g}/\text{cm}^2$)	Results	References
HRIPT	5.3% in 75% DEP/25% alcohol	4000	No reactions (0/201)	RIFM, 2003
HRIPT	15% in 75% alcohol/ 25% DEP	8264	No reactions (1/109)	RIFM, 1999a
HRIPT	5% in 95% ethanol	3876	No reactions (0/39)	RIFM, 1964a
HRIPT	5% in 95% ethanol	3876	No reactions (0/38)	RIFM, 1964b
HRIPT	1% in water	NA	No reactions (0/50)	RIFM, 1958
MAX	10% in petrolatum	6896	No reactions (0/25)	RIFM, 1977

A human repeated insult patch test was conducted with HMPCC on 201 volunteers (54 males and 147 females). A 0.3 ml dose of a 5.3% solution of HMPCC in 75% DEP/25% alcohol was applied to a webriil/adhesive patch (Kendall Healthcare Products Company Patch # 4022) resulting in a dose/unit area of $4000 \mu\text{g}/\text{cm}^2$. The test material was applied to each designated patch approximately 10-20 minutes prior to application of the patch to the designated test site. The patches were then applied to the back under occlusion. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Reactions were read 1-2 days after patch removal just prior to application of the next patch. Reactions were scored according to the modified scoring scale of the ICDRG (Fisher, 1986). Patches were applied three times a week, on a Monday-Wednesday-Friday schedule. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, an occluded challenge patch was applied to a site not previously exposed and removed after 1 day. Reactions to challenge were read at patch removal and 1, 2 and 3 days after patch removal. No reactions were observed (RIFM, 2003).

Ref.: 41

Another human repeated insult patch test was conducted with HMPCC on 109 volunteers (18 males and 91 females). A 0.2 ml dose of a 15% solution of HMPCC in 75% alcohol SD39C/25%

DEP was applied to a 3.63 cm² area patch (equivalent to a dose/unit area of 8264 µg/cm²), which consisted of a 1.9 cm x 1.9 cm gauze square on an adhesive dressing – Manufactured by TruMed Technologies, Inc., Burnsville, MN, and allowed to volatilize for approximately 30 minutes. The patches were then applied to the upper back under occlusion. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week. Reactions were read 1-2 days after patch removal just prior to application of the next patch. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, an occluded challenge patch was applied to a site not previously exposed and removed after 1 day. Reactions to challenge were read at patch removal and 1 and 2 days after patch removal. Two subjects reacted at challenge.

These two subjects were re-challenged approximately 5-6 weeks after the primary challenge. Re-challenge consisted of a 1 day semi-occluded patch and a single open patch application. One subject did not react and was not considered to be sensitized to HMPCC. The second subject reacted at the re-challenge. This subject was re-challenged for a second time approximately one month after the first re-challenge application. The second re-challenge consisted of a 1 day semi-occluded patch and open applications, twice daily to virgin sites on the forearms for 3 consecutive days. The subject reacted at both semi-occluded and open applications of HMPCC. This subject was re-challenged a third time approximately 5 months after the second re-challenge application. Both open and occluded patch applications were used. The subject again reacted to HMPCC at both open and occluded applications.

The subject who reacted had psoriasis and his medical history included a mild to moderate reaction to a soap product and a mild reaction to a deodorant product during the challenge phase of an HRIPT. Follow up tests were conducted with the soap and the deodorant. The subject did not react again to the soap product, however, a mild response to the deodorant product was again observed.

15% HMPCC (8264 µg/cm²) induced sensitization in 1 of 109 volunteers (RIFM, 1999a).

Ref.: 36

A third human repeated insult patch test was conducted with HMPCC on 39 volunteers (6 males and 33 females). A 0.5 ml dose of a 5% solution of HMPCC in 95% ethanol was applied to a 1 inch square Webril pad affixed to a 1 x 2 inch adhesive bandage (equivalent to a dose/unit area of 3876 µg/cm²) which was then applied to the upper arm under semi-occlusion. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Reactions were read 1-2 days after patch removal just prior to application of the next patch. A total of nine applications were made over a three week period. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1 and 3 days after patch removal. No sensitization reactions were produced (RIFM, 1964a).

