Human & Environmental Risk Assessment on ingredients of European household cleaning products

HYDROXYCITRONELLAL
3,7-dimethyl-7-hydroxyoctanal
(CAS 107-75-5)

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1. CONTENT

2. EXECUTIVE SUMMARY

Hydroxycitronellal, a fragrance ingredient used to impart a pleasant floral odour to numerous consumer products, has been chosen for a full risk assessment principally because of its known skin sensitising properties. While this risk assessment attempts to address all possible endpoints, the low volumes of use of hydroxycitronellal and low levels of inclusion in these consumer products have led to this risk assessment giving a preponderant emphasis to dermal sensitisation.

Hydroxycitronellal (3,7-dimethyl -7 hydroxyoctanal) CAS 107-75-5, EINECS 203-518-7 is low molecular weight (172) substance that is generally a viscous liquid although it congeals at temperatures below 23°C. It has considerable water solubility (23.8 g/l) and low lipophilicity (log Pow: 1.5-2.2). It has a low estimated vapour pressure of 0.76 Pa at 25°C and a low calculated Henry's constant (log H: -2.26).

Hydroxycitronellal is used as an ingredient in fragrances and is found in a wide variety of consumer products. These include cosmetics like soaps, shampoos, detergents, cosmetics and perfumes and household cleaning and maintenance products. Maximum levels of use in these latter products are 70 ppm in laundry detergents, 70 ppm in fabric conditioners, 90 ppm in dish-washing products and less than 10 ppm in toilet cleaners and hard surface cleaners.

Hydroxycitronellal used in Europe is produced primarily inside the European Union in quantities estimated to be 88,000 kg/year. It is estimated that 35% of this (35,000 kg/year) is used in household cleaning and maintenance products.

Environmental Assessment

Exposure: The current risk assessment is made according to the "HERA detergent scenario" and the EUSES regional methodology. Highest regional levels were calculated to be c. 1.6×10^{-5} mg /kg in sediments, 8×10^{-6} mg/l in surface water and 1.4×10^{-10} mg/m^3 in air.

Hazards: Hydroxycitronellal is readily biodegradable. Acute toxicity studies showed that it was harmful to algae (96h- EC50: 68 mg/l) but not to daphnids (48h-EC50: 410 mg/l).

Possible no effect levels: In the absence of test data, assessment factors and QSARs have been used to give PNECS of 7.8 µg/l for aquatic organisms, 9.6 µg/kg bw for terrestrial organisms and 17 µg/kg bw for sediment-dwelling organisms.

Risk characterisation: Margins of exposure are well below 1 for all environmental compartments (1×10^{-3} for aquatic organisms in regional water, 1.2×10^{-6} for terrestrial organisms and 9×10^{-4} for organisms in sediments. Even if the total usage volume of 88 tonnes/year (i.e. including use in cosmetics), these regional risk characterisation ratios are all less than 1×10^{-3}.

Conclusion: Current use levels and volumes of hydroxycitronellall in household cleaning products does not concern with regard to possible effects on the environment.

Human Health Assessment

Consumer exposure: This risk assessment has been restricted to direct or indirect exposure to consumers arising from the use of laundry detergents, fabric conditioners, hard surface cleaners,

toilet cleaners, cleaning sprays and dish-washing products. In addition to considering exposure in terms of the quantities potentially entering the body, this assessment has focused on exposure in terms of the quantity likely to be deposited on the skin surface as this is the exposure factor that is critical to the induction of allergic contact dermatitis.

Highest exposures: Accidental exposure from splashes of spills (0.9 μg/cm^2), represent the highest potential skin exposure doses likely to induce or elicit allergic contact sensitization. Hand washing using laundry pre-treatment liquids is estimated to give the highest levels of direct or indirect exposure in terms of quantities penetrating the skin (0.14 μg/kg bw/day). Total aggregate systemic exposure from all routes and all exposure scenarios is estimated to not exceed 0.17 μg/kg bw/day.

Hazards: Studies on animals and humans demonstrate that hydroxycitronellal is a skin sensitiser. This is substantiated by clinical data that show widespread under-lying allergy to hydroxycitronellal although very few cases of allergy are clearly attributable to the presence of hydroxycitronelal in any specific consumer products.

Hydroxycitronellal has a low order of acute toxicity by the oral and dermal routes. Inhalation is not considered a significant route of exposure. Systemic toxicity studies have shown that levels of 400 mg/kg/day are well tolerated by rats over two years although these studies do not meet modern testing requirements. Hydroxycitronellal is negative in bacterial and mammalian genotoxicity screens and was not tumorigenic in the above-mentioned 24-month feeding study.

Hydroxycitronellal shows low to moderate skin and eye irritancy while a limited inhalation study has shown some irritancy by this route too.

Critical end-points and threshold levels: Skin sensitisation and systemic toxicity were considered to be the critical end-points. A No Expected Sensitization Level (NESL) of 2.95 mg/cm^2 has been determined using a “weight of evidence” approach from a large number of predictive tests carried out on human and animal subjects. There is evidence to show that although the threshold for elicitation of allergic responses in prior-sensitised individuals may be as low as 1 μg/cm^2, these endpoints cannot be used in risk assessment as they are neither reliable nor unique determinants of elicitation.

In the absence of a reliable NOAEL for systemic toxicity, two measures were taken as a basis for risk assessment. One was a NOEL estimated by JECFA to be 250 mg/kg bw/day. The other was the Threshold of Toxicological Concern (TTC) of 30 μg/kg bw/day based on a large data set NOAELs of substances that have been similarly classified chemical structures.

Risk characterisation: Margins of exposure for skin sensitization induction from different exposure scenarios were found to vary between over a million and 3,000. Aggregate margins of exposure for systemic effects from all products combined were over one million based on the NOEL and above 170 based on the TTC (which already incorporates other safety factors).

