EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE

(HICC)

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

The SCCP adopted this opinion at its 13th plenary

of 13-14 December 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

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

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1. BACKGROUND

The commercial abbreviation HICC has the chemical name 4-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde) with the CAS no. 31906-04-4 and EC No 250-863-4. However, this is not a pure substance as it also contains 3-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde) with the CAS no. 51414-25-6 and the EC No 257-187-9.

The INCI name is Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (CAS no. 31906-04-4), but this compound has the technical name of the 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde indicating that it is a mixture of the 2 compounds.

The current regulation of "Hydroxymethylpentylcyclohexenecarboxaldehyde (CAS No 31906-04-4)" in Annex III, entry 79, of the Cosmetics Directive was introduced with the 7thamendment of the Cosmetics Directive (2003/15/EC). It stipulates that the presence of the substance must be indicated in the list of ingredients of the cosmetic product when its concentration exceeds 0.001% in leave-on products or 0.01% in rinse-off products.

The first opinion (SCCNFP/0743/03) on HICC was adopted by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) on 9th December 2003. This first opinion on HICC was based on publicly available data and answered the questions:

Is 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1 carboxaldehyde safe for use in cosmetic products taking into account the data provided?

"The available data clearly demonstrate that 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1 carboxaldehyde is an important contact allergen. In recent large European surveys, it has been shown that in patients with eczema 1.9 – 2.7% react to 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1 carboxaldehyde 5% in petrolatum on routine testing. The allergy is often relevant.
The frequency of contact allergy in the general population is unknown. The proportion of individuals with eczema who are evaluated by diagnostic patch testing will depend on the accessibility of appropriate facilities within their geographical location in Europe.
Therefore, the current use levels of 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1carboxaldehyde are unsafe as current use levels have both caused the induction and elicitation of contact allergy to it.
Additionally, although the presence of it in a finished cosmetic product will be identified on ingredient labels if present at 10ppm (0.001%) in leave on products or 100 ppm (0.01%) in rinse off cosmetic products, only that unknown proportion of individuals who have been clinically tested will be able to avoid cosmetics that are potentially harmful to them.
Industry has recommended that 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde should not be used at a level greater than 1.5% in a finished cosmetic product. This recommended level far exceeds levels known to be a risk to the consumer."

If not, does the SCCNFP consider 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1 carboxaldehyde is safe if used up to a maximum concentration in cosmetic products and do the data provided indicate such a concentration?

"Results from the experimental data above, and a risk assessment model, suggest that a safe level of exposure for the consumer would be in the range of 0.9 µg /cm2 to 10 µg/cm²."

And/or does the SCCNFP 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?

"Based on the information presently available, a concentration of up to 0.02% in a finished cosmetic product will have a low potential to induce sensitisation, or elicit allergic contact reactions in those consumers already sensitised to this fragrance chemical.
Although strictly a risk management matter, because of the importance of 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1 carboxaldehyde as an allergen for the consumer, a more easily recognised INCI name than hydroxyisohexyl 3-cyclohexene carboxaldehyde may be of assistance to the consumer."

The second opinion (SCCP/0838/04), based on data submitted by Industry, was adopted by the Scientific Committee on Consumer Products (SCCP) on 7th December 2004. Only the sensitisation aspect was considered in this opinion with the conclusion:

"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."

The present submission II was submitted by EFFA[1] in June 2009. EFFA proposes to restrict the use of HICC according to the IFRA[2] standard 2009 e.g. 0.02% for use in lip products, deodorants and antiperspirants and 0.2% in all other cosmetic products except for use in oral care products.

2. TERMS OF REFERENCE

1. *Does the SCCS consider, with the data provided that 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde is safe for the consumers, when exposed to 0.02% 3- and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde in lip products, deodorants and antiperspirants and 0.2% 3- and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde in other cosmetic products except oral products?*

2. *Does the SCCS have any other scientific concerns of the use of HICC in cosmetic products based the data provided?*

[1] EFFA – European Flavour & Fragrance Association
[2] IFRA – International Fragrance Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

The INCI name of 4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde is hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). The INCI name will be used throughout this opinion.

3.1.1.1. Primary name and/or INCI name

Hydroxyisohexyl 3-cyclohexene carboxaldehyde

This is a mixture of two isomers:

A: 4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carboxaldehyde, and
B: 3-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carboxaldehyde

The isomer ratio A:B is approximately 2:1

3.1.1.2. Chemical names

3-Cyclohexen-1-carboxaldehyde,4-(4-hydroxy-4-methylpentyl)-
4-Cyclohexen-1-carboxaldehyde,4-(4-hydroxy-4-methylpentyl)-

3.1.1.3. Trade names and abbreviations

HICC
HICC
Lyral®
Kovanol®

3.1.1.4. CAS / EC number

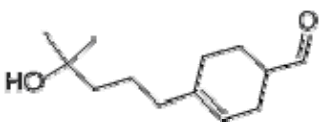
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carboxaldehyde

CAS: 31906-04-4
EC: 250-863-4

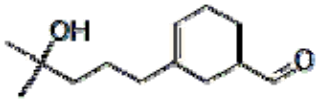
3-(4-Hydroxy-4-methylpentyl)cyclohex-3-ene carboxaldehyde

CAS: 51414-25-6
EC: 257-187-9

3.1.1.5. Structural formula



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



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

3.1.1.6.	Empirical formula

Formula: $C_{13}H_{22}O_2$

3.1.2.	Physical form

A colourless viscous liquid with a delicately sweet, light and floral odour

3.1.3.	Molecular weight

Molecular weight: 210.32 g/mol

3.1.4.	Purity, composition and substance codes

Minimum 98%

3.1.5.	Impurities / accompanying contaminants

No data submitted

3.1.6.	Solubility

Water: 184.6 mg/l at 25 °C (calculated)

3.1.7.	Partition coefficient (Log P_{ow})

Log K_{ow}: 3.32 (calculated)

3.1.8.	Additional physical and chemical specifications

Melting point:	/
Boiling point:	318 °C
Flash point:	135.1 °C
Vapour pressure:	< 0.001 mm Hg at 20 °C
Specific gravity:	0.990 – 0.994
Viscosity:	/
Acid value:	0.31
Refractive index:	1.527

3.1.9.	Homogeneity and Stability

HICC formulations used for the reproductive toxicity were checked for homogeneity and stability. HICC formulated in peanut oil at a target concentration 2.5 mg/ml were stable at 22°C±5°C for at least 26 hours and at 5°C±3°C for at least 13 days (maximum variation 3.9%). The relative standard deviation (RSD) of the mean average concentration values for the top middle and bottom of each formulation, prepared at the start of the dosage period, was ≤5%. Thus, the formulations were considered homogeneous.

HICC formulations used for the repeated dose toxicity were checked for homogeneity and stability. HICC formulations at a target concentration 125 mg/ml were stable at 22°C±5°C for at least 24 hours and at 5°C±3°C for at least 14 days (maximum variation ≤ 10%). The relative standard deviation (RSD) of the mean average concentration values for the top middle and bottom of each formulation, prepared at the start of the dosage period, was ≤6.4%. Thus, the formulations were considered homogeneous.

General comments concerning physico-chemical specifications

- Water solubility and Log P_{ow} of HICC are reported as calculated values, but not determined according to EC Methods A.6 and A.8 respectively.
- Calculated Log P_{ow} values are not acceptable. The Log P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

3.2. Function and uses (copied from 2004 opinion)

HICC is a fragrance ingredient used to perfume both cosmetic products and non-cosmetic products such as household cleaners and detergents. Its worldwide use was in the region of 1000 metric tonnes per annum (SCCP 2004).

According to EU Cosmetic Directive, presence of HICC in rinse-off cosmetic products should be labelled when its concentration in the product is >100 ppm; and its labelling in a leave-on cosmetic product is required at a concentration >10 ppm.
HICC needs to be labelled when present at concentrations exceeding 0.01 % by weight in detergents, according to the EU Detergent Regulation.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: not specified
Species/strain: rats Sprague-Dawley
Group size: n= 2 /sex/dose
Test substance: HICC
Batch:
Purity:
Vehicle:
Dose levels: 0.5, 1.6 and 5.0 g/kg
Dose volume:
Route: oral
Administration:
GLP:
Study period:

In this dose-range finding study, no death occurred at the dose levels of 0.5 and 1.6 g/kg while one of four rats died at the 5.0 g/kg dose level. Decreased activity, flaccid body tone, ptosis, abnormal stance, hunched body position, red exudate around the nasal area chromodacryorrhoea, piloerection and vasodilatation were reported.

Guideline: not specified
Species/strain: rats Sprague-Dawley
Group size: n= 5 /sex/dose
Test substance: HICC
Batch:
Purity:
Vehicle:
Dose levels: 4.0, 4.5, 5.0, 5.5 and 6.0 g/kg
Dose volume:
Route: oral
Administration:
GLP: yes
Study period:

In this acute oral toxicity study, 3/10 rats at the dose of 4.0 and 4.5 g/kg died, 4/10 at the dose of 5.0 and 5.5 g/kg and 5/10 at the dose of 6.0 g/kg. Decreased activity, flaccid body tone, ptosis, abnormal stance and prostration were reported.
The oral LD50 was reported to be > 5.0 g/kg

Ref. 63

Guideline: not specified
Species/strain: rats strain not specified
Group size: n= 5 /sex/dose
Test substance: 76-377, Lyral®
Batch:
Purity:
Vehicle:
Dose levels: 5.0 g/kg
Dose volume:

Route: oral
Administration:
GLP: not specified
Study period:

Two rats died on day 1 following oral administration.
The oral LD50 was reported to be > 5.0 g/kg

Ref. 53

3.3.1.2. Acute dermal toxicity

Guideline: not specified
Species/strain: rabbits
Group size: n= 10
Test substance: 76-377, Lyral®
Batch:
Purity:
Vehicle:
Dose levels: 5.0 g/kg
Dose volume:
Route: dermal
Administration: under occlusion
GLP: not specified
Study period:

One rabbit died on day 7 and another on day 13 following dermal exposure. Skin irritation was observed in all animals: severe in one rabbit and slight or moderate in others.
The dermal LD50 was reported to be > 5.0 g/kg

Ref. 53

The acute toxicity of HICC is low by oral route (LD 50 > 4 g/kg in rats) and by dermal route (LD 50 > 5 g/kg in rabbits). Signs of dermal irritation were reported in all treated rabbits.