Ref.: 27

A fourth human repeated insult patch test was conducted with HMPCC on 38 volunteers (6 males and 32 females). A 0.5 ml dose of a 5% solution of HMPCC in 95% ethanol was applied to a 1 inch square Webril pad affixed to a 1 x 2 inch adhesive bandage (equivalent to a dose/unit

area of 3876 $\mu\text{g}/\text{cm}^2$) which was then applied to the upper arm under semi-occlusion. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Reactions were read 1-2 days after patch removal just prior to application of the next patch. A total of nine applications were made over a three week period. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1 and 3 days after patch removal. No sensitization reactions were produced (RIFM, 1964b).

Ref.: 28

A fifth human repeated insult patch test was conducted with HMPCC on 50 female volunteers. A 1/2 inch square of clean white blotting paper was saturated with a 1% solution of HMPCC in water and was then applied to a test site on the upper back and covered with an Elasto-patch plaster. These patches were removed 2 days after application. The sites were then scored and another patch was re-applied at the same test site. Five alternate-day 2 day semi-occluded induction applications were made. After a rest period of one week, subjects were challenged with a 2 day semi-occluded patch application. Reactions were read at patch removal. No sensitization reactions were produced (RIFM, 1958).

Ref.: 25

A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 10% HMPCC in petrolatum (equivalent to a dose/unit area of 6896 $\mu\text{g}/\text{cm}^2$) on 12 male and 13 female volunteers. Application was under occlusion to the same site on the volar forearms or backs of all subjects for five alternate-day 2 day periods. Patch test sites were pretreated for 1 day with 2.5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a ten-day rest period, a challenge patch was applied to a fresh site for 2 days under occlusion. The challenge sites were pretreated for one hour with 5%-10% aqueous SLS under occlusion. Reactions to challenge were read at patch removal and 1 day after patch removal.

No reactions were observed that were considered significantly irritant or allergic (RIFM, 1977).

From 1995 to 2002, several repeated insult patch tests were conducted with fragrance compounds that contained HMPCC. They are described below and summarized in Table 3.

Table 3: Summary of human skin sensitization studies with fragrance compounds that contain HMPCC

Fragrance Compound	HMPCC Level in HRIPT	HRIPT Conditions (ml; cm^2)	Dose/unit area ($\mu\text{g}/\text{cm}^2$)	Results	HRIPT Date
A	2.5%	0.3; 3.14	1592	0/117	1995a
B	1.79%	0.3; 3.14	1137	0/112	1997
C	2.75%	0.3; 4	1375	0/111	2000
D	1.88%	0.3; 4	938	0/102	2002
E	1.18%	0.2; 2	1181	0/103	1995b
F	1.12%	0.3; 2	1118	0/101	1996
G	1.56%	0.2; 2	1563	0/103	1995c

Ref.: 29

A human repeated insult patch test was conducted on 117 male and female volunteers with a fragrance compound (Fragrance A) that contained 2.5% HMPCC (equivalent to a dose unit/area of 1592 $\mu\text{g}/\text{cm}^2$). A 0.3 ml dose of the fragrance compound was applied to a 3.14 cm^2 area patch (HTR Webril System -patch consisted of a 2.5 cm diameter Webril pad with a 5 cm^2 Micropore[®] tape backing) and allowed to volatilize for approximately 30-60 minutes. The patches were then applied to the upper arm under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 2 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 1995a).

Ref.: 31

A human repeated insult patch test was conducted on 112 male and female volunteers with a fragrance compound (Fragrance B) that contained 1.79% HMPCC (equivalent to a dose/unit area of 1137 $\mu\text{g}/\text{cm}^2$). A 0.3 ml dose of the fragrance compound was applied to a 3.14 cm^2 area patch (2.0 cm diameter Webril cotton pad with a 4.5 cm^2 Micropore[®] tape backing) and allowed to volatilize for approximately 20-40 minutes. The patches were then applied to the upper arm under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day hour rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 2 and 4 days after application.

Sensitization was not observed (See Table 3) (RIFM, 1997).

Ref.: 35

A human repeated insult patch test was conducted on 14 male and 97 female volunteers with a fragrance compound (Fragrance C) that contained 2.75% HMPCC (equivalent to a dose/unit area of 1375 $\mu\text{g}/\text{cm}^2$). A 0.3 ml dose of the fragrance compound was applied to a 4 cm^2 area patch (2 cm^2 Webril pad affixed to a strip of Micropore[®]) and allowed to volatilize for approximately 40-60 minutes. The patches were then applied to the upper arm under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1, 2, 3 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 2000).