Conclusion: The use of hydroxycitronellal at current levels in household cleaning products does not raise any safety concerns with regard to its potential to cause allergic contact dermatitis and adverse systemic effects.

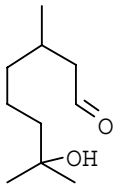
3. SUBSTANCE CHARACTERISATION

3.1. CAS No and grouping information

Hydroxycitronellal is an aldehyde saturated which also contains a tertiary alcohol. Although hydroxycitronellal is not very substantive, it is commonly used in fragrance formulations for household products such as: detergents, fabric conditioners and other cleaning products. On the other hand, hydroxycitronnellal is an important ingredient for cosmetics because of its typical sweet, floral and lily-type odor.

The chemical structure, CAS number and chemical name is in table 1:

Table 1. Identification

INCI name: Hydroxycitronellal	CAS: 107-75-5 EINECS: 203-518-7
Chemical structure: $C_{10}H_{20}O_2$  Physical state: colorless to very pale yellow liquid at room temperature.	Other names: Octanal, 7-hydroxy-3,7-dimethyl (CAS) Citronellalhydrate Laurinal, Laurine, Oxydihydrocitronellal, 3,7-dimethyl-7-hydroxyoctanal

3.1 Chemical structure and composition

The environmental behaviour of a substance is determined by the physical chemical properties. These include the solubility in water, vapour pressure and the octanol/water partition coefficient. Some of these properties were estimated by so-called QSARs (EPIWIN). The estimation is based on molecular fragments. The reliability of the data can be further improved by empirical data.

The high water solubility and low partition coefficient of hydroxycitronellal would suggest low potential for bioaccumulation and moderate concerns for the environmental compartment.

Table 2. General properties

Molecular weight	172.27				
Melting point	23°C			Calculated	Epiwin
Boiling point	241°C			Measured	FMA
Flash point	104°C			Measured	BBA, 1993
Vapour pressure	0.76 Pa	At 25°C		Calculated	Epiwin

Log Pow *Log Pow*	1.5 *2.17*	 *At 40°C*		Measured *Measured*	*(Procter and Gamble Company, 1996)* *Firmenich, 2003 (non GLP)*
Water solubility *Water solubility*	8913 mg/L *23800 mg/L*	At 25°C *At 23°C*		Measured *Measured*	Givaudan *Firmenich, 2003 (non GLP)*
Density *Density*	0.920 – 0.925 *0.918 – 0.923*	At 20°C *At 25°C*		Measured *Measured*	FMA *FMA*

Values in italic are only indicative and are not used in the risk assessment.

The Henry's constant: Molecular Weight * Vapour Pressure/water solubility = 0.0147

Log H = -1.83

3.3 Manufacturing & production/volume

The volume of hydroxycitronellal is based on a survey conducted on volumes used in compounding, carried out by the International Fragrance Association (IFRA) in 2002. This study was further restricted to compounding intended for sale in the current 15 member countries of the European Union as well as Norway and Switzerland.

Responding manufacturers were asked to give a value for total hydroxycitronellal use in all fragrance formulations and also for use in the following household laundry and cleaning products: laundry detergents, laundry pre-treatment products, fabric softeners, hard-surface cleaners, hand dishwashing products and toilet cleaners: this was estimated by IFRA to be 35% of the total volume (W.W.Emmons and J.G.Marks, 1985).

Table 3. Use volumes in Europe (IFRA survey, 2002)

Year	IFRA global volume	Average percentage used in household & detergent product (IFRA)	Household & detergent volume
2002	88 tonnes/year	35%	31 tonnes/year

The majority of the total European hydroxycitronellal tonnage, which includes uses outside the scope of HERA, is ultimately released down-the-drain, where depending on treatment it may reach the environment. Thus this risk assessment also includes an overall assessment using the total European usage estimate of 88'000 kg/year.

3.4 Use applications summary

Hydroxycitronellal is used as an ingredient in commercial preparations intended to be used as fragrances in a wide variety of consumer products such as perfumes, cosmetics, household and laundry cleaning products and air fresheners. These commercial preparations are not sold retail. The level of hydroxycitronellal in household cleaning and laundry products is limited by the low level of fragrance used in these products and by its low "substantivity" by which its high water solubility makes it easily rinsed off the surfaces being cleaned. Maximum levels of hydroxycitronellal in household cleaning products have been collected from major producers of these products and are 70 ppm (0.007%) in laundry detergents, 70 ppm (0.007%) in fabric conditioners, 90 ppm (0.009%) in dishwashing products and less than 10 ppm (0.0001%) in surface cleaners and toilet cleaners (AISE and HERA, 2004).

The IFRA has applied a risk management quantitative limit of 1% of hydroxycitronellal in the final consumer products (cosmetics, household cleaning and laundry products and other fragranced consumer products) (IFRA, 2004).

4. ENVIRONMENTAL ASSESSMENT

4.1. Environmental exposure assessment

The following risk assessment is based on the estimated tonnage of 31'000 kg/year in HERA applications (W.W.Emmons and J.G.Marks, 1985).

It is recognized that the majority of the total European tonnage is ultimately released in the same way as the HERA volume, down-the-drain to the environment. As such, although not within the scope of HERA, a more conservative assessment using the total European usage estimate (88’000 kg/year) is also presented in an addendum.

Exposure Pathways and Detergent Scenario

The "HERA detergent scenario" was used for the environmental exposure calculations. The entire tonnage was assumed to follow the domestic down-the-drain pathway to sewage treatment and to the environment. Releases from production and formulation activities fall outside of the scope of HERA and were not explicitly considered, at the local level, although both production and formulation losses are included in the regional risk assessment. For the calculation of the EUSES (European Union System for the Evaluation of Substances) regional tonnage, 7% of the EU tonnage was assigned to the region (replacing the default 10%), and the local emissions were not increased by the default factor 4, but by a factor of 1.5. (Chapter 2.6 of the HERA methodology document. - www.heraproject.com).