3.3.1.3. Acute inhalation toxicity

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Summary of HICC human skin irritation studies

Method	Dose (%)	Exposure time	Results	Reference
Induction phase of an HRIPT	5.3% in DEP/EtOH (75%/25%)	Nine 24-hour exposures	Mild transient irritation observed in 9/201 subjects	84
Induction phase of an HRIPT	15% in 75% alcohol SD39C/25% DEP	Nine 24-hour exposures	Scattered, barely perceptible to moderate irritation in 4/109 subjects	76
Induction phase of an HRIPT	5% in ethanol	Nine 24-hour exposures	Little or no irritation in 39 subjects	47
Induction phase of an HRIPT	5% in ethanol	Nine 24-hour exposures	Little or no irritation in 38 subjects	48
HRIPT pre-screen	1% in water and Tween 20	Five 48 hour exposures	No irritation in 50 subjects	43

Method	Dose (%)	Exposure time	Results	Reference
Maximization pre-test	10% in petrolatum	48 hours	No irritation in 25 subjects	54
Irritation test	2%, 5%, 10% and 20% in lanolin	24 hours	No irritation in 28 subjects	81
Patch test	5% in petrolatum	24 hours	No irritation in 30 subjects	64

Summary of HICC animal skin irritation studies

Study Type	Species	Results	Reference
Intradermal pre-screen for maximization test	Guinea pigs	Irritation observed at 0.5%, 0.75% and 1% in Dobs/saline No irritation with 0.25% in Dobs/saline	67
Pre-screen for maximization test (24-hour occluded patch)	Guinea pigs	No irritation observed at 25% and 50% in acetone/PEG/saline or with neat HICC	67
Intradermal pre-screen for maximization test	Guinea pigs	Irritation observed at 0.75% in Dobs/saline No irritation at 0.1, 0.25, and 0.5% in Dobs/saline	55
Intradermal pre-screen for maximization test	Guinea pigs	Irritation observed at 0.5 and 1% in Dobs/saline, but not at 0.1 and 0.25%	68
Pre-screen for maximization test (24-hour occluded patch)	Guinea pigs	No irritation with 2.5% in ethanol Very slight irritation in 1/4 at 5% in ethanol Very slight irritation in 2/4 at 10% in ethanol	55
Pre-screen for maximization test (24-hour occluded patch)	Guinea pigs	No irritation with 2.5% in ethanol Very slight irritation in 2/4 at 5% in ethanol Very slight irritation in 3/4 at 10% in ethanol Very slight irritation in 2/8 at 25% in ethanol Very slight irritation in 2/4 at 50% in ethanol	68
Irritation evaluated as part of a phototoxicity test	Guinea pig	No irritation with 10, 30 or 50% (0/5)	77
Skin irritation (open application)	Rabbit	Slight erythema in 3/3 rabbits (24 hr.)	44
Primary irritation test	Rabbit	HICC was classified as a non-irritant	65
Primary irritation test	Rabbit	HICC was classified as a non-irritant.	66

Study Type	Species	Results	Reference
Primary irritation test	Rabbit	Slight to moderate erythema and oedema observed in 8/8 with neat material, and skin cracking (5/8 at 72 hr.)	56
Primary irritation test	Rabbit	0.4% in alcohol produced no irritation (0/3)	51
Skin irritation	Rabbit	A 5.0 g/kg dose of neat HICC produced slight erythema in 5/10 rabbits, moderate erythema in 4/10 rabbits, and severe erythema in 1/10 rabbits. Oedema was described as slight in 2/10 rabbits and moderate in 8/10 rabbits.	53

Summary skin irritation
Little to no irritation was observed in the majority of subjects in human repeated insult patch tests with HICC at concentrations ranging from 1 to 15%. Scattered, barely perceptible to moderate patch test responses were observed in 4/119 subjects during induction with 15% HICC in 75% alcohol /25% DEP, and mild to moderate irritation was observed in repeated insult patch tests conducted with fragrance compounds that contained various concentrations of HICC.
With the exception of an acute dermal toxicity study in rabbits, little to no irritation was seen in animal studies, and HICC was generally considered to be a non-irritant or mild irritant.

Comment
Although HICC at higher exposures may have some irritant potential, under conditions of actual use, no irritant effect is to be expected.

3.3.2.2. Mucous membrane irritation

Summary of eye irritation studies in rabbits

Dose	Number of animals	Results	References
100%	6	Corneal opacity, conjunctival irritation	57
100%	3	Corneal opacity, corneal swelling, conjunctival irritation, iritis	58
10% in Tween 80	3	Corneal opacity and conjunctival irritation	60
0.4% in alcohol	3	no reactions	52
5% (vehicle not reported)	3	Corneal opacity and conjunctival irritation	49
5% HICC (vehicle not reported)	3	Conjunctival irritation with chemosis and discharge	50
2% HICC in base concentrate	9	Iritis, conjunctival irritation with chemosis, discharge and vessel injection	45

Instillation of HICC into the eyes of rabbits produced corneal opacity, iritis and conjunctival irritation with chemosis and discharge. At a dose of 0.4% HICC was not irritating to the rabbit eye.

Comment

Although HICC is irritant to the rabbit eye, under anticipated exposure conditions, irritant effects are not expected.

3.3.3. Skin sensitisation

Animal studies

Summary of animal skin sensitisation studies with HICC

Test Method	Concentration (induction)	Subjects	Results (elicitation concentration)	References
Maximisation test	10% in acetone/PEG/saline 100%	Guinea pig	0/10 reactions at 10% 4/10 reaction at 100%	67
Maximisation test	5, 10, 20 and 40% in propylene glycol and acetone	Guinea pig	4/5 reactions at 5% 4/5 reactions at 10% 4/5 reactions at 20% 4/5 reactions at 40%	77
Intradermal test	0.1% in physiological saline	Guinea pig	0/8 reactions	46
LLNA	1, 2.5, 5, 10 and 25% in acetone/olive oil	Mice	Sensitisation effects were observed at 25%	82

HICC 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 HICC 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% HICC in Dobs/saline; 2 injections of 0.1 ml of a suspension of 0.5% HICC 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 HICC). 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% HICC. The third challenge application was made with 100% HICC and also with 10% HICC in 6% acetone/20% polyethylene glycol 400/0.9% physiological saline. One sensitization (1/10) reaction was observed at the first challenge with 100% HICC; 4/10 reactions plus two questionable reactions were observed at the second challenge with 100% HICC; 4/10 reactions plus one questionable reaction were observed at the third challenge with 100% HICC; no allergic reactions were observed at the third challenge with 10% HICC.

Ref. 67

HICC 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% HICC 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% HICC 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.

Ref. 77

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% HICC 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 cm^2 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% HICC in physiological saline was administered. Reactions were read 1 day after application. Sensitization was not observed.

Ref. 46

Sensitization was evaluated in a Local Lymph Node Assay (LLNA). Groups of four female CBA/CaOlaHsd mice were tested with HICC 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 HICC 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 ^{3}H-methyl thymidine (^{3}HTdR). 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. ^{3}HTdR 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% HICC.

The EC3 value was reported to be 17.1% or 4275 ug/cm^2.

Ref. 82

Human Predictive (induction) Studies

Summary of human skin sensitization studies with HICC

Test Method	Test Concentration	Dose/unit area (µg/cm^2)	Results	References
HRIPT	5.3% in 75% DEP/25% alcohol	4000	No reactions (0/201)	84
HRIPT	15% in 75% alcohol/ 25% DEP	8264	No reactions (1/109)	76
HRIPT	5% in 95% ethanol	3876	No reactions (0/39)	47
HRIPT	5% in 95% ethanol	3876	No reactions (0/38)	48
HRIPT	1% in water	NA	No reactions (0/50)	43
MAX	10% in petrolatum	6896	No reactions (0/25)	53

A human repeated insult patch test was conducted with HICC on 201 volunteers (54 males and 147 females). A 0.3 ml dose of a 5.3% solution of HICC in 75% DEP/25% alcohol was applied to a Webril/adhesive patch (Kendall Healthcare Products Company Patch # 4022) resulting in a dose/unit area of 4000 µg/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.

Ref. 84

Another human repeated insult patch test was conducted with HICC on 109 volunteers (18 males and 91 females). A 0.2 ml dose of a 15% solution of HICC in 75% alcohol SD39C/25% DEP was applied to a 3.63 cm^2 area patch (equivalent to a dose/unit area of 8264 µg/cm^2), 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 volatize 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 HICC. 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 HICC. This subject was re-challenged a third time approximately 5 months after the second rechallenge application. Both open and occluded patch applications were used. The subject again reacted to HICC 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% HICC (8264 µg/cm^2) induced sensitization in 1 of 109 volunteers.

Ref. 76

A third human repeated insult patch test was conducted with HICC on 39 volunteers (6 males and 33 females). A 0.5 ml dose of a 5% solution of HICC 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/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.

Ref. 47

A fourth human repeated insult patch test was conducted with HICC on 38 volunteers (6 males and 32 females). A 0.5 ml dose of a 5% solution of HICC 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/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.

Ref. 48

A fifth human repeated insult patch test was conducted with HICC on 50 female volunteers. A 1/2 inch square of clean white blotting paper was saturated with a 1% solution of HICC 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.

Ref. 43

A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 10% HICC in petrolatum (equivalent to a dose/unit area of 6896 μg/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.

Ref. 53

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

Summary of human skin sensitization studies with fragrance compounds that contain HICC

Fragrance Compound	HICC Level in HRIPT	HRIPT Conditions (ml; cm^2)	Dose/unit area ($\mu g/cm^2$)	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. 54

A human repeated insult patch test was conducted on 117 male and female volunteers with a fragrance compound (Fragrance A) that contained 2.5% HICC (equivalent to a dose unit/area of 1592 $\mu g/cm^2$). 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 volatize 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 2).

Ref. 69

A human repeated insult patch test was conducted on 112 male and female volunteers with a fragrance compound (Fragrance B) that contained 1.79% HICC (equivalent to a dose/unit area of 1137 $\mu g/cm^2$). 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 volatize 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 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.

Ref. 73

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% HICC (equivalent to a dose/unit area of 1375 $\mu g/cm^2$). 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 volatize 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.

Ref. 80

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% HICC (equivalent to a dose/unit area of 938 µg/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 volatize 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 2).

Ref. 83

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% HICC (equivalent to a dose/unit area of 1181 µg/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 volatize 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.

Ref. 70

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% HICC (equivalent to a dose/unit area of 1118 µg/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 volatize 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.