Ref.: 38

A human repeated insult patch test was conducted on 18 male and 84 female volunteers with a fragrance compound (Fragrance D) that contained 1.88% HMPCC (equivalent to a dose/unit area of 938 $\mu\text{g}/\text{cm}^2$). A 0.3 ml dose of the fragrance compound was applied to a 4 cm^2 area patch (2 cm^2 Webril pad affixed to a strip of Micropore[®]) and allowed to volatilize for approximately 20-40 minutes. The patches were then applied to the upper back under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1, 3, 3 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 2002).

Ref.: 40

A human repeated insult patch test was conducted on 26 male and 77 female volunteers with a fragrance compound (Fragrance E) that contained 1.18% HMPCC (equivalent to a dose/unit area of 1181 $\mu\text{g}/\text{cm}^2$). A 0.2 ml dose of the fragrance compound was applied to a 2 cm^2 area patch (2 cm^2 Webril adhesive patch) and allowed to volatilize for approximately 30-60 minutes. The patches were then applied to the upper arm or upper back under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1, 2, 3 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 1995b).

Ref.: 32

A human repeated insult patch test was conducted on 23 male and 78 female volunteers with a fragrance compound (Fragrance F) that contained 1.12% HMPCC (equivalent to a dose/unit area of 1118 $\mu\text{g}/\text{cm}^2$). A 0.3 ml dose of the fragrance compound was applied to a 2 cm^2 area patch (2 cm^2 Webril adhesive patch) and allowed to volatilize for approximately 30-60 minutes. The patches were then applied to the upper arm under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1, 2, 3 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 1996).

Ref.: 34

A human repeated insult patch test was conducted on 27 male and 74 female volunteers with a fragrance compound (Fragrance G) that contained 1.56% HMPCC (equivalent to a dose/unit area of 1563 $\mu\text{g}/\text{cm}^2$). A 0.2 ml dose of the fragrance compound was applied to a 2 cm^2 area patch (2 cm^2 Webril adhesive patch) and allowed to volatilize for approximately 30-60 minutes. The patches were then applied to the upper arm or the upper back under semi-occlusive conditions. These patches were removed 1 day after application. After a 1-2 day rest period, subjects were

again patched at the same site. Patches were applied three times a week for 3 weeks. Reactions were read 2-3 days after application. A total of nine applications were made over a three week period. Approximately two weeks after the application of the last induction patch, a semi-occluded challenge patch was applied to the original site and to a site not previously exposed and removed after 1 day. Reactions to challenge were read at 1, 2, 3 and 4 days after application. Sensitization was not observed (See Table 3) (RIFM, 1995c).

Ref.: 33

Human Elicitation Studies

The elicitation potential of HMPCC was evaluated by Johansen *et al.* (2003). Eighteen eczema patients (2 male and 16 female) who previously reacted to 5% HMPCC on patch testing were patch tested with a serial dilution of HMPCC and also subjected to a Repeated Open Application Test (ROAT). Seven control subjects (2 male and 5 female) who had not previously reacted to 5% HMPCC in a patch test were included in the Repeated Open Application Test.

Patch tests were conducted using a 10-fold serial dilution of HMPCC from 0.0006% to 6% in ethanol. A 15 µl dose of HMPCC in ethanol was applied to each patch. These patches were then applied for 2 days to a 0.5 cm² area on the upper back using Finn Chambers[®] on Scanpor[®]. Reactions to the patch test were read on days 2, 3 and 7 using the ICDRG's scale. Seventeen (17/18) patients reacted to HMPCC. The dose of HMPCC eliciting a reaction in 10% of the patients was 0.9 µg/cm² and the dose eliciting a reaction to 50% of the patients was 20 µg/cm² (Johansen *et al.*, 2003).