4.1.1. Environmental fate

The review of degradation data was based on proprietary test data submitted to the Research Institute for Fragrance Materials Inc. As the quality of the reports is variable, standard criteria were applied to determine the quality of data obtained from these study reports (Klimisch *et al.*, 1997).

Biodegradation Properties

The two available test indicate that hydroxycitronellal is readily biodegradable:

1. The ready biodegradability was determined using a CO_2 evolution test (OECD 301 B). The test was carried out on hydroxycitronellal at 10.0 mg/l and gave 93.7% biodegradation after 28 days. The pass level for biodegradability is 60% of $ThCO_2$ production. Hence, it was concluded that hydroxycitronellal is readily biodegradable (Quest Int.Ltd., 1994). This test follows the OECD guideline 301B and can be classified as reliable 1 according to Klimisch scoring (Klimisch *et al.*, 1997).

In another study, hydroxycitronellal at an initial dose of 52.5 mg DOC/l , was incubated with activated sludge from a local sewage works for 19 days. This study was conducted per Method F in The Assessment of Biodegradability (1981) in the "Blue Book" series and the progression of degradation was measured in terms of dissolved organic carbon. By this method, 99.8% biodegradation had occurred by day 19 (Bush Boake Allen, 1990). No further details were given in the RIFM database. However, this result confirms the ready biodegradability given by the OECD test above. A score of 2 according to Klimisch is attributed to this test [reliable with restriction (Klimisch *et al.*, 1997)]

4.1.2. Removal

SimpleTreat™ calculation

Due to the absence of measured data on the removal of hydroxycitronellal in sewage treatment plants, only the tier-1 estimate of removal could be used. This follows the default EUSES calculation that uses SimpleTreat™ model.

A SimpleTreat™ calculation was used to determine removal of hydroxycitronellal in waste-water treatment as well as its partitioning between air, water and sludge by taking relevant physico-chemical parameters detailed in section 3.2 into account. These calculations were based on the default rates assigned for readily biodegradable chemicals.

Table 4. Fate of chemicals in a wastewater treatment plant based on the Simple Treat Model

Fraction of WWTP emission to				
	Air	Surface water	Sludge	Degraded
Hydroxycitronellal	0%	13%	0%	87%

4.1.3. Monitoring

No data exist from the monitoring of concentrations of hydroxycitronellal.

4.1.4. PEC Calculations

EUSES was applied to calculate the regional and local exposure to hydroxycitronellal using the following parameters:

Industry category:	005 Personal / domestic use
Use category:	009 Cleaning/washing agents and additives
Fraction of tonnage for application:	100% to use as cleaning products
Fraction of chemical in formulation	1% for cleaning products
Production:	No
Formulation:	No
Processing:	No
Private use:	Yes
Recovery:	No

Use Pattern: Private Use - cleaning products

Fraction of tonnage released to air:	0
Fraction of tonnage released to waste water:	1
Fraction of tonnage released to surface water:	0
Fraction of tonnage released to industrial soil:	0
Fraction of main local source:	7.5×10^{-4} (EUSES default value)
Number of emission days:	365

Predicted Continental and Regional Environmental Concentrations (PECs):

As explained in the HERA methodology document, use of production tonnage for HERA means that the losses to the region during formulation are automatically included when 100% of the production tonnage is released to the environment. The regional and local PECs are as follows:

Table 5: Local and Regional PECs

	PECLocal	PECRegional
Surface water (total) [mg/l]	4.93×10^{-5}	7.91×10^{-6}
Air [mg/m^3]	1.41×10^{-10}	1.41×10^{-10}
Agricultural soil (total) [mg/kg]	3.48×10^{-8}	1.15×10^{-8}
Sediment (total) [mg/kg]	1.06×10^{-4}	1.57×10^{-5}

Sewage (effluent) [mg/l]	4.14×10^{-4}	Not Applicable

Indirect Exposure to Humans:

For the calculation of indirect human exposure via drinking water, the EUSES calculations for indirect uptake via regional exposure can be used (taking into account that drinking water will not be sourced immediately downstream of wastewater emissions). These are shown in table 6, with the calculated uptake from a local source given for comparison. The total human uptake calculated by EUSES is also shown in the table, though known inadequacies with the current model for plant uptake mean that these calculated values will considerably overestimate the uptake from food. Thus these total regional uptake values may not be considered to be acceptably realistic for the HERA Human Health Assessment.

Table 6: Hydroxycitronellal uptake by Humans – as calculated with EUSES

	Regional [mg/kg/day]		Local [mg/kg/day]	
	Drinking Water	Total Food + Water Uptake	Drinking Water	Total Food + Water Uptake
Hydroxycitronellal	2.26×10^{-7}	2.75×10^{-7}	1.41×10^{-6}	1.71×10^{-6}

4.2. Environmental effects assessment

4.2.1. Toxicity

A review of ecotoxicity data was based on reports from BASF. EPIWIN™ calculations were used to complete the data. Here too, standard criteria were applied to determine the quality of data obtained from these study reports (Klimisch *et al.*, 1997).

4.2.1.1 Acute toxicity of hydroxycitronellal to aquatic organisms

The tests describe in this section were conducted by BASF. The reports do not give sufficient experimental details for a score 1 and hence score 2 according to Klimisch criteria [reliability with restriction were assigned to these reports (Klimisch *et al.*, 1997)].

a) Algae EC_{50}

In a study on the acute effects to the alga Scenedesmus subspicatus the following effect levels in terms of concentrations of hydroxycitronellal were determined after 72 hours exposure: EC20 28.9 mg/L, EC50 68 mg/L, and EC90 200.7 mg/L (BASF, 1990).

b) Daphnid EC_{50}

A study on Daphnia magna Straus was carried out at pH 8.0 at 293.7 K using method C2 of Annex V to Directive 79/831/EEC. The following effect levels in terms of concentrations of

hydroxycitronellal were recorded after 48 hours exposure: EC0: 250 mg/L, EC50: 410 mg/L, EC100: greater than or equal to 500 mg/L (BASF, 1989).

c) Fish LC_{50}

No data were located.

d) Other data

In a growth inhibition test according to the method of Bringmann and Kühn, the cell multiplication inhibition test was carried out on Pseudomonas putida. EC10: 625 mg/l, EC50: 950 mg/l and EC90: 1800 mg/l after 17 hours. The toxic limit levels in terms of the concentration of hydroxycitronellal was determined to be 625 mg/l (corresponding to the EC10) (BASF, 1988).