Ref. 72

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% HICC (equivalent to a dose/unit area of 1563 µg/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 volatize 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.
Ref. 71

Comment
HICC is clearly demonstrated to be a contact allergen in animal models. The EC3 value of 17.1% categorises it as a moderate skin sensitizer. This result, and the results of the several human RIPT experiments, need to be viewed with consideration of the epidemiology of contact allergy to HICC (section 3.3.11).

The SCCS considers that human RIPT/maximisation studies are unethical. In addition, the predictive value is shown to be poor in this particular case.

3.3.4. Dermal / percutaneous absorption

Guideline:	/
Species/strain:	female human breast and abdominal skin from cosmetic surgery
Group size:	12 membranes from 6 donor for each experiment and 3 controls
Membrane integrity	tritiated water
Membrane surface area	1.2 cm^2
Test substance:	HICC
Batch:	RA00788545
Purity:	99.3%
Radiolabel	[methyl-^{14}C]HICC
Batch	CFQ14480 Batch 1; purity 99.6%
Vehicle:	70/30 (v/v) ethanol/water
Test item:	1.5% HIPCC
Dose volume:	5.0µl/cm^2
Receptor	PBS
Solubility in receptor	>500µg/ml at 25°C
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	2006

An *in vitro* human skin absorption study on HICC was conducted under both occluded and non-occluded conditions.
Twelve dosed diffusion cells were prepared (utilizing skin from 6 donors) for both the occluded and non-occluded applications. Three control cells were also prepared.
Permeation of HICC, from a 5 µl/cm2 target dose of a 1.5% (w/v) solution in 70/30 (v/v) ethanol/water, was measured at 12 time-points over 24 hours, using a 6% PBS receptor phase.
For the occluded group, a glass cover slip was place over the donor chamber immediately after dosing.
At 24 hours, the epidermal membranes were wiped, tape stripped 10 times and the HICC content of the wipes, strips and remaining epidermis determined. The filter paper skin supports were extracted and the diffusion cell donor chambers washed and wiped. Potential evaporative loss of HICC was estimated by measuring the loss from PTFE sheets under the same experimental conditions.

The assessment of evaporation of HICC from PTFE sheets showed minimal evaporative loss, ~5% of the applied dose had evaporated over the 24 hours.

Following 24 hours of exposure, 5.54 ± 2.15 µg/cm^2 and 18.6 ± 6.4 µg/cm^2 HICC (mean ± standard deviation, SD), corresponding to 7.37 ± 2.86 and 24.8 ± 8.5% of the applied dose had permeated for the unoccluded and occluded groups, respectively. Overall recoveries of the applied HICC at 24 hours were good at 86.2 ± SD 6.7% and 90.5 ± SD 2.3% under unoccluded and occluded conditions, respectively.

The overall skin absorption values for HICC, defined as amounts in the receptor phase, amounts in the epidermis (therefore excluding tape strips) and on the filter support, were 14.3 ± SD 2.98% and 36.4 ± SD 8.5% of the applied dose under unoccluded and occluded conditions, respectively.

Recoveries UNOCCLUDED CONDITIONS µg/cm^2

Cell	24h wipe	Donor chamber	Strip 1	Strips 2-3	Strips 4-6	Strips 7-10	Epider-mis	Filter paper	Receptor phase	Total recovered
1	37.9	8.86	1.89	1.91	1.10	0.737	5.62	0.334	3.28	61.7
2	33.4	19.3	1.50	1.64	0.907	0.273	3.44	0.537	2.82	63.8
3	26.0	14.5	1.36	1.14	0.801	0.685	6.58	0.548	4.16	55.8
4	31.0	18.8	1.44	1.32	1.27	0.914	4.74	1.17	6.81	67.5
5	27.7	19.7	1.87	1.66	0.591	0.306	2.84	0.626	7.96	63.2
6	31.7	15.9	1.89	2.08	1.03	0.448	4.10	1.33	4.11	62.5
7	22.2	16.3	1.55	2.31	1.83	1.63	7.64	1.13	6.14	60.6
8	25.3	18.1	2.96	3.03	1.30	0.471	2.92	0.626	9.15	63.9
9	42.3	13.2	2.10	3.00	1.48	0.732	4.19	0.241	4.33	71.6
10	38.5	17.3	1.50	3.33	1.90	1.54	4.27	0.227	4.24	72.8
11	43.3	9.17	2.16	1.45	0.781	0.280	3.79	0.261	7.95	69.1
12	31.4	10.3	1.58	1.83	1.11	1.12	8.37	0.363	10.6	66.7
Mean	32.6	15.1	1.82	2.06	1.17	0.761	4.87	0.616	5.96	64.9
SD	6.8	3.9	0.45	0.72	0.41	0.465	1.81	0.388	2.51	4.8
SE	2.0	1.1	0.13	0.21	0.12	0.134	0.52	0.112	0.73	1.4
Mean*	**32.7**	**15.6**	**1.84**	**2.08**	**1.18**	**0.728**	**4.56**	**0.639**	**5.54**	**64.8**
SD*	**7.1**	**3.8**	**0.46**	**0.75**	**0.42**	**0.472**	**1.51**	**0.398**	**2.15**	**5.0**
SE*	**2.1**	**1.1**	**0.14**	**0.23**	**0.13**	**0.142**	**0.45**	**0.120**	**0.65**	**1.5**

* - data excluding cell 12

% of the applied dose recovered UNOCCLUDED CONDITIONS

Cell	24h wipe	Donor chamber	Strip 1	Strips 2-3	Strips 4-6	Strips 7-10	Epider-mis	Filter paper	Receptor phase	Total recovered
1	50.5	11.8	2.51	2.54	1.46	0.980	7.47	0.444	4.37	82.0
2	44.5	25.7	1.99	2.19	1.21	0.363	4.58	0.714	3.75	84.9
3	34.6	19.3	1.81	1.52	1.07	0.912	8.75	0.729	5.53	74.2
4	41.3	24.9	1.92	1.76	1.69	1.22	6.30	1.56	9.06	89.7
5	36.8	26.2	2.49	2.21	0.79	0.407	3.77	0.833	10.6	84.1
6	42.1	21.1	2.52	2.76	1.37	0.596	5.45	1.77	5.46	83.2
7	29.5	21.6	2.06	3.07	2.43	2.17	10.2	1.50	8.17	80.7
8	33.7	24.1	3.93	4.03	1.73	0.626	3.88	0.832	12.2	85.0
9	56.2	17.6	2.79	3.99	1.96	0.973	5.57	0.320	5.76	95.2
10	51.2	23.1	2.00	4.43	2.53	2.04	5.68	0.302	5.63	96.9
11	57.5	12.2	2.87	1.92	1.04	0.372	5.05	0.347	10.6	91.9
12	41.8	13.7	2.10	2.43	1.48	1.49	11.1	0.483	14.1	88.7
Mean	43.3	20.1	2.42	2.74	1.56	1.01	6.48	0.820	7.93	86.4
SD	9.0	5.2	0.59	0.96	0.54	0.62	2.41	0.516	3.34	6.4
SE	2.6	1.5	0.17	0.28	0.16	0.18	0.69	0.149	0.97	1.9
Mean*	**43.4**	**20.7**	**2.44**	**2.77**	**1.57**	**0.969**	**6.06**	**0.850**	**7.37**	**86.2**
SD*	**9.4**	**5.0**	**0.61**	**1.00**	**0.56**	**0.628**	**2.00**	**0.530**	**2.86**	**6.7**
SE*	**2.8**	**1.5**	**0.19**	**0.30**	**0.17**	**0.189**	**0.60**	**0.160**	**0.86**	**2.0**

* - data excluding cell 12

Recoveries OCCLUDED CONDITIONS µg/cm²

Cell	24h wipe	Donor chamber	Strip 1	Strips 2-3	Strips 4-6	Strips 7-10	Epider-mis	Filter paper	Receptor phase	Total recovered
13	20.9	15.4	0.870	1.17	0.813	0.752	5.85	3.16	20.4	69.4
14	24.8	15.6	0.950	1.35	1.24	1.18	6.71	1.58	13.6	67.0
15	20.5	11.9	0.707	1.25	0.980	0.772	7.39	3.09	19.5	66.1
16	28.8	15.7	1.45	1.75	1.15	1.31	6.49	0.859	10.0	67.5
17	15.4	12.4	1.76	1.49	1.03	0.721	6.30	1.57	28.0	68.6
18	20.2	11.8	0.914	1.10	0.844	0.973	7.50	2.76	20.6	66.7
19	17.0	12.5	0.808	1.35	1.01	0.771	9.87	6.63	17.1	67.1
20	27.0	13.5	2.58	1.99	1.60	1.61	6.23	2.17	10.3	67.0
21	27.2	10.5	1.78	1.99	1.04	0.794	4.40	1.58	20.9	70.1
22	19.3	9.80	1.23	1.18	0.738	0.554	6.26	1.43	27.6	68.0
23	23.7	11.3	0.468	0.641	0.385	0.305	4.12	1.17	24.9	66.9
24	36.2	10.8	1.10	2.00	1.57	1.91	7.25	0.502	10.6	71.9
Mean	**23.4**	**12.6**	**1.22**	**1.44**	**1.03**	**0.971**	**6.53**	**2.21**	**18.6**	**68.0**
SD	**5.8**	**2.0**	**0.59**	**0.42**	**0.34**	**0.457**	**1.49**	**1.63**	**6.4**	**1.7**
SE	**1.7**	**0.6**	**0.17**	**0.12**	**0.10**	**0.132**	**0.43**	**0.47**	**1.9**	**0.5**

% of the applied dose recovered OCCLUDED CONDITIONS

Cell	24h wipe	Donor chamber	Strip 1	Strips 2-3	Strips 4-6	Strips 7-10	Epider-mis	Filter paper	Receptor phase	Total recovered
13	27.8	20.5	1.16	1.55	1.08	1.00	7.77	4.21	27.2	92.3
14	33.0	20.7	1.26	1.79	1.64	1.57	8.93	2.10	18.2	89.2
15	27.3	15.8	0.941	1.66	1.30	1.03	9.82	4.11	25.9	87.9
16	38.4	20.8	1.93	2.32	1.53	1.74	8.63	1.14	13.4	89.9
17	20.5	16.4	2.35	1.98	1.37	0.959	8.38	2.09	37.2	91.3
18	26.9	15.7	1.22	1.46	1.12	1.29	9.98	3.67	27.3	88.7
19	22.7	16.7	1.08	1.79	1.35	1.03	13.1	8.82	22.7	89.2
20	35.9	18.0	3.43	2.65	2.12	2.14	8.29	2.89	13.7	89.1
21	36.1	13.9	2.36	2.64	1.39	1.06	5.85	2.11	27.8	93.3
22	25.7	13.0	1.63	1.57	0.982	0.737	8.32	1.90	36.7	90.5
23	31.5	15.0	0.622	0.852	0.513	0.405	5.49	1.56	33.1	89.0
24	48.1	14.4	1.46	2.66	2.09	2.54	9.65	0.668	14.1	95.7
Mean	**31.2**	**16.8**	**1.62**	**1.91**	**1.37**	**1.29**	**8.69**	**2.94**	**24.8**	**90.5**
SD	**7.7**	**2.7**	**0.78**	**0.56**	**0.45**	**0.61**	**1.98**	**2.16**	**8.5**	**2.3**
SE	**2.2**	**0.8**	**0.23**	**0.16**	**0.13**	**0.18**	**0.57**	**0.62**	**2.5**	**0.7**

Ref. 85

Comment

The overall skin absorption values for HICC, (amounts permeated and amounts in the epidermis), were 14.3 ±SD 2.98% and 36.4 ±SD 8.5% of the applied dose under unoccluded and occluded conditions, respectively.