In the Repeated Open Application Test, two drops, (equivalent to 30 mg) were applied to 3 cm² area on the volar aspect of the lower arm. A concentration of 0.5% HMPCC in ethanol was applied to one area, twice daily, for 2 weeks. If no reactions occurred, applications were continued with 3% HMPCC in ethanol for the next 2 weeks. Ethanol was applied on the contralateral site. Test sites were evaluated weekly, and new sets of bottles with test solution and vehicle were issued. If no reactions occurred, the study was terminated in 4-weeks. A positive use test developed in 16/18 patients. Eleven patients were positive to 0.5% HMPCC. In these 11 patients, the median amount applied was 15.3 µg HMPCC/cm²/application (range 3.4-22.2). Five patients were positive to 3% HMPCC; the median amount applied to these 5 patients was 126.2 µg HMPCC/cm²/application (range 40.5-226.2). The median day of termination due to a positive use test was day 9. There were no reactions to HMPCC in the 7 control subjects and there were no reactions to the vehicle control in either the patients or the control subjects (Johansen *et al.*, 2003).

Ref.: 19

A Use Test was conducted by Heydorn *et al.* (2003a) using an experimental exposure model that simulated real-life exposure to a dishwashing liquid diluted with water. Both patients in this study had previously been diagnosed with hand eczema of at least 3 months duration and also had previously reacted to HMPCC in a patch test in the 12 months prior to the Use Test. Patch tests were conducted with 5% HMPCC in petrolatum during the first week of immersion to confirm reactivity to HMPCC. Patch tests were applied to the upper back for 2 days using Finn chambers[®] on Scanpor[®]. Reactions were read on day 2 and/or days 3-4 and on day 7 according to ICDRG recommendations (Heydorn *et al.*, 2003a).

To stimulate real-life exposures during the immersion study, each subject immersed a finger from one hand for 10 minutes in a solution with HMPCC. A finger from the other hand was immersed in a solution that did not contain HMPCC and served as a control. After the immersion, the fingers were air-dried with no washing or use of moisturizers for the next 30 minutes. In the first two weeks of the study, patients were exposed to a solution of 0.001% HMPCC in ethanol in water 10% (v/v). If no reactions occurred, patients were exposed to a solution of 0.025% HMPCC in ethanol in water 10% (v/v) for the next two weeks. If no reactions occurred, the study was terminated in 4-weeks. Test sites were evaluated on day 1 prior to immersion and once weekly thereafter. Evaluation was made using a clinical scale and laser Doppler flow meter. Both patients were observed to have a clinically visible reaction to the finger immersed in the control solution; one subject was also observed to have a clinically visible reaction on the finger immersed in the solution with HMPCC. Analysis of the laser Doppler measurements of blood flow did not detect differences between reactions to the control solution or reactions to the solution containing HMPCC. The authors concluded that there was no association between immersion of a finger in a solution containing HMPCC and development of clinically visible eczema (Heydorn *et al.*, 2003a).

Ref.: 15

Appropriate patch test concentrations for HMPCC was determined in dermatitis patients prior to patients being tested in a Use Test. Threshold levels were determined using 2 day patch tests with A1 Test[®] patches with Scanpor[®] under a 0.5 inch diameter cellulose disk. Reactions were read at 2, 3 and 4 days. One male and eleven female dermatitis patients with a history of dermatologic problems and with pre-existing sensitivities to either geraniol or hydroxycitronellal (previously determined in a patch test) were tested. Fourteen female control subjects were also tested. To determine their threshold level, subjects were patch tested with a 0.5% - 5% (in petrolatum) concentration series. To help establish the threshold level, another concentration series (doses were not reported for this series) was tested six weeks after the original series was tested. One patient reacted to HMPCC at concentrations greater than 0.25%. No other reactions were observed in the remaining 11 dermatitis patients and no reactions were observed in the 14 control subjects who were patch tested with 5% HMPCC in petrolatum (Benke and Larsen, 1984)

Eight to ten weeks after patch test thresholds were determined in the above 12 patients, the patients were patch tested with mixtures of geraniol, hydroxycitronellal and HMPCC. Patch tests were conducted using A1 Test[®] patches with Scanpor[®] under a 0.5 inch diameter cellulose disk. The one patient who had reacted to HMPCC at concentrations greater than 0.25%, now reacted to mixtures of hydroxycitronellal and geraniol, hydroxycitronellal and HMPCC and hydroxycitronellal, geraniol and HMPCC. Two other patients who had not reacted to HMPCC, now reacted to mixtures containing HMPCC, geraniol and hydroxycitronellal (Benke and Larsen, 1984).