As only two acute toxicity tests to aquatic organisms were available, a comparision was made with EPIWIN calculation. The most sensitive species was derived from this comparision: A summary of the data is given in table 7:

Table 7: Ecotoxicological dataset – determination of the most sensitive species

		BASF result		EPIWIN calculation	Most sensitive species
Acute toxicity to Algae	72-h EC_{50}	68 mg/l	96-h EC_{50}	68.4 mg/l	68 mg/l
Acute toxicity to Daphnid	48-h EC_{50}	410 mg/l	48-h LC_{50}	7.9 mg/l	**7.9 mg/l**
Acute toxicity to Fish		Not available	96-h LC_{50}	13.3 mg/l	13.3 mg/L

Based on the above comparision, the EPIWIN result for acute toxicity to Daphnid was taken as the most sensitive species. This was considered as acceptable with a conservative tier 1 approach.

4.2.1.2 Ecotoxicity – Aquatic: chronic test results

No chronic aquatic data were found/ available

4.2.1.3 Terrestrial – acute test results

No acute terrestrial data were found/ available

4.2.1.4 Terrestrial – chronic test results

No chronic terrestrial data were found/ available

4.2.1.5 Micro-organisms e.g. in Wastewater Treatment

No data were located.

4.2.2. PNEC calculations

Due to a general lack of data on long-term aquatic, terrestrial and sediment toxicity, the EUSES equilibrium partitioning method was used to derive the PNECs for these compartmentsThis was based on the most sensitive species derived in the table 7. This approximation is considered acceptable as a worst case scenario in a tier 1 risk assessment.

Table 8: PNECs

	PNEC
Aquatic organism [mg/l]	7.8×10^{-3}
Terrestrial [mg/kg]	9.6×10^{-3}
Sediment [mg/kg]	0.0168
Sewage (effluent) [mg/l]	95

4.3. Environmental risk characterisation

In the table below, the PEC/PNEC ratios (= Risk Characterization Ratios: RCR) (calculated with EUSES) are given below, based on the different exposure scenarios :

Table 9:Risk Characterization Ratios

	PEC/PNECLocal	PEC/PNECRegional
Aquatic organism [mg/l]	6.32×10^{-3}	1.01×10^{-3}
Terrestrial [mg/kg]	3.62×10^{-6}	1.20×10^{-6}
Sediment [mg/kg]	6.32×10^{-3}	9.35×10^{-4}
Sewage (effluent) [mg/l]	4.36×10^{-6}	Not defined

4.4. Discussion and conclusions

The absence of environmental concerns can be shown for current use levels of hydroxycitronellal in HERA products. The Risk Characterization ratios (PEC/PNEC) are well below 1 for all environmental compartments. These are largely driven by the low volume of use (i.e. tonnage distributed into the environment) of hydroxycitronellal as well as its high water solubility and low octanol/water partition coefficient. In view of the conservative nature of these calculations, it can be assumed that hydroxycitronellal presents a low risk to the environment.

In summary, this environmental risk assessment has demonstrated that the use of hydroxycitronellal in household laundry and cleaning products is safe for the environment and does not cause concern with regard to consumer use.

The tier 1 used is a rough estimate of the overall risk for the environment. This approach was used due to the fact that only very few data were available for hydroxycitronellal and that some were of limited reliability. Hence, the missing data were derived from calculation. Even if the reliability of the estimated data could be questioned, the probability of under-estimation of the risk is low considering the assessment factor of 1000 used to derive the PNECs.

We can most likely consider this tier 1 approach is relevant for hydroxycitronellal as a conservative picture of the overall risk on the environment and say that at this stage there is no need to conduct a tier 2 assessment which would require further testing.

4.5 Addendum – "Total Tonnage" Scenario

4.5.1 Environmental risk characterization

The total tonnage used in Europe is 88 tonnes/year (IFRA survey). An alternative more conservative exposure scenario was included in this risk assessment by assuming this entire tonnage is disposed of down-the-drain. The PEC/PNEC ratios for the HERA tonnage could be extrapolated to the overall tonnage by multiplying the PEC by the appropriate factor (2.8). This approach is valid from a mathematical point of view because of the linearity of the EUSES model.

Table 10: Risk Characterization Ratios

	PEC/PNECLocal	PEC/PNECRegional
Aquatic organism [mg/l]	1.79×10^{-2}	2.87×10^{-3}
Terrestrial [mg/kg]	3.62×10^{-6}	3.41×10^{-6}
Sediment [mg/kg]	1.02×10^{-1}	2.65×10^{-3}
Sewage (effluent) [mg/l]	3.37×10^{-6}	Not defined

5. HUMAN HEALTH ASSESSMENT

5.1. Consumer Exposure

5.1.1. Product Types

In keeping with the scope of the HERA initiative, this human health assessment focuses only on household cleaning products. Hydroxycitronellal is only used as an ingredient of fragrances that are themselves relatively minor ingredients in these types of products (0.8 - 0.2% by weight). As a result of its relatively high water solubility, hydroxycitronellal is not a major building block of the fragrances used in these types of products as it tends to be lost in the rinse water. None the less, it is used in all of the different categories. These include most notably laundry powders (maximum concentration: 70 ppm in the final consumer product), laundry liquids (maximum concentration: 70 ppm in the final product), dish-washing liquids (maximum concentration: 90 ppm in the final product), hard surface cleaning products (maximum concentration: less than 10 ppm in the final product) and toilet cleaning products (maximum concentration: less than 10 ppm in the final product) (AISE and HERA, 2004).