The vehicle for application indicates a model for a hydroalcoholic formulation and the 1.5% HICC is indicative of that to which the consumer has been exposed. The experiments do not provide information on the availability of HICC that may occur from its use in other formulation (product) types. No justification for the position of the radiolabel in the molecule was provided and no information on the metabolism of the substance is available.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose oral toxicity

Guideline: not specified
Species/strain: rats, Sprague Dawley Cr:CD(SD) IGS BR
Group size: n= 3 /sex/dose
Test substance: HICC
Batch: RA00765283
Purity:
Vehicle: peanut oil
Dose levels: 500 or 1000 mg/kg bw/d
Dose volume: 4 ml/kg
Route: oral
Administration: gavage
GLP: not specified
Study period:

In this preliminary 14 days repeated dose oral (gavage) range-finding toxicity study in rats, no clinical signs, body weight changes or histopathological lesions were observed at the dose of 500 mg/kg/d of HICC. At the dose of 1000 mg/kg/d some minor effects such as reduction in body weight gain were observed on day 4 only.

Ref. 87

Guideline: OECD 407
Species/strain: rat, Sprague Dawley Cr:CD(SD) IGS BR
Group size: n = 5/sex/dose
Test substance: HICC
Batch: RA00765283
Purity:
Vehicle: peanut oil
Dose levels: 0, 15, 150, or 1000 mg/kg bw/d
Dose volume: 4 ml/kg
Route: oral
Administration: gavage
GLP: Yes
Study period: July 2005 – April 2006

In this 28 days oral (gavage) toxicity study in rats, no treatment-related deaths were observed. Rats at the dose of 1000 mg/kg bw/d showed respiratory pattern changes, hunched posture and other isolated incidents of red/brown staining around the mouth and scab formation in males only.

A reduction in body weight gain during week 1 and in food consumption was observed in male rats from the high dose group. Respiratory symptoms, hunched posture, modifications of haematological parameters (plasma enzymes, albumin…), increases in absolute and relative liver and kidney weights and treatment-related histopathological changes in the liver (centrilobular inflammation and necrosis and hepatocyte enlargement in males and females) and kidney (increased density of the proximal tubular epithelium in males) were also observed at the highest dose of 1000 mg/kg bw/d in males and females.

At the dose of 150 mg/kg bw/d, increase in liver weights (absolute and relative) was also observed in male rats and hepatocyte enlargement in 3 males. Females treated at this dose showed a statistically significant reduction in absolute and relative kidney weight. In females treated with 150 mg/kg bw/d, alanine aminotransferase and alkaline phosphatase were increased. Males treated at this dose level, showed increased albumin level and

reduction in cholesterol and glucose. These observations are considered to be related to an adaptive metabolic activation and of no toxicological importance.

The applicant considered the NOAEL to be 150 mg/kg bw/d.

Ref. 86

Comment
The NOAEL is considered by the SCCS to be 15 mg/kg bw/d, based on modifications of biochemical parameters observed in males and females at the dose of 150 mg/kg bw/d and the effects on liver (increase in liver weight and hepatocyte enlargement). These modifications may be considered as early indicators of liver toxicity observed at higher doses.

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

No data submitted

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	OECD 471
Species/Strain:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, TA1538 and *Escherichia coli* WP2 *uvrA*
Replicates:	duplicates
Test substance:	Kovanol
Batch:	/
Purity:	/
Vehicle:	DMSO
Concentration:	10, 50, 100, 500, 1000 and 5000 µg/plate
Treatment:	/
GLP:	in compliance
Study period:	20 February 1984 – 6 March 1984

Kovanol was investigated for the induction of gene mutations in strains of *S. typhimurium* and *E. coli*. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was evaluated as the level of inhibition of the growth of the bacterial lawn. Negative and positive controls were in accordance with the OECD guideline.

Results
Kovanol caused a reduction in the growth of the bacterial lawn at a concentration of 5000 µg/plate in all strains of *S. typhimurium* and *E. coli* tested.
Both without and with metabolic activation a biologically relevant increase in the number of revertants was not observed in any of the tester strains used.

Conclusions
Under the test conditions used, it is concluded that Kovanol is not mutagenic in the gene mutation tests in bacteria.

Ref.74

Comment: Purity, batch number and way of treatment were not mentioned.
Guideline: OECD 471

Species/Strain:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 *uvrA*
Replicates:	triplicate cultures in two independent experiments
Test substance:	3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
Batch:	/
Purity:	/
Vehicle:	DMSO
Concentration:	75, 200, 600, 1800 and 5000 µg/plate both without and with S9-mix
Treatment:	direct plate incorporation method with 48 to 72 h incubation without and with S9-mix
GLP:	in compliance
Study period:	30 December 1998 - 4 October 1999

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde was investigated for the induction of gene mutations in strains of *S. typhimurium* and *E. coli*. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a preliminary toxicity test with all strains. Toxicity was evaluated 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. Both experiments were performed according to the direct plate-incorporation method. In the main tests, the condition of the bacterial back ground lawn was evaluated by using a dissection microscope; precipitation by visual examination. Negative and positive controls were in accordance with the OECD guideline.

Results
In the preliminary toxicity test no precipitation was observed whereas toxicity was noted for the top dose of 5000 µg/plate for TA98 and TA1537 in the absence of S9-mix.
In both experiments neither a biologically relevant nor a concentration dependent increase in the number of revertant colonies was observed. However, in the absence of S9-mix a non-concentration dependent increase was seen for TA98 (both experiments) and TA100 (experiment 1). As the maximum revertant count was within the normal historical vehicle control range these increases are not considered to be biologically relevant.

Conclusions
Under the test conditions used, it is concluded that 3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde is not mutagenic in this gene mutation test in bacteria.
Ref. 8, 75

Comment
Although not mentioned in the report other then a citation in the reference list of the study protocol, the experiments are performed according to the OECD 471 guideline. Batch and purity are not mentioned.

***In vitro* Mammalian Chromosome Aberration Test**

Guideline:	/
Species/strain:	CHO-K_1 cells
Replicates:	duplicate cultures
Test substance:	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
Batch:	/
Purity:	/
Vehicle:	DMSO
Concentrations:	4 h treatment without S9-mix: 200, 400 and 600 µg/ml 20 h treatment without S9-mix: 100, 200 and 400 µg/ml 4 h treatment with S9-mix: 200, 800 and 900 µg/ml
Treatment:	4 h or 20 treatment without S9-mix; harvest time 20 h after start of treatment. 4 h treatment with S9-mix; harvest time 20 h after the start of treatment.
GLP:	in compliance
Study period:	11 August 1999 - 19 May 2000

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde was investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO-K_1 cells. Liver S9-fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a preliminary toxicity test, performed identically to the main experiment, with concentrations up to 2100 µg/ml in the absence and presence of S9-mix. Cell counts and cellular viability was measured to determine the cell growth inhibition relative to the solvent control. In the main experiment cells were treated for 4 h (without and with S9-mix) or for 20 h (without S9-mix) and harvested 20 h after the start of treatment. Approximately 2 h before harvest, each culture was treated with Colcemid® (final concentration 0.1 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with 5% Giemsa and examined microscopically for chromosomal aberrations and the mitotic index. Negative and positive controls were in accordance with the OECD guideline.

Results
In the main test cell growth inhibition relative to the solvent control was about 60% as required according the OECD guideline.
In the absence of S9-mix and 4 h treatment, a statistically significant increase in the number of cells with chromosomal aberrations was observed at the mid concentration. However, the increase was only 1% outside the range of the historical control data and in the absence of concentration dependency this single increase was considered not biologically relevant. In the absence of S9-mix and 20 h treatment, a biologically relevant increase in the number of cells with chromosomal aberrations was not found. The statistically significant increase in cells with chromosomal aberrations found at the top concentration was within the range of the negative control data and considered not biologically relevant.
In the presence of S9-mix, a concentration-dependent and statistically significant increase in the number of cells with chromosomal aberrations was observed.
Both in the absence and presence of S9-mix an increases in the number of cells with numerical chromosome aberrations (polyploidy and/or endo-reduplication) was not observed.

Conclusion
Under the experimental conditions used, 3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde was genotoxic (clastogenic) in this chromosome aberration test.