A Use Test program was then conducted with these 12 dermatitis patients and the 14 control subjects to determine the level of reactivity to shampoos containing fragrance mixtures of HMPCC, geraniol and hydroxycitronellal. A fragrance mixture prepared from equal amounts of HMPCC, geraniol and hydroxycitronellal was added at various levels to a shampoo without fragrance, colour or colour stabilizers. The shampoo was then distributed for *ad libitum* use. Patch test threshold levels were used to select the initial fragrance levels for the shampoo. Test

subjects were provided with a shampoo containing 25-40% (or less) of their 2 day patch test threshold level. Doses were increased every 2-weeks, with a 3.0-3.3 fold higher level, until they reached a maximum of 5% of each material in the shampoo. The overall concentrations of the fragrance mixture in the shampoos were 0.03%, 0.09%, 0.3%, 0.9%, 3%, 9% and 15%. The 0.03% fragrance mixture contained 0.01% of HMPCC, 0.01% of geraniol and 0.01% of hydroxycitronellal, the 0.09% fragrance mixture contained 0.03% each of the 3 materials, 0.3% contained 0.1% each of the three materials, 0.9% contained 0.3% each of the 3 individual ingredients, 3% contained 1% each of the three materials, 9% contained 3% each of the individual ingredients and the 15% fragrance mixture contained 5% each of the 3 individual ingredients. The 14 control subjects used a shampoo containing 15% of the fragrance mixture for 6-weeks. One patient reacted to a shampoo containing the 15% fragrance mixture (which contained 5% HMPCC, 5% geraniol, 5% hydroxycitronellal) however this subject used a medicated shampoo to treat her seborrheic dermatitis/dandruff and the reaction appeared to be related to this condition rather than to a contact allergic response. A second patient reported a burning sensation to the shampoo containing the 15% fragrance mixture but no visible skin reactions were observed. Two control subjects reported a stinging sensation to the shampoo containing the 15% fragrance mixture but no visible reactions were observed (Benke and Larsen, 1984).

Ref.: 2

Beginning in January 2003, the North American Contact Dermatitis Group (NACDG) started evaluating HMPCC in dermatitis patients and will continue to do so until January 2004. As of July 2003, 400 patients had been tested with 0.5%, 1.5% and 5% HMPCC in petrolatum. No reactions were observed with 0.5% and 1.5% HMPCC. Reactions to 5% HMPCC were observed in 2 patients; both reactions were 1⁺; one reaction was possibly relevant and the other reaction was possibly past relevant. Four of the patients who were tested with HMPCC had multiple fragrance allergies (3 or more), but none of these patients reacted to HMPCC. The 2 patients who did react to HMPCC were also tested with the fragrance mix, balsam of Peru, jasmine, cinnamic aldehyde, ylang ylang oil and tea tree oil and did not react to any of these materials (NACDG).

Ref.: NACDG

The German Contact Dermatitis Research Group (DKG) conducted a multicentre trial to assess the frequency of contact allergy to HMPCC and to examine concomitant reactions to HMPCC and the fragrance mix. From March 2000 to February 2001, 5% HMPCC in petrolatum was tested in 3245 consecutive patients along with the fragrance mix in 20 departments of dermatology. Patch tests were conducted according to DKG guidelines and were read at least until day 3. Reactions were scored according to ICDRG recommendations with slight changes by the DKG. In 739 patients the patch test exposure time was 24-hours and in 2506 patients the patch test exposure time was 48-hours. Reactions to HMPCC were observed in 1.9% (62/3245) of the patients. In 3185 patients who were tested with 5% HMPCC in parallel with the fragrance mix, 300 patients reacted to the fragrance mix and 59 reacted to HMPCC. Positive reactions were observed to both the fragrance mix and HMPCC in 40 patients (Geier *et al.*, 2002).