5.1.2. Consumer Contact Scenarios

Based on the product types, the following consumer exposure routes were identified and assessed:

1. Direct skin contact with neat (laundry pre-treatment) or diluted consumer product (hand-washed laundry, hand dish-washing, hard surface cleaning);

2. Indirect skin contact via release from clothes fibres to skin;

3. Inhalation of detergent dust and of the fragrance emanating during product use and afterwards, from cleaned surfaces of fabrics, kitchen-ware and hard surfaces;

4. Oral ingestion of residues deposited on dishes;

5. Oral ingestion of residues in drinking water;

6. Accidental or intentional over-exposure

A key aim of this risk assessment is to examine the possibility of allergic contact dermatitis arising from the use of these products when they contain the highest levels of hydroxycitronellal reported. Contact allergy is induced by single or multiple dermal exposures to substances. The exposure conditions that are critical to the acquisition and elicitation of contact allergies are not those that are taken into account when evaluating the risk of systemic toxicity. There is now an extensive body of evidence to show that the critical “dose” for contact allergy is best expressed in terms of quantity per unit area (Boukhman and Maibach, 2001), (Rees *et al.*, 1990), (Friedmann *et al.*, 1990), (White *et al.*, 1986), (Fowler and Finley, 1995), (Upadhye and Maibach, 1992). This applies unless the area is less than a square centimeter (Rees *et al.*, 1990). For this reason, a separate section of the consumer exposure estimates given below for each type of product, expresses exposure in terms of the quantity of hydroxycitronellal deposited per unit area on the skin. Although penetration into the epidermis is a critical preliminary step in the production of allergic reactions, there is no need to take account of dermal penetration because the tests that are most useful in assessing this risk, all

involve placing hydroxycitronellal on the outer surface of the skin. Where possible, the dose levels that are critical to assessing this risk are therefore expressed in terms of the quantity of hydroxycitronellal per unit area of skin surface. The products examined here that lead most skin contact all contain surfactants and involve dilution and rinsing. Although it can be expected that the rinsing process will lead to a significant reduction in the amount of hydroxycitronellal retained on the skin [for instance, rinsing is considered to reduce exposure to ingredients of shampoos and other "rinse-off products" by a factor of 100 (SCCNFP, 2003)], rinsing has not been taken into account in estimating exposure arising from each use event. Instead, it is assumed that a film of the product remains on the skin after use, thereby permitting hydroxycitronellal to penetrate the skin. It is however assumed that in cases where products are used several times in one day, successive exposures need not be summated because subsequent uses of the product will remove residual hydroxycitronellal remaining on the skin from the previous use.

Estimates of systemic exposure are expressed as quantity of hydroxycitronellal penetrating the skin per unit of body weight per day. For this purpose, it is necessary to use estimates of the dermal penetration flux.

5.1.3. Consumer Exposure Estimates

These are based in part on exposure factors given in the Technical Guidance Document provided by the European Commission for the risk assessment of newly notified substances **(TGD, 1996)** and on a consolidated overview concerning habits and practices of use of detergents and surface cleaners in Western Europe that was issued by the European Soap and Detergent Industry Association, AISE (AISE and HERA, 2004). This table reflects consumers' use of detergents in g/cup, tasks/week, duration of task and other uses of products and is largely the basis for the exposure estimates in the following paragraphs. In some instances, e.g. habits & practices (H&P) of pre-treatment of clothes, additional H&P information for a targeted exposure assessment was directly provided by the member companies of AISE.

For systemic exposure, the dermal penetration coefficient has been derived from *in vitro* studies carried out under occlusion (Tonge, 1995). In calculating this coefficient, the absorption of hydroxycitronellal into the skin (but not passing through it) has also been considered in the interest of conservatism, despite recent evidence (Yourick *et al.*, 2004) showing that substances absorbed into the skin in these *in vitro* studies should not be automatically considered as being ultimately systemically available.

5.1.3.1 **Direct skin contact from hand-washed laundry**

Hand-washed laundry is a common consumer habit. During this procedure, the Hydroxycitronellal containing laundry solution with an estimated product concentration of 10 mg/ml comes in direct contact with the skin of hands and forearms. A hand-washing task typically takes 10 minutes (Table of Habits and Practices - (Barron *et al.*, 1996)). This table also reports a maximum frequency of 18 times per week (3 times/day) when using laundry powder, which seems highly exaggerated but nevertheless is used here as a worst case scenario. The table gives a lower frequency of hand washing with laundry liquid of 10 times per week (1.43 times/day), which still seems exaggerated.

A. Estimation of potential systemic exposure to hydroxycitronellal (Exp_{sys}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00007** (70 ppm) (AISE and HERA, 2004)
C	product concentration:	**0.01** (10 mg/ml) (AISE/HERA, 2002)
Kp	dermal penetration coefficient	**1.2×10^{-3} cm/h*** (Tonge, 1995)
t	duration of exposure or contact	**10 min (0.167h)** (AISE/HERA, 2002)
S_{der}	surface area of exposed skin	**1980 cm^2** (TGD, 1996).
n	product use frequency (tasks per day)	**3** (AISE/HERA, 2002)
BW	body weight	**60 kg**

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* *The dermal penetration coefficient was calculated from the dermal flux (0.22 mg/cm2) which was determined in an in vitro dermal penetration (Tonge, 1995) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution);*

$Kp = (0.22\ mg/cm^2)/(1h \times 184\ mg/cm^3) = 1.2 \times 10^{-3}\ cm/h$

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$Exp_{sys} = F_1 \times C \times S_{der} \times Kp \times t \times n / BW$