Ref. 8, 79

Comment
Although not mentioned in the report other then a citation in the reference list, the experiments are performed according to the OECD 473 guideline. Batch and purity are not mentioned.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline:	/
Species/strain:	ICR mice
Group size:	5 mice/sex/group
Test substance:	3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
Batch:	/
Purity:	/
Vehicle:	corn oil
Dose level:	0, 225, 450 or 900 mg/kg
Route:	intraperitoneal injection
Sacrifice times:	24 h and 48 h (controls and high dose only) after treatment.
GLP:	in compliance
Study period:	2 December 1999- 30 June 2000

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde was investigated for induction of micronuclei in bone marrow cells of male and female mice. Dose levels were based on the results of a pilot study followed by a toxicity study in male and female mice on clinical signs and mortality recorded over a period of 3 days. Body weights were recorded prior to dose administration and 1 and 3 days thereafter.
In the main experiment mice were exposed orally to 0, 225, 450 or 900 mg/kg bw. Erythrocytes were collected 24 h or 48 h (controls and high dose only) after dosing. All mice were observed after dosing for clinical signs. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Bone marrow preparations were stained with May-Grünwald-Giemsa and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pilot assay, all mice dosed with 2000 mg/kg bw and 50% of the mice treated with 1000 mg/kg bw died. Clinical signs at these doses included lethargy, piloection, irregular breathing, hunched position, crusty eyes and prostration. Animals treated with lower doses appeared normal throughout the observation period.
In the following toxicity assay, mice were treated up to 800 mg/kg bw. No mortality was found. Clinical signs at these doses included lethargy and piloerection. Mice dosed with 200 mg/kg bw appeared normal throughout the observation period.
In the main experiment, one female of the 900 mg/kg bw group died (but was replaced).
Clinical signs included lethargy and piloerection at all dose and irregular breathing at 900 mg/kg bw. A more or less dose dependent decrease in the PCE/TE ratio has been observed indicating to bioavailability of 3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde to the bone marrow cells. A biological relevant and dose dependent increase in the number of polychromatic erythrocytes with micronuclei over the concurrent vehicle control was not observed.

Conclusions
Under the experimental conditions used, 3 and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde did not induce an increase in the number of micronucleated polychromatic erythrocytes of treated mice and, consequently, 3 and 4-(4-hydroxy-4-

methylpentyl)-3-cyclohexene-1-carboxaldehyde is not genotoxic (clastogenic and/or aneugenic) in polychromatic erythrocytes of mice

Ref. 8, 78

Comment
Although not mentioned in the report other then a citation in the reference list, the experiments are performed according to the OECD 474 guideline. Batch and purity are not mentioned.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

Guideline:	OECD 415 "One generation reproduction toxicity study"
Species/strain:	rats Sprague-Dawley
Group size:	n = 24 rats /sex/dose
Test substance:	HICC
Batch:	RA00765283
Purity:	/
Vehicle:	peanut oil
Dose levels:	0, 25, 100 and 500 mg/kg bw/d
Dose volume:	
Route:	oral
Administration:	gavage, 10 weeks in males and 19 weeks in females
Positive control:	none
GLP statement:	yes
Study period:	March 2005- August 2007

The reproductive toxicity of HICC was studied in rats by oral route at the dose of 25, 100 and 500 mg/kg bw/d following OECD guideline n° 415 "One generation Reproduction Toxicity Study".

Mortality was reported in the high dose group (1 male and 3 females). No clinical signs were observed in surviving males at all doses. In females, hunched postures, pilo-erection and tiptoe were observed during the last week of gestation in the high dose group. Reduced body weights were recorded in males from the high dose group. Reduced food consumption was observed in females from the high dose group throughout lactation but not in males.

There were no treatment related effects on female oestrus cycles, mating or fertility, gestation or parturition indices. At 100 and 500 mg/kg bw/d, the duration of gestation was increased.

6 females had a litter loss in the high dose group between birth and PND (Post Natal Day) 1. Reduced body weights were observed in pups born in the high dose group until weaning. Retardation in ossification was also observed in this group.

Skin sloughing was observed in offspring during the first week of lactation on all treatment groups with ridges along the tail in the 100 and 500 mg/kg bw/d groups. Swollen ears,

premature opening of eyes and sparse fur coverage were also observed. No such effects were detected in the 25 mg/kg bw/d litters.

Acanthosis and hyperkeratosis were observed in relation to treatment for the skin of male and female offspring in the 100 and 500 mg/kg bw/d litters. The observed skin effects occurred several days after birth; after skin shedding, the pups appeared normal.

No other significant effects were recorded.

25 mg/kg bw/d was considered as a NOAEL for the maternal toxicity based on increased duration of gestation at higher doses. 25 mg/kg bw/d was also considered as the developmental NOAEL based on skin peeling, acanthosis and hyperkeratosis observed at the dose of 100 et 500 mg/kg bw/d. Reduced pup viability and body weights of the pups were also observed at the dose of 500 mg/kg bw/d, possibly as a result of the prolongation of gestation.

Ref. 87

Guideline:
Species/strain: rats Sprague-Dawley - female
Group size: n = 10 in groups Ia, Ib, II and III; n= 5 in groups IV and V
Test substance: HICC
Batch: 559-010
Purity: 99.3%
Vehicle: peanut oil
Dose levels: 0 or 500 mg/kg bw/d
Dose volume: 4 ml/kg
Route: oral gavage
Positive control: none
GLP statement: in compliance
Study period: Jan 2007 – Apr 2009

The applicant considered that the presence of skin peeling, acanthosis and hyperkeratosis in the offspring of female rats dosed with 100 and 500 mg/kg bw/d in the one generation reproduction toxicity study described above may indicate that HICC affects dermal development directly or by altering nutrition. The hypothesis of a zinc deficiency in the dams was also discussed. Consequently, an exploratory repeated dose toxicity study to clarify the cause for the skin effects was conducted on rats, with postnatal evaluations to evaluate: 1) whether the pup dermal effects observed in the one- generation oral gavage reproduction study were due to pre- or post-natal exposure to HICC; and 2) whether HICC produced a functional zinc deficiency in the dams, thereby producing the skin sloughing (i.e., shedding or separation of necrotic tissue from viable tissue), peeling (i.e., loss of epidermis) and/or flaking (i.e., barely perceptible to pronounced scaling, resulting in a denuded portion of the epidermis) in pups.

Dose Group	Dosage (mg/kg/day)	Number of Rats	Dose Administration
Ia	0 (vehicle)	10	GD 0 through DG 21 or 24
Ib	0 (vehicle)	10	LD 1 through 21
II	500	10	GD 0 through DG 21 or 24
III	500	10	LD 1 through 21
IV	0 (vehicle)	5	GD 0 through 14
V	500	5	GD 0 through 14

Different groups of rats were treated during gestation (throughout gestation or only from GD0 to GD14) or throughout lactation with vehicle alone or with HICC at the dose of 500 mg/kg bw/d.

Dams were euthanized by CO_2 asphyxiation on GD 15 (Groups IV and V) or LD 21 (Groups Ia, Ib, II, and III). F0 and F1 generations pups were examined. Zinc and metallothionein levels were measured in dams. Groups IV-V (n = 5) comprised the satellite part of the study, intended to evaluate the role, if any, of zinc in the observed effects.

The dose of 500 mg/kg bw/d was confirmed to be toxic (reduced maternal body weight gains, dystocia, modifications of haematological parameters) to dams and pups (perinatal mortality, reduced live litter size and transient skin flaking) when administered during gestation or lactation.
The numbers of litters with pups with flaking and/or peeling skin were increased in the 500 mg/kg/day dosage group treated throughout gestation or lactation (Group II and Group III). Skin flaking was transient in the group II but more severe and not reversible at the end of the lactation period in the Group III.

According to the applicant, the data suggest that skin effects observed in pups from dams treated with 500 mg/kg bw/d are postnatal effects probably due to residual HICC available to the pups *via* maternal milk. Treatment of dams with HICC during gestation or lactation had no biologically important effect on zinc or metallothionein levels.

Ref. 88

Guideline:	
Species/strain:	rats Sprague-Dawley
Group size:	4 dosage groups – n= 10 per group
Test substance:	HICC
Batch:	RD00559-18
Purity:	96.6%
Vehicle:	Peanut oil
Dose levels:	10, 25 or 500 mg/kg bw/d
Dose volume:	4 ml/kg
Route:	oral
Administration:	gavage, from day of lactation 1 (DL1) through 21 (DL 21)
Positive control:	
GLP statement:	in compliance
Study period:	Dec 2008 – Feb 2009

The purpose of this study was to detect adverse effects of HICC treatment of Crl:CD(SD) female rats during lactation and weaning on lactation and maternal behaviour in female rats and on the development of the offspring of the treated female rats.

To check the hypothesis that residual HICC in the maternal milk is responsible for the skin effects observed in the pup, a repeated-dose toxicity study at the doses of 10, 25 and 500 mg/kg bw/d with exposure of pups to HICC via lactation, postnatal evaluations and a recovery period of seven weeks was conducted in rats.

No adverse effects were observed in female F0 rats with any dosage of HICC, except for transient reductions in maternal body weight gain early on during the lactation period and reduction in feed consumption.

In the F1 generation, reduced body weights were observed (greater in female pups than in the male) at the dose of 500 mg/kg bw/d. Five postweaning deaths at 500 mg/kg bw/d in the F1 pups were observed and attributed to a failure to thrive and were considered an effect of maternal treatment with the test article. At 500 mg/kg bw/d, skin peeling and cold

to touch were attributed to maternal treatment. This clinical sign was first observed as early as day 8 postpartum, and it persisted to necropsy on day 15 postpartum or through weaning and into the postweaning recovery period. It resolved in both sexes by day 35 postpartum. Skin peeling occurred more frequently in the F1 female rats than in the F1 male rats during the recovery period.

No HICC was detected in the milk of rats treated with 0 (Vehicle), 10 or 25 mg/kg/day HICC on either day 14 or day 21 postpartum. In the 500 mg/kg/day dosage group, HICC was quantifiable in the milk of two of 10 rats on day 14 postpartum with an average concentration of 73.5 ng/ml and four of five rats on day 21 postpartum with an average concentration of 59.85 ng/ml.
In conclusion, treatments of the dams with 500 mg/kg bw/day dosages of HICC during the lactation period resulted in quantifiable levels of HICC in the milk which was associated with increased incidences of skin peeling in the F1 generation pups. A NOAEL of 25 mg/kg bw/d based on pups viability and growth was derived from this study.

Ref. 99

3.3.8.2. Teratogenicity

No teratogenicity study was provided

General comment on reproduction toxicity
The SCCS concludes that, based on the reproductive toxicity studies, the NOAEL for adult rats is 100 mg/kg bw/day. The NOAEL for reproductive and developmental toxicity was considered to be 25 mg/kg/day. Treatment of the dams with 500 mg/kg bw/day HICC only during the lactation period resulted in skin peeling in all F1 generation pups. No observations of skin peeling were observed in F1 generation pups from dams treated with 10 or 25 mg/kg bw/d HICC during the lactation period.