Ref.: 10

A multicentre study was conducted in Europe between October 1997 and October 1998. The study tested 1855 consecutive patients from contact dermatitis clinics at 6 dermatology departments. Patch tests were applied to the back for 2 days using Finn Chambers[®] on Scanpor[®] or van der Bend chambers. Reactions were read at most centres on days 2 and 4; readings on day 3 or day 4 were used for overall evaluation of positive results. HMPCC was tested at 5% in petrolatum and produced reactions in 2.7% (50/1855) of the patients; doubtful reactions were also observed in 1.1% (20/1855) of the patients. (Frosch *et al.*, 1999; Frosch *et al.*, 2002). Thirty-seven out of the 50 patients who had reacted to 5% HMPCC in petrolatum were retested with HMPCC at a lower concentration, 1% HMPCC in petrolatum. Of these 37 patients who were retested, 25 reacted to 1% HMPCC (Frosch *et al.*, 1999).

Ref.: 8

Baxter *et al* (2003) reported the results of patch testing in 766 consecutive patients over a 12-month period. The test materials were applied using Finn Chambers[®] on Scanpor[®] and reactions were read on day 2 and day 4. Sixteen of the patients reacted to HMPCC. Of these 16, ten also reacted to the Fragrance Mix.

Ref.: 1

Analysis of 59 products intended for hand exposure found that fragrance materials which are not present in the Fragrance Mix are frequently used. Fourteen of these fragrance materials were tested on 658 (254 males and 404 females) consecutive hand eczema patients who were suspected of having allergic contact dermatitis. Patch tests were applied to the skin of the upper back for 2 days using Finn Chambers[®] on Scanpor[®]. Reactions were read on day 2 and/or days 3-4 and on day 7 according to ICDRG recommendations. Fourteen patients reacted to 5% HMPCC in petrolatum (Heydorn *et al.*, 2002; Heydorn *et al.*, 2003b)

Ref.: 14, 16

Frosch *et al* (1995) reported the results of a multicentre study on patch tests with 48 fragrance materials. HMPCC, 1% and 5% in petrolatum, was tested in 22 male and 84 female patients. The material was applied to the back for 2 days using Finn chambers[®] on Scanpor[®]. Reactions were assessed per ICDRG guidelines on days 2 and 3 or on days 2 and 4. One allergic reaction was observed at 1%; three allergic reactions were observed at 5%.

Ref.: 7

A multicentre study from March 1986 to July 1987 was conducted to determine the causative allergens in cosmetic products. One hundred and nineteen (119) cosmetic sensitive patients (17 male and 102 female) were tested about 8-10 weeks after their initial diagnosis of cosmetic allergy. Patch tests were carried out with 2% HMPCC in petrolatum using Van der Bend patch chambers and acrylate tape. The patch was removed after 2 days and the reactions were read 20 minutes later and again 24 or 48 hours later. One patient reacted (DeGroot *et al.*, 1988).

Ref.: 5

Patch tests were conducted from 1981-1986 on 1781 patients with contact dermatitis to determine contact allergy to cosmetics. Seventy-five patients were identified with contact

allergy to cosmetics. Thirty-five of these 75 patients were patch tested with all of the ingredients of the cosmetics to which they had reacted. Patch tests were conducted with Silver Patch Testers or with van der Bend patch test chambers which were fixed on Leukosilk and covered with acrylate tape. Patch tests were conducted according to ICDRG recommendations. One subject reacted to HMPCC (no dose reported) which was present in a deodorant cream (DeGroot, 1987).

Ref.: 4

In human patch test data from the period 1978-1980, 5% HMPCC produced no reactions in 16 patients with cosmetic dermatitis and no reactions in 27 patients with non-cosmetic eczema and dermatitis. No reactions were observed in 10 control subjects (Ishihara *et al.*, 1981). In human patch test data from 1977, Ishihara *et al.* (1979) reported that 5% HMPCC in petrolatum did not produce allergic reactions in 7 facial melanosis patients or in 31 cosmetic dermatitis patients or in 17 non-cosmetic dermatitis and eczema patients or in 9 control subjects.

Ref.: 18

A 50-year-old female with a severe eczema of 5-months duration was patch tested with the fragrance mix and with her own cosmetic products. Patch tests were conducted with Finn chambers[®]. Reactions were read on days 2, 4 and 7. She reacted strongly to her eau de toilette and was then further tested with the components of the eau de toilette. She reacted very strongly to 2% and 5% HMPCC in petrolatum (Gimenez-Arnau *et al.*, 2002).