Exp_{sys} = [(0.00007) x (10 mg/ml) x (0.0012 cm/h) x (0.167h) x 3 x (1980 cm^2)]/ 60

= 0.014 μg/kg bw/day

B: Estimation of <u>potentially skin-sensitizing</u> exposure to hydroxycitronellal (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00007** (70 ppm) (AISE and HERA, 2004)
C	product concentration:	**10 mg/ml** (AISE/HERA, 2002)
T_{der}	film thickness on skin	**0.01cm** (TGD, 1996), (Vermeire and et al., 1993)

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The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$Exp_{sens} = F_1 \times C \times T_{der}$$

$$Exp_{sens} = [(0.00007) \times (10\ mg/ml) \times (0.01cm)] = \mathbf{0.007\ \mu g/cm^2}$$

5.1.3.2 <u>Direct skin contact from laundry tablets and laundry powder</u>

Placing tablets into the dispenser of the washing machine is unlikely to involve any significant transfer of hydroxycitronellal from the tablet to the skin due to the encapsulated solid form of the product. Furthermore, contact time and contact with a very small area of the palm skin generally regarded as relatively impermeable (Wester and Maibach, 2002). As a result, dermal exposure to hydroxycitronellal from this use is considered to be relatively insignificant.

5.1.3.3 <u>Laundry pre-treatment of clothes</u>

Consumers typically spot-treat clothing stains by hand using either a detergent paste (i.e. water/laundry powder = 1:1) or a laundry liquid, which is applied undiluted (i.e. concentration = 1000 mg/ml) directly on the garment. In this exposure scenario, only the skin surface of the hand (~ 840 cm2) is exposed.

The exposure to Hydroxycitronellal is estimated according to the same algorithm from the HERA guidance document as is used in 5.1.3.1 above using the liquid detergent since this is the highest concentration of Hydroxycitronellal.

A: Estimation of <u>systemic exposure</u> to hydroxycitronellal (Exp_{sys}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00007** (70 ppm) (AISE and HERA, 2004)
C	product concentration:	**1000 mg/ml** (100%) (AISE/HERA, 2002)
Kp	dermal penetration coefficient	**1.2×10^{-3} cm/h*** (Tonge, 1995)
t	duration of exposure or contact	**10 min (0.167h)** (AISE/HERA, 2002)
S_{der}	surface area of exposed skin	**840 cm^2** (TGD, 1996)
n	product use frequency (tasks per day)	**0.71 (= 5/7)**
BW	body weight	**60 kg**

** The dermal penetration coefficient was calculated from the dermal flux (0.22 mg/cm2) which was determined in an in vitro dermal penetration (Tonge, 1995) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution);*

$Kp = (0.22\ mg/cm^2)/(1h \times 184\ mg/cm^3) = 1.2 \times 10^{-3}\ cm/h$

The following algorithm is used to calculate exposure relevant to this end-point:

$Exp_{sys} = F_1 \times C \times S_{der} \times Kp \times t \times n / BW$

Exp_{sys} = $[7 \times 10^{-5} \times (1000\ mg/ml) \times (840\ cm^2) \times (0.0012\ cm/h) \times (0.167h) \times 0.71]/60$

= 0.139 μg/kg bw/day

This exposure estimate is very conservative in that it does not recognize use of water to dilute the detergent, a common practice and the fact that only a fraction of the surface of both hands will actually be exposed.

B: Estimation of potentially skin-sensitizing exposure to hydroxycitronellal (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00007** (70 ppm) (AISE and HERA, 2004)
C	product concentration:	**1000 mg/ml** (100%) (AISE/HERA, 2002)
T_{der}	film thickness on skin	**0.01 cm** (TGD, 1996), (Vermeire and et al., 1993)

The following algorithm is used to calculate exposure relevant to this end-point:

$$Exp_{sens} = F_1 \times C \times T_{der}$$

$$Exp_{sens} = [7 \times 10^{-5} \times (1000\ mg/ml) \times (0.01\ cm)] = 0.7\ \mu g/cm^2$$

5.1.3.4 Direct skin contact from hand dishwashing

A: Estimation of systemic exposure to hydroxycitronellal (Exp_{sys}):

The determination of Hydroxycitronellal exposure from hand dishwashing also uses the algorithm discussed in chapter 5.1.3.1 is used to calculate the dermal exposure to Hydroxycitronellal from hand dishwashing. The following assumptions have been made to address a reasonable worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00009** (90 ppm) (AISE and HERA, 2004)

C	product concentration:	**(2.0 mg/ml)** (AISE/HERA, 2002)
S_{der}	surface area of exposed skin	**1980 cm^2** (TGD, 1996)
Kp	dermal penetration coefficient	**1.2 x 10^{-3} cm/h*** (Tonge, 1995)
t	duration of exposure or contact	**45 min (0.75 h)** (AISE/HERA, 2002)
n	product use frequency (tasks per day)	**3** (AISE/HERA, 2002)
BW	body weight	**60 kg**

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** The dermal penetration coefficient was calculated from the dermal flux (0.22 mg/cm2) which was determined in an in vitro dermal penetration (Tonge, 1995) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution);*

Kp = (0.22 mg/cm^2)/(1h x 184 mg/cm^3) = 1.2 x 10^{-3} cm/h

The following algorithm is used to calculate exposure relevant to this end-point:

Exp_{sys} = F_1 x C x S_{der} x Kp x t x n /BW

Exp_{sys} = [9 x 10^{-5} x (2 mg/ml) x (1980 cm^2) x (0.0012 cm/h) x (0.75h) x 3]/60

= 0.016 μg/kg bw/day

B: Estimation of potentially <u>skin-sensitizing exposure</u> to hydroxycitronellal (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1 weight fraction of hydroxycitronellal in the product **0.00009** (90 ppm)