3.3.9. Toxicokinetics

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Summary of HICC Human Phototoxicity Study

Study Type	Species/System	Endpoint/Results	Reference
Phototoxicity	Human	No phototoxic effects were reported with 13.75% HICC.	62, 97

HICC (13.75%) was applied to the skin of the 10 subjects using semi-occlusive patches. Twenty-four hours later, the patches were removed and the sites were wiped clean of any excess test material. The test sites were then exposed to UVA from a 150 watt Xenon-arc Solar Simulator with a Schott WG 345 filter (320-400 nm, intensity of 31.5 mW/cm^2) for 12 minutes. Two control sites were included; a second site was treated with the test material but was not irradiated, while an untreated site was irradiated. Test sites were evaluated 24 and 48 hours after irradiation. No evidence of phototoxicity was observed.

Ref. 62; 97

Summary of HICC Animal Phototoxicity Studies:

Study Type	Species/System	Endpoint/Results	Reference
Phototoxicity	Guinea pig	No phototoxic effects at 10, 30 or 50%.	77
Phototoxicity	Guinea pigs	No phototoxic effects at 25%	94

Five female Hartley albino guinea pigs were clipped free of hair and four hours later HICC in acetone was applied on a circle of skin 1.5 cm in diameter on both sides of the animal. A total of 6 applications were made, one spot each of a 10%, 30%, and 50% solution on each side of the animal. Immediately after application, one side was covered with aluminium foil. The test sites on the other side were then exposed to UVA (14 J/cm^2) for 70 minutes at a distance of 10 cm from five Toshiba model FL-40 BLB lamps (320-400 nm) with window glass filters to eliminate wavelengths below 320 nm. Test sites were observed for reaction 24 and 48 hours after irradiation. No phototoxic effects were observed. HICC was classified as non-phototoxic at concentrations up to 50%.

Ref. 77

Female Hartley albino guinea pigs animals were clipped free of hair and a 0.2 ml aliquot of 25% HICC (vehicle not reported) was applied to a 1.8 cm^2 area and allowed to dry for 30 minutes. The test sites of 5 animals were then exposed to UVA from Westinghouse F40BL black light tubes (320-400 nm, peak 350 nm) for 1 hour at a distance of 31 cm. UV flux ranged from 1.2-1.8 mW/cm^2. Five animals were not irradiated and served as controls. No phototoxic effects were observed.

Ref. 94

Summary of HICC Miscellaneous Phototoxicity Studies:

Study Type	Species/System	Endpoint/Results	Reference
In vitro phototoxicity	Fleischman's Baker's Yeast	No phototoxic activity was seen at concentrations of 0.1 and 1% HICC, however a phototoxic effect was seen with 10% HICC.	61, 97
In vitro phototoxicity	*Saccharomyces cerevisiae*	HICC was considered to have a positive phototoxic response with 0.004% of the phototoxic activity of 8-MOP in the 18-hour assay.	94
In vitro phototoxicity	*Saccharomyces cerevisiae*	HICC was observed to have 0.01% of the phototoxic activity of 8-MOP in a 7-hour assay. The addition of benzophenones and sunscreen agents to the vehicle reduced or eliminated the phototoxic activity of HICC.	11
In vitro phototoxicity (3T3 NRU phototoxicity test and photohaemolysis test)	Not specified	HICC showed phototoxicity in the photohaemolysis test but not the 3T3 NRU test.	12

An *in vitro* study on Fleischman's active dry yeast using an agar overlay technique was conducted to determine the phototoxicity of 0.1%, 1.0% and 10% HICC in methanol. An air dried paper disc impregnated with 40 µl of the test material was added to a microplate

along with the yeast suspension, and the plate was irradiated with UV light for 18 hours from Sylvania F15T8 BLB lamps, (emission spectrum, 320-400 nm, peak 370 nm) with a UV surface flux at the plate of 1.5-2 mW/cm^2. Zones of inhibition were measured at 48 hours after inoculation or when the contrast is adequate. No phototoxic effects were observed with 0.1% and 1.0% HICC in methanol; a phototoxic effect was observed with 10% HICC in methanol.

Ref. 61; 97

The phototoxicity of HICC was evaluated *in vitro* in Saccharomyces cerevisiae using a quantitative assay by measuring zones of growth inhibition around treated discs. Activity of fragrance materials was compared to that of 8-methoxypsoralen (8-MOP). S. cerevisiae was used in an agar overlay technique with 3 ml of a 1% suspension of yeast in molten plate count agar was added to a 20 ml agar plate. Quarter-inch paper discs treated with 25 µl of 5% HICC in methanol were dried for 15 minutes and placed on the surface of agar plates. Three plates containing the test material were exposed to UVA for 18 hours at a distance of 31 cm and then incubated at 31-35 °C for 48 hours. An additional plate was not irradiated but incubated for 48 hours and served as a control. HICC was considered to have a positive phototoxic response and was observed to have 0.004% of the phototoxic activity of 8-MOP in the 18-hour assay.

Ref. 94

A second *in vitro* phototoxicity assay was performed in S. cerevisiae in which the UVA exposure time was reduced to 7 hours and the distance from the light source to the plates was decreased to 10.5 cm. HICC was observed to have 0.01% of the phototoxic activity of 8-MOP in this 7-hour assay. The addition of benzophenones and sunscreen agents to the vehicle in the 7-hour assay reduced or eliminated the phototoxic activity of HICC.
Ref. DiNardo et al., 1985

HICC was evaluated in two *in vitro* phototoxicity tests. The 3T3 NRU phototoxicity test is a screening method for DNA and cellular damage while the photohaemolysis test is a screening method for phototoxic chemicals that cause oxygen-dependent membrane damage. HICC reportedly showed phototoxicity in the photohaemolysis test but not in the 3T3 NRU test. (Reported on proceedings abstract. no full paper).

Ref. 12

Comment
HICC has some phototoxic potential but there is no evidence of such effects under conditions of use.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

3.3.11. Human data

The elicitation potential of HICC was evaluated by Johansen *et al*. (2003). Eighteen eczema patients (2 male and 16 female) who previously reacted to 5% HICC on patch testing were patch tested with a serial dilution of HICC 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% HICC in a patch test were included in the Repeated Open Application Test.

Patch tests were conducted using a 10-fold serial dilution of HICC from 0.0006% to 6% in ethanol. A 15 µl dose of HICC in ethanol was applied to each patch. These patches were then applied for 2 days to a 0.5 cm^2 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 HICC.

The dose of HICC eliciting a reaction in 10% of the patients was 0.9 µg/cm^2 and the dose eliciting a reaction to 50% of the patients was 20 µg/cm^2 (Johansen *et al*., 2003).

In the Repeated Open Application Test, two drops, (equivalent to 30 mg) were applied to 3 cm^2 area on the volar aspect of the lower arm. A concentration of 0.5% HICC in ethanol was applied to one area, twice daily, for 2 weeks. If no reactions occurred, applications were continued with 3% HICC 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% HICC. In these 11 patients, the median amount applied was 15.3 µg HICC/cm^2/application (range 3.4-22.2). Five patients were positive to 3%HICC; the median amount applied to these 5 patients was 126.2 µg HICC/cm^2/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 HICC in the 7 control subjects and there were no reactions to the vehicle control in either the patients or the control subjects.

Ref. 37

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 HICC in a patch test in the 12 months prior to the Use Test. Patch tests were conducted with 5% HICC in petrolatum during the first week of immersion to confirm reactivity to HICC. 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 HICC. A finger from the other hand was immersed in a solution that did not contain HICC 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% HICC in ethanol in water 10% (v/v). If no reactions occurred, patients were exposed to a solution of 0.025% HICC 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 HICC. 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 HICC. The authors concluded that there was no association between immersion of a finger in a solution containing HICC and development of clinically visible eczema.

Ref. 27

Patch test studies with HICC

Test	Concentration	Subjects	Results	Reference
Multicentre study conducted over a 4-year period by the IVDK, a network of > 40 departments of dermatology in Austria, Germany and Switzerland.	5% in petrolatum	35 582 eczema patients	n=836 (2.3%) positive patch test reactions	AR.6
The frequency of contact allergy to HICC among patients tested by the Danish Contact Dermatitis Group from 2003-2007.	5% in petrolatum	18 789 eczema patients (12 301 female, 6488 male)	the prevalence of reactions ranged from 2.1% (2003) to 2.8% (2007)	6
Patch tests were conducted between January 2002 and May 2006.	5% in petrolatum	53 females with chronic anogenital complaints	2/53 reactions were observed	96
Multicentre study conducted over a 2-year period by the IVDK, a network of 40 departments of dermatology in Austria, Germany and Switzerland.	5%	21 325 patients	502 patients reacted	93
A multicentre trial was conducted in 6 centres in North America between January 1 and December 31, 2003	0.5%, 1.5% and 5% in petrolatum	1603 patients (67% were female) with eczematous dermatitis	7/1603 (0.4%) reactions to with 5% 6/1603 (0.3%) reactions with 1.5% 3/1603 (0.2%) reactions with 0.5%	4
Patch tests were conducted over a 2-month period.	5% in petrolatum	170 cosmetic dermatitis patients	2/170 reactions	25
Multicentre trial conducted in 6 centres in Europe.	0.5%, 2.5% and 5% in petrolatum	dermatitis patients	1/22 reactions to 0.5% 18/49 reactions to 2.5% plus 10 questionable reactions 26/70 reactions to 5% plus 10 questionable reactions	18 19 17
The IVDK analyzed patch test results from 2001-2002.	no dose reported	220 female hairdressers with occupational dermatitis and 303 female dermatitis patients	a total of 2.9% of the hairdressers reacted to HICC [95% CI - 3.7% (0.0-7.5%)] a total of 2.2% of the non-hairdressers	95

Test	Concentration	Subjects	Results	Reference
		who had never been hairdressers	reacted to HICC [95% CI - 1.6% (0.2-3%)]	
Patch tests were conducted in 9 dermatology departments and 1 cosmetic company over a one year period.	no dose reported (no further details reported)	422 male and female dermatitis patients (83% were female)	HICC showed high positive responses (no further details reported)	12 1
Patch tests conducted over a 12-month period.	5% in petrolatum	766 consecutive dermatitis patients	16/766 reactions	3
Multicentre using consecutive eczema patients.	5% in petrolatum	254 males and 404 female consecutive hand eczema patients were tested with fragrance components from 59 products intended for hand exposure	14/658 reactions	28 26
Multicentre conducted in 6 centres in Europe.	5% in petrolatum	1855 consecutive dermatitis patients	50 reactions were observed 20 questionable reactions were also observed	16 15
Multicentre in 20 departments of dermatology in Germany.	5% in petrolatum	3245 consecutive dermatitis patients	62/3245 reactions	20
Patch test	1% in petrolatum	dermatitis patients who previously reacted to 5% HICC in a closed patch test	25/37 reactions were observed	15
A multicentre study conducted to determine the causative allergens in cosmetic products.	2% in petrolatum	17 male and 102 female dermatitis patients were tested 8-10 weeks after initial diagnosis of cosmetic contact dermatitis	1/119 reactions	10
Multicentre study on 48 fragrance materials.	1% and 5% in petrolatum	22 male and 84 female dermatitis patients tested with a fragrance tray	1/106 reactions at 1% 3/106 reactions at 5%	14
Patch test conducted according to ICDRG recommendations.	no dose reported	patients with cosmetic contact dermatitis	1/35 reactions	9
Closed patch test.	5% in petrolatum	31 cosmetic dermatitis patients 7 facial melanosis patients 17 non-cosmetic dermatitis/eczema	no reactions in cosmetic dermatitis patients (0/31) no reactions in facial melanosis patients (0/7)	33