Ref.: 11

A 37-year-old female with cosmetic allergic contact dermatitis was tested with the components of several fragrances. She reacted to 1% HMPCC in petrolatum (LeCoz and Goldberg, 2002).

Ref.: 23

A 28-year old male who developed dermatitis of both axillae from 2 deodorants was tested with the components of the 2 deodorants and reacted to the perfume (which contained 0.075% HMPCC) in one deodorant and also to the perfume in the second deodorant which also contained HMPCC. He was later tested with 6.5% HMPCC in dipropylene glycol and 0.125% and 0.25% HMPCC in petrolatum and reacted to all three concentrations. Twenty control subjects were also tested with 0.25% HMPCC in petrolatum and 6.5% HMPCC in dipropylene glycol and no reactions were observed (Handley and Burrows, 1994).

Ref.: 12

A 20-year old female with a 5-month history of severe dermatitis in both axillae which was related to the use of her underarm deodorant was tested with the components of the deodorant including the ingredients of the fragrance in the deodorant. The patient reacted to 10% HMPCC in petrolatum (Hendriks *et al.*, 1999).

Ref.: 13

A 22-year-old male with a history of dermatitis in the axillary area which developed after using a solid roll-on antiperspirant was tested with the components of the antiperspirant and also with a perfume screening series. Patient did not react to 5% HMPCC (vehicle not reported) (Larsen, 1983).

Ref.: 22

Animal Studies

Table 4: Summary of Animal Skin Sensitization Studies with HMPCC

Test Method	Concentration (induction)	Subjects	Results (elicitation concentration)	References
Maximization test	10% in acetone/ PEG/saline 100%	guinea pigs	0/10 reactions at 10% 4/10 reactions at 100%	RIFM, 1988
Maximization test	5%, 10%, 20% and 40% in propylene glycol and acetone	guinea pigs	4/5 reactions at 5% 4/5 reactions at 10% 4/5 reactions at 20% 4/5 reactions at 40%	RIFM, 1999b
Intradermal test	0.1% in physiological saline	guinea pigs	0/8 reactions	RIFM, 1963
LLNA	1%, 2.5%, 5%, 10% and 25% in acetone/olive oil	mice	sensitization effects were observed at 25%	RIFM, 2001

Ref.: 26, 30, 37, 39

HMPCC was tested in a maximization test (Magnusson and Kligman, 1969) in 10 albino Dunkin/Hartley strain guinea pigs (6 male/4 female), weighing between 313-362 grams. Induction consisted of two stages, intradermal injection followed one week later by a 2 day occluded patch application (patch consisted of 2 cm x 4 cm filter paper saturated with HMPCC attached to an adhesive dressing then placed over a 2 cm x 4 cm shaved site). A total of 6 intradermal injections were administered. They comprised: 2 injections of 0.1 ml of 50% Freund's Complete Adjuvant in Dobs/saline; 2 injections of 0.1 ml of a solution of 0.5% HMPCC in Dobs/saline; 2 injections of 0.1 ml of a suspension of 0.5% HMPCC in Dobs/saline emulsified with Freund's Complete Adjuvant (50:50). The topical induction concentration was 100%. Challenge application was made two weeks after the topical induction application. The guinea pigs were challenged on the shaved flank by an occluded 1 day patch (patch consisted of an 8 mm filter paper patch in an 11 mm aluminium patch test cup saturated with HMPCC). At the same time, the challenge treatment was applied to 4 control animals that had not been treated before. The treatment sites were examined for evidence of sensitization 1 and 2 days after patch removal. Two further challenge applications were made at weekly intervals on alternate flanks. Challenge concentration for the first and second challenge applications was 100% HMPCC. The third challenge application was made with 100% HMPCC and also with 10% HMPCC in 6% acetone/20% polyethylene glycol 400/0.9% physiological saline. One sensitization (1/10) reaction was observed at the first challenge with 100% HMPCC; 4/10 reactions plus two questionable reactions were observed at the second challenge with 100% HMPCC; 4/10 reactions plus one questionable reaction were observed at the third challenge with 100% HMPCC; no allergic reactions were observed at the third challenge with 10% HMPCC (RIFM, 1988).