(AISE and HERA, 2004)

C product concentration: **(1.0 mg/ml)**
(AISE/HERA, 2002)

$\mathbf{T_{der}}$ film thickness on skin **0.01cm** (TGD, 1996),
(Vermeire and et al., 1993)

_____

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sens} = F_1 \times C \times T_{der}}$$

$\mathbf{Exp_{sens}}$ = [0.00009 x (1.0 mg/ml) x (0.01 cm)] = **0.0009 μg/cm^2**

5.1.3.5 Direct skin contact from hard surface cleaning

A: Estimation of systemic exposure to hydroxycitronellal ($\mathbf{Exp_{sys}}$):

During this procedure, the Hydroxycitronellal -containing hard surface cleaning solution comes in direct contact with the skin of the hands. A hard surface-cleaning task takes at maximum 20 minutes (AISE/HERA, 2002). The exposure to Hydroxycitronellal is estimated according to the following algorithm from the HERA guidance document:

$$\mathbf{Exp_{sys} = F_1 \times C \times Kp \times t \times S_{der} \times n / BW}$$

For this exposure estimate, the terms are defined with following values for the calculation considering a worst-case scenario:

$\mathbf{F_1}$ weight fraction of hydroxycitronellal in the product **0.00001** (10 ppm)
(AISE and HERA, 2004)

C product concentration: **(12 mg/ml)**
(AISE/HERA, 2002)

Kp	dermal penetration coefficient	**1.2 x 10^{-3} cm/h*** (Tonge, 1995)
t	duration of exposure or contact	**20 min (0.334 h)** (AISE/HERA, 2002)
S_{der}	surface area of exposed skin	**840 cm^2** (TGD, 1996)
n	product use frequency (tasks per day)	**1** (AISE/HERA, 2002)
BW	body weight	**60 kg**

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* *The dermal penetration coefficient was calculated from the dermal flux (0.22 mg/cm2) which was determined in an in vitro dermal penetration (Tonge, 1995) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution);*

Kp = (0.22 mg/cm^2)/(1h x 184 mg/cm^3) = 1.2 x 10^{-3} cm/h

Exp_{sys} = [0.00001 x 0.012 x 840 x (1.2 x 10^{-3} cm/h) x (0.334 h) x 1] /60

= 0.00067 μg/kg bw/day

Estimation of potentially skin-sensitizing exposure to hydroxycitronellal (Exp_{sens}):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00001** (10 ppm) (AISE and HERA, 2004)
C	product concentration:	**(12 mg/ml)** (AISE/HERA, 2002)
T_{der}	film thickness on skin	**0.01 cm** (TGD, 1996), (Vermeire and et al., 1993)

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sens} = F_1 \times C \times T_{der}}$$

$$\mathbf{Exp_{sens}} = [0.00001 \times (12 \text{ mg/ml}) \times (0.01 \text{ cm})] = \mathbf{0.0012\ \mu g/cm^2}$$

5.1.3.6 Indirect skin contact from wearing clothes

Residues of components of laundry detergents may remain on textiles after washing and can transfer from the textile to the skin. There are no data available showing how much Hydroxycitronellal is deposited on the fabric following a wash process. If 1 kg of clothes retains 600 ml rinse water (Henkel, 2002) and that rinse water contains 2.5 % (ZVEI and IKW, 1999) of the detergent (and thus Hydroxycitronellal) used then the concentration of Hydroxycitronellal in that rinse water can be calculated: 600 ml x 10 mg/ml x 2.5% x 0.007% = 0.01 mg.

If 100% is transferred to the 1 kg of fabric, then the concentration in the fabric will be 0.01 mg/kg. Given the fabric density of 10 mg/cm2 (Procter and Gamble Company, 1996), it can be calculated that the Hydroxycitronellal is present at 1×10^{-7} mg/cm^2.

A: Estimation of systemic exposure to hydroxycitronellal (Exp_{sys}):

On this basis, the following algorithm recommended in the HERA guidance document can be used to estimate the dermal exposure to detergent residues in the fabric:

$$\mathbf{Exp_{sys} = F_1 \times C \times S_{der} \times n \times F_{2\,x}\, F_{3\,x}\, F_4/BW}$$

For the exposure estimate, the terms are defined with the following values for the calculation:

$\mathbf{F_1}$	proportion transferred	**100%**
C	fabric (Hydroxycitronellal) load:	**1×10^{-7} mg/cm^2**
$\mathbf{S_{der}}$	Area of exposed skin:	**17'600 cm^2** (TGD, 2003).
$\mathbf{F_2}$	fraction transfered to the skin	**1%**

(Vermeire and et al., 1993)

F_3	percent weight fraction remaining on skin	**100%** (worst case)
F_4	percent weight fraction absorbed via skin	**50%** (0.052) for 24 hr (Tonge, 1995)*
BW	body weight	**60 kg**

* *the percentage weight fraction absorbed via the skin in 24 hours is taken as 50% based on the in vitro studies (Tonge, 1995) .*

Exp_{sys} = [100% x (1 x 10^{-7} mg/cm^2) x (17,600 cm2) x 1% x 100% x 50%] / 60

= **1.5 x 10^{-7} µg /kg bw day**

B. Estimation of potentially skin-sensitizing exposure to hydroxycitronellal (Exp_{sens}):

C	fabric (Hydroxycitronellal) load:	**1 x 10^{-7}** mg/cm^2
S_{der}	Area of exposed skin:	**17'600** cm^2 (TGD, 2003)
F_2	fraction transfered to the skin	**1%** (Vermeire and et al., 1993)

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$\mathbf{Exp_{sens} = C \times F_2 / S_{der}}$$

$$\mathbf{Exp_{sens}} = [(1 \times 10^{-7}\ mg/cm^2) \times 1\%] / (17'600\ cm^2)$$

$$\mathbf{= 5.6 \times 10^{-11}\ \mu g/cm^2}$$

5.1.3.7 Inhalation of detergent dust during washing processes

According to studies on the release of dust per cup of laundry powder (van de Plassche et al., 1998) on average about 0.27 μg dust is released during consumer manipulation during machine laundering. Taking the worst case assumption that all released dust is inhaled and washing of laundry occurs 3 times daily, the exposure to hydroxycitronellal of an adult with a body weight of 60 kg would be as follows:

$$\mathbf{Exp_{sys} = 7 \times 10^{-5} \times 270 \times 3 / 60 = 9.5 \times 10^{-7}\ \mu g/\ kg\ bw/day}$$

5.1.3.8 Inhalation of aerosols from cleaning sprays

Hydroxycitronellal is present in surface cleaning sprays at concentrations below 10 ppm. The HERA guidance document specifies the algorithm to be used for calculation of consumers' worst-case exposure to Hydroxycitronellal –containing aerosols generated by the spray cleaner.

There is no significant dermal exposure from this type of exposure.

Estimation of systemically exposure to hydroxycitronellal (Exp_{sys}):

F_1	weight fraction of hydroxycitronellal in the product	**0.00001** (10 ppm) (AISE and HERA, 2004)
C'	product concentration in air:	**0.35 mg/m³** * (Procter and Gamble Company, 1996)
Q_{inh}	ventilation rate	**0.8 m³ /h**
t	duration of exposure	**0.17 h** (10 min)

(AISE/HERA, 2002)

n	product use frequency (tasks per day)	**1.0** (AISE/HERA, 2002)
F_7	weight fraction of respirable particles	**1.0** (100% - worst case)
F_8	weight fraction absorbed or bioavailable	**75%**
BW	body weight	**60 kg**

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* *C' was determined by experimental measurements of the concentration of aerosol particles smaller than 6.4 microns in size which are generated upon spraying with typical surface cleaning spray products.*

For systemic exposure, the algorithm is as follows:

$$\mathbf{Exp_{sys} = F_1 \times C' \times Q_{inh} \times t \times n \times F_7 \times F_8 / BW}$$

$$\mathbf{Exp_{sys}} =$$

$$[0.00001 \times (0.35\ mg/m^3) \times (0.8\ m^3/h) \times (0.17\ h) \times 1.0 \times 1.0 \times (75\ \%)] / 60\ kg$$

$$= \mathbf{6 \times 10^{-7}\ \mu g/kg\ bw/day}$$

5.1.3.9 Oral exposure

Oral exposure to hydroxycitronellal can originate from residues on eating utensils and dishes washed in hand dish-washing detergents and from hydroxycitronellal residues taken up via food and drinking water.

A. Oral exposure from food and drinking water

In addition to the described consumer exposure scenarios, oral exposures to FWA-1 can be assumed to originate also from drinking water or milk as well as eating of fish or other aquatic organisms, meat and plant products. Modeling of the oral intake from food and drinking water using EUSES software (European Union System for Evaluation of Substances – see Table 6 in Section 4.1.4) has estimated the human total daily intake via food and drinking water for a male adult (70 kg):

$$\mathbf{Exp_{sys(oral\ via\ food\ \&\ drinking\ water)} = 2.75 \times 10^{-4}\ \mu g/kg\ bw/day}$$

In reality, this exposure estimate must be regarded as overly conservative. A considerable fraction of hydroxycitronellal will be removed from surface water due to biodegradation and further purification during the drinking water treatment process.

B. Indirect exposure via dishwashing residues (hypothetical misuse)

Oral exposure to hydroxycitronellal can originate from residues on eating utensils and dishes washed in hand dish-washing detergents and from hydroxycitronellal residues taken up via drinking water.

The daily exposure hydroxycitronellal from eating with utensils and dishware that have been washed in hand dish-washing detergents can be estimated according to the following factors:

F_1	weight fraction of hydroxycitronellal in the product	**0.00009** (90 ppm) (AISE and HERA, 2004)
C'	concentration of the product in dish wash solutions	**1.0 mg/cm^3** (AISE/HERA, 2002)
$T_{a'}$	amount of water left on dishes after rinsing	**5.5 x 10^{-5} ml/cm^2** (Schmitz, 1973).
S_a	area of dishes in daily contact with food	**5400 cm^2** (FRANCE, 1990)
BW	body weight	**60 kg**

Using these factors the following algorithm gives the exposure:

$$\mathbf{Exp_{sys(oral\ dish\ deposition)} = F_1 \times C' \times T_{a'} \times S_a / BW}$$

$$= [0.00009 \times (1.0\ mg/cm^3) \times (5.5 \times 10^{-5}\ ml/cm^2) \times (5400\ cm^2)]/60\ kg$$

$$= \mathbf{4.5 \times 10^{-4}\ \mu g/kg\ bw/day}$$

5.1.3.10 Accidental or intentional over-exposure

Accidental or intentional over-exposure can occur to all of the product types containing hydroxycitronellal but would not be a factor for repetitive, long-term systemic exposure. Accidental exposure to the skin may occur due to accidental splashes or spills of undiluted formulated products and this could have significance to skin sensitization even though such contact would not be expected to occur in a repeated manner.

Estimation of potentially skin-sensitizing exposure to hydroxycitronellal ($Exp_{sens(accid./\ miss\text{-}use)}$):

For this exposure estimate, the terms are defined with the following values for the calculation of exposure in a worst-case scenario:

F_1	weight fraction of hydroxycitronellal in the product	**0.00009** (90 ppm) (AISE and HERA, 2004)
C	product concentration:	**1.0** (undiluted product)
T_{der}	film thickness on skin	**0.01 cm** (TGD, 1996), (Vermeire and et al., 1993).

The following algorithm is used to calculate exposure relevant to this end-point (assuming a specific gravity of 1.0 for both the product and the solution):

$$Exp