Test	Concentration	Subjects	Results	Reference
		patients 9 control subjects were also tested	 no reactions in non-cosmetic dermatitis patients (0/17) no reactions in control subjects (0/9)	
Closed patch tests conducted from 1978-1980.	5% vehicle not reported	16 cosmetic dermatitis patients 27 eczema and dermatitis patients 10 control subjects were also tested	no reactions in cosmetic dermatitis patients (0/16) no reactions in eczema and dermatitis patients (0/27) no reactions in control subjects (0/10)	34
Patch test.	5% vehicle not reported	a 22-year-old man who developed dermatitis in the axillary area after using a solid roll-on antiperspirant was tested with the constituents of the antiperspirant	patient did not react to HICC	40
Patch test.	0.075%, 0.125% and 0.25% in petrolatum and 6.5% in dipropylene glycol	a 28-year old male subject who had reacted to a deodorant was tested with constituents of the deodorant 20 control subjects were also tested.	subject reacted to HICC at all dose levels no reactions were observed in the control subjects (0/20)	23
Patch test.	10% in petrolatum	a 20-year old female with severe dermatitis in both axillae related to the use of an underarm deodorant was tested with the components of the deodorant	patient reacted to HICC	24
Patch test.	5% in petrolatum vehicle not reported	76 year old female with pruritic chronic dermatitis of the neck and face	patient reacted to HICC	98
Patch test.	2% in petrolatum	a 50-year old female with severe eczema produced by an eau de toilette was tested with the	patient reacted to HICC	22

Test	Concentration	Subjects	Results	Reference
		components of the eau de toilette		
Patch test.	1% in petrolatum	a 37-year old female with cosmetic allergic contact dermatitis	patient reacted to HICC	41
Patch test.	no dose reported	1 geriatric nurse	patient reacted to HICC	106

Krautheim et al. report patch test results with HICC (5% pet.) in 37 270 eczema patient from the German/Swiss/Austrian IVDK network. Overall, 836 (2.4%) positive patch test reactions were observed, of which 108 would not have been detected if only fragrance mix II (which contains HICC at 2.5%) had been tested.

Ref. AR.6

The frequency of contact allergy to HICC among 18 789 eczema patients (12 301 female, 6488 male) patients tested by the Danish Contact Dermatitis Group from 2003-2007 was reported. Patch tests were conducted with 5% HICC in petrolatum using Finn Chambers® (Epitest, Tuusula, Finland) on Scanpor® (Norgesplaster A/S, Vennesla, Norway). Patches were applied for 2 days and reactions were read at a minimum on day 3. The prevalence of reactions ranged from 2.1% (2003) to 2.8% (2007).

Ref. 6

Schnuch et al. reported the results of studies conducted by the IVDK. During a 2-year period, from January 2003 and December 2004, 21 325 patients were patch tested with 5% HICC. The materials were applied for 24 or 48 hours. Reactions were read until at least 72 hours based on international standards. Reactions to HICC were observed in 502 patients.

Ref. 92

A multicentre trial was conducted in 6 centres in North America between January 1 and December 31, 2003. Patch tests were conducted on a total of 1603 patients (67% were female) with eczematous dermatitis. The test materials were applied to the back for 2 days using Finn Chambers® on Scanpor® tape. Reactions were read 48 hours after application and again at 4 to 7 days after application. The NACDG baseline series and HICC (99.7% pure) were tested. HICC was tested at 0.5%, 1.5% and 5% in petrolatum. Allergic reactions were observed in 7/1603 (0.4%) patients with 5% HICC; in 6/1603 (0.3%) patients with 1.5% HICC and in 3/1603 (0.2%) patients with 0.5% HICC

Ref. 4

Patch tests were conducted on cosmetic dermatitis patients over a 2-month period. HICC at 5% in petrolatum was applied to the back for 48 hours using Curatest®. Reactions were read according to ICDRG at 48 and 96 hours. Reactions were observed in 2/170 patients.

Ref. 25

A multicentre trial was conducted in 6 centres in Europe between October 2002 and June 2003. Patch tests were conducted on 1701 consecutive patients. The test materials were applied to the back for 2 days using Finn Chambers® on Scanpor® tape. Reactions were read at most centres on day 2 and 4; the second reading usually at day 3 or 4 was used to evaluate positive results. The baseline series and Fragrance Mix II (FM II), which contained

HICC, were tested. Three different formulations of FM II were testing using various concentrations of the constituents. Patients reacting to FM II were tested with the constituents of the mix. In patients who had reacted to FM II, HICC was tested at 0.5%, 2.5% and 5% in petrolatum. One reaction was observed in 22 patients that were tested with 0.5% HICC; 18/49 reactions plus 10 questionable reactions were observed with 2.5% HICC and 26/70 reactions plus 10 questionable reactions were observed with 5% HICC.

Ref. 17, 18, 19

A multicentre study in Korea to determine the frequency of responses to selected fragrances in patients with suspected contact allergy to fragrances. From April 2002 to June 2003, 5% HICC was tested in 422 patients (70 male/352 female) as part of a fragrance series being tested in 9 university hospitals. Patch tests were conducted using Finn Chambers® on Scanpor® tape and reactions were scored according to ICDRG recommendations. Reactions to HICC were observed in 1.7% (7/422) of the patients.

Ref. 1, 12

The IVDK analyzed patch test results from 2001-2002 in patients suspected of having contact allergy from hair cosmetics to determine if the pattern of sensitization was different between hairdressers and clients. Hairdressers were defined as hairdressers who had currently or in the past been diagnosed with occupational dermatitis; clients (303 females) were defined as patients who had never been hairdressers. Both groups had been tested with a hairdresser series. In the 220 female hairdressers, reactions to HICC (no dose reported) were observed in 2.9%; in the 303 clients, reactions to HICC (no dose reported) were observed in 2.2%.

Ref. 95

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

Ref. 105

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% HICC in petrolatum.

Ref. 26, 28

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 HICC. Of these 16, ten also reacted to the Fragrance Mix.

Ref. 3

The German Contact Dermatitis Research Group (DKG) conducted a multicentre trial to assess the frequency of contact allergy to HICC and to examine concomitant reactions to HICC and the fragrance mix. From March 2000 to February 2001, 5% HICC 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 as suggested 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 HICC were observed in 1.9% (62/3245) of the patients. In 3185 patients who were tested with 5% HICC in parallel with the fragrance mix, 300 patients reacted to the fragrance mix and 59 reacted to HICC. Positive reactions were observed to both the fragrance mix and HICC in 40 patients.

Ref. 20

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% HICC in petrolatum.

Ref. 22

A 37-year-old female with cosmetic allergic contact dermatitis was tested with the components of several fragrances. She reacted to 1% HICC in petrolatum.

Ref. 41

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% HICC in petrolatum.

Ref. 24

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. HICC 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. (15, 16). Thirty-seven out of the 50 patients who had reacted to 5% HICC in petrolatum were retested with HICC at a lower concentration, 1% HICC in petrolatum. Of these 37 patients who were retested, 25 reacted to 1% HICC.

Ref. 15

Frosch *et al.* (1995) reported the results of a multicentre study on patch tests with 48 fragrance materials. HICC, 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. 14

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% HICC) in one deodorant and also to the perfume in the second deodorant which also contained HICC. He was later tested with 6.5% HICC in dipropylene glycol and 0.125% and 0.25% HICC in petrolatum and reacted to all three concentrations. Twenty control subjects were also tested with 0.25% HICC in petrolatum and 6.5% HICC in dipropylene glycol and no reactions were observed.

Ref. 23

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% HICC 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.

Ref. 10

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 HICC (no dose reported) which was present in a deodorant cream.

Ref. 9

Appropriate patch test concentrations for HICC 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 HICC 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% HICC 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 HICC. 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 HICC at concentrations greater than 0.25%, now reacted to mixtures of hydroxycitronellal and geraniol, hydroxycitronellal and HICC and hydroxycitronellal, geraniol and HICC. Two other patients who had not reacted to HICC, now reacted to mixtures containing HICC, 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 HICC, geraniol and hydroxycitronellal. A fragrance mixture prepared from equal

amounts of HICC, 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 HICC, 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% HICC, 5% geraniol, 5% hydroxycitronellal) however this subject used a medicated shampoo to treat her seborrhoeic 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.

Ref. 5

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% HICC (vehicle not reported).

Ref. 40

In human patch test data from the period 1978-1980, 5% HICC 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% HICC 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. 34

Comment

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the SCCNFP's 1999 opinion on fragrance allergy in consumers (AR.4). In total, reports of about 1500 cases have been published in the scientific literature. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

In patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (without a decreasing trend from 2003 to 2007; in 70% of the cases the reaction was of current relevance, i.e. causing disease (AR.1)). This is in agreement with the results of a recent German study with HICC, 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (92, AR.2). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (38).

On the basis of the high frequency of allergy to HICC, in 2003 the SCCNFP recommended 0.02% (200 ppm) as the maximum amount of HICC in cosmetic products (AR.5). This was not implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry (IFRA) has its own safety guidelines. Up to 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to a risk assessment model applied by the fragrance industry to different levels in 11 different product types derived from the quantitative risk assessment (QRA). Limits from 0.11% in lip products to 1.5% in hair styling was given; in 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material." (AR.3). An overview of the IFRA restrictions is given in the table below.