Ref.: 30

HMPCC was tested in a second guinea pig maximization test (Magnusson and Kligman, 1969) using 5 female Hartley albino guinea pigs weighing 330-345 grams. Induction consisted of two stages, intradermal injection followed one week later by an occluded patch application. Challenge application was made two weeks after the topical induction application. Intradermal induction injections were made with 10% HMPCC in Freund's Complete Adjuvant (FCA) with and without physiological saline (1:1). The topical induction concentration was 10% in FCA. The guinea pigs were challenged with 5%, 10%, 20% and 40% HMPCC in a mixture of propylene glycol and acetone (1:1). Reactions were scored according to Draize at 1 day. Sensitization was observed in 4/5 guinea pigs at every dose level (RIFM, 1999b)

Ref.: 37

Eight male guinea pigs weighing 300-400 grams were tested in a guinea pig intradermal injection test consisting of intradermal induction injections followed two weeks later by an intradermal challenge application. Induction applications were given every other day until a total of ten intradermal induction injections had been made. A 0.05 ml dose of a suspension of 0.1% HMPCC in physiological saline was used for the first induction injection. Subsequent induction injections were made with a 0.1 ml dose. An area 3-4 cm² was used for the site of the injections. Two weeks after the final induction injection, an intradermal challenge injection with a 0.05 ml dose of a freshly prepared suspension of 0.1% HMPCC in physiological saline was administered. Reactions were read 1 day after application. Sensitization was not observed (RIFM, 1963)

Ref.: 26

Sensitization was evaluated in a Local Lymph Node Assay (LLNA). Groups of four female CBA/CaOlaHsd mice were tested with HMPCC at dose levels of 1%, 2.5%, 5%, 10% and 25% in acetone/olive oil (4:1). Each animal received a daily topical application of 25 µl of one concentration of HMPCC on the dorsal surface of each ear for 3 consecutive days. A positive control group of animals was treated with α -hexylcinnamaldehyde and a vehicle control group was treated with the vehicle alone. Five days after the first application all mice were injected intravenously through the tail vein with 250 µl of 20.81 µCi ³H-methyl thymidine (³HTdR). All mice were sacrificed approximately five hours after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left overnight at 4°C. The samples were then re-suspended in TCA and then transferred to a scintillation cocktail. ³HTdR incorporation was then measured by β -scintillation counting and stimulation indices were determined for each experimental group. Sensitization effects were observed; the Stimulation Index was 4.9 with 25% HMPCC. The EC3 value was reported to be 17.1% or 4275 µg/cm² (RIFM, 2001).

Ref.: 39

3.3.4. Dermal / percutaneous absorption

/

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

/

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

/

3.3.5.3. Chronic (> 12 months) toxicity

/

3.3.6. Mutagenicity / Genotoxicity

/

3.3.7. Carcinogenicity

/

3.3.8. Reproductive toxicity

/

3.3.9. Toxicokinetics

/

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

3.3.11. Human data

See point 3.3.3

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)
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CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

The HRIPT data indicates that the no-effect level (NOEL) for the induction of sensitization is about 4000 $\mu\text{g}/\text{cm}^2$. However, this may be an overestimate because of the large volume applied. The murine local lymph node assay showed an EC3 value of 17.1% (4275 $\mu\text{g}/\text{cm}^2$).

It is understood that COLIPA is conducting a study on exposure, which will provide current exposure data specific to Europe. Therefore, the exposure data provided in table 1 is not considered conclusive.

The provided experimental data must be viewed against the present epidemiology of contact allergy to HMPCC in Europe (opinion n° SCCNFP/0743/03) which demonstrates that recent consumer exposure to HMPCC has caused a high rate of contact allergy to it. As current/recent usage levels of HMPCC have caused sensitisation, information on the actual use of HMPCC in Europe is required.

4. CONCLUSION

In response to the questions asked, the SCCP is of the opinion that:

Current epidemiological data demonstrates that contact allergy to Hydroxyisohexyl 3-cyclohexene carboxaldehyde is a problem in Europe. The provided experimental data does not demonstrate the highest level for the safe use of Hydroxyisohexyl 3-cyclohexene carboxaldehyde in cosmetics.

Because of the widespread use and potential exposure to Hydroxyisohexyl 3-cyclohexene carboxaldehyde, data for all toxicological end-points should be provided to enable a full risk assessment.

5. MINORITY OPINION

Not applicable

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