Table 3-1: **Restriction for HICC independent of the QRA according to AR.3**

IFRA QRA CATEGORY	Product type that drives the category	consumer exposure level 2003–2008	IFRA Standard July 2008	IFRA Standard July 2009
Category 1	Lip products	1.5%	0.11%	0.02%
Category 2	Deodorants/ antiperspirants	1.5%	0.15%	0.02%
Category 3	Hydroalcoholics for shaved skin	1.5%	0.60%	0.2%
Category 4	Hydroalcoholics for unshaved skin	1.5%	1.5%	0.2%
Category 5	Hand cream	1.5%	1.0%	0.2%
Category 6	Mouthwash	1.5%	1.5%	Not applicable*
Category 7	Intimate wipes	1.5%	0.3%	0.02%
Category 8	Hair styling aids	1.5%	1.5%	0.2%
Category 9	Rinse-off hair conditioners	1.5%	1.5%	0.2%
Category 10	Hard surface cleaners	1.5%	1.5%	0.2%
Category 11	Incidental or non-skin contact	15%	Not restricted	Not restricted

HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; QRA, quantitative risk assessment.
*Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the initial version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany, AR.7) and 2003-2011 (Danish contact dermatitits group, AR.8), respectively. The first analysis identified a slight decrease, which was considered "not overwhelming in absolute terms", namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

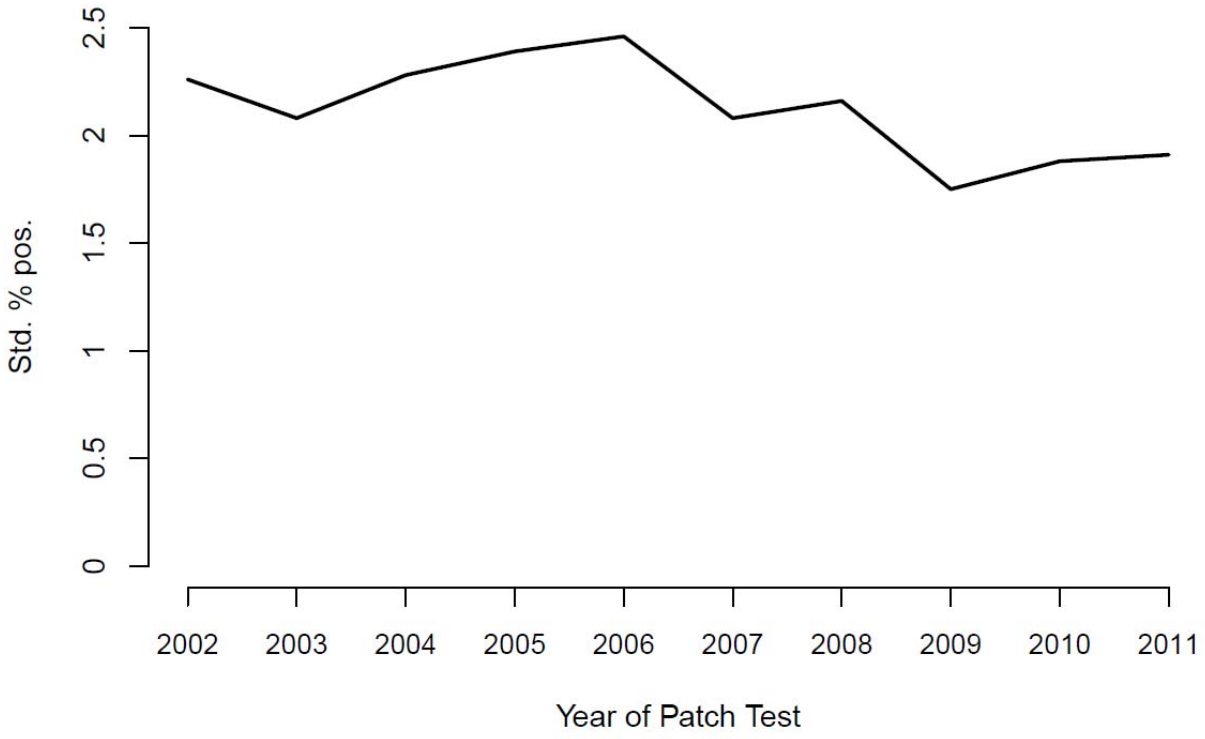


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

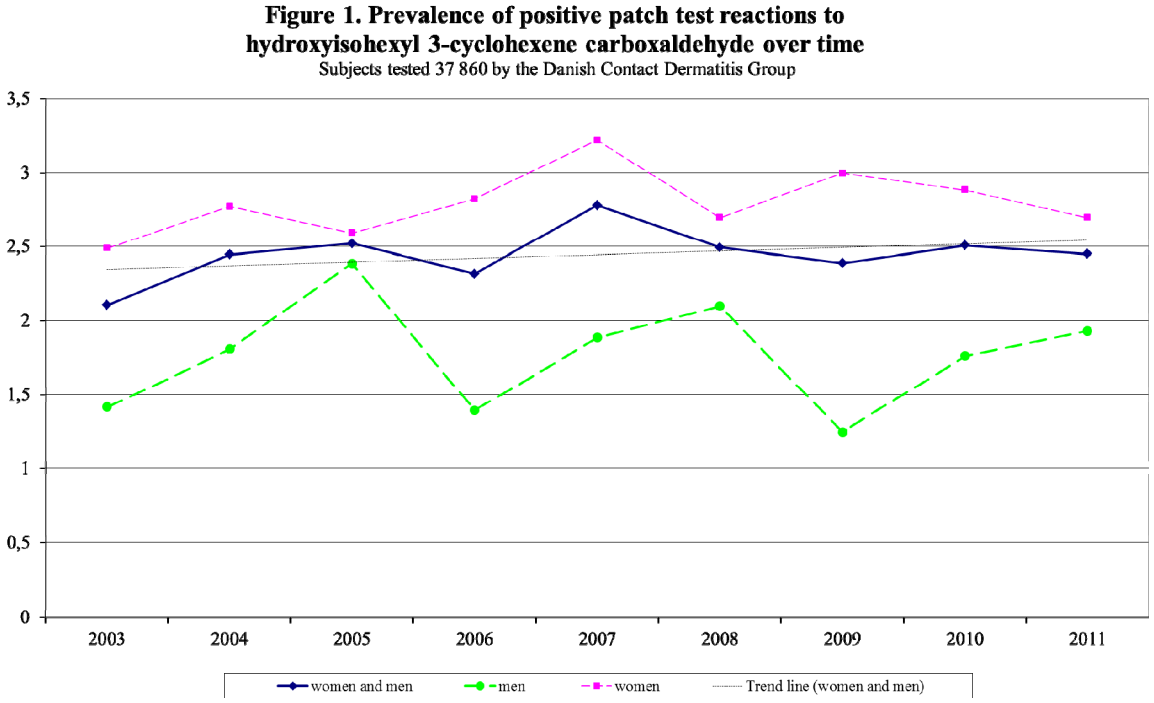


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

AR. 7, 8

3.3.12. Special investigations

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

HICC is a fragrance ingredient used to perfume both cosmetic products and non-cosmetic products such as household cleaners and detergents.

Irritation, sensitisation
Although HICC at higher exposures may have some irritant potential for skin and eye, under conditions of actual use, no irritant effect is to be expected. It has some phototoxic potential, which may not be relevant for the exposures encountered from cosmetic products HICC is clearly demonstrated to be a contact allergen in experimental models. The EC3 value of 17.1% categorises it as a moderate skin sensitizer. However, this result has to be viewed with consideration of the epidemiology of contact allergy to it.
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention. In the 2003 opinion of the SCCP, it was stated that 200 ppm of the substance would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation. Unfortunately, up to the present, the prevalence of contact allergy to HICC remains high in the European consumer.

Dermal absorption
The overall skin absorption values for 1.5% HICC in ethanol/water (70/30), (amounts permeated and amounts in the epidermis), were 14.3 ±SD 2.98% and 36.4 ±SD 8.5% of the applied dose under unoccluded and occluded conditions, respectively. These data show a high dermal absorption from an hydroalcoholic formulation. Absorption may be even higher from other cosmetic formulation types; this is of concern. No MoS calculation was calculated, because the vehicle and concentration were not representative.

General toxicity
The acute toxicity of HICC is low by oral route (LD 50 > 4 g/kg bw in rats) and by dermal route (LD 50 > 5 g/kg bw in rabbits). Signs of dermal irritation were reported in all treated rabbits.

In a 28 days oral (gavage) toxicity study in rats, the NOAEL is considered by the SCCS to be 15 mg/kg bw/d, based on modifications of biochemical parameters observed in males and females at the dose of 150 mg/kg bw/d and the effects on liver (increase in liver weight and hepatocyte enlargement). These modifications may be considered as early indicators of liver toxicity observed at higher doses. A adjustment factor of 3 is used by the SCCS to take into account the duration of the study.

No subchronic or chronic toxicity study was provided.

Based on the reproductive toxicity studies, the NOAEL for maternal toxicity is 100 mg/kg bw/day. The NOAEL for reproductive and developmental toxicity was considered to be 25

mg/kg/day. Treatment of the dams with 500 mg/kg bw/day HICC only during the lactation period resulted in quantifiable levels of HICC in the milk which was associated with increased incidences of skin peeling in the F1 generation pups. The clinical observation of skin peeling resolved by postnatal day 35, two weeks into the post weaning recovery period.

No teratogenicity study was provided

Mutagenicity
Overall, the genotoxicity of hydroxyisohexyl 3-cyclohexene carboxaldehyde is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Hydroxyisohexyl 3-cyclohexene carboxaldehyde, tested in 2 independent gene mutation tests in bacteria, did not induce an increase in the mutant frequency. A gene mutation test in mammalian cells was not performed. In an *in vitro* chromosome aberration test hydroxyisohexyl 3-cyclohexene carboxaldehyde induced an increase in cells with chromosome aberrations.
The positive result in the *in vitro* chromosome aberration test could not be confirmed in an *in vivo* assay in mice covering the same genotoxic endpoint. Consequently, hydroxyisohexyl 3-cyclohexene carboxaldehyde can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No carcinogenicity study was provided.

4. CONCLUSION

- *Does the SCCS consider, with the data provided that 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde is safe for the consumers, when exposed to 0.02% 3- and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde in lip products, deodorants and antiperspirants and 0.2% 3- and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde in other cosmetic products except oral products?*

HICC has for more than 10 years been recognized as an important contact allergen in humans with more cases of contact allergy documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC also had reactions to cosmetics, which contained or were likely to contain HICC.

Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient.

The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

Does the SCCS have any other scientific concerns of the use of HICC in cosmetic products based the data provided?

/

5. MINORITY OPINION

6. REFERENCES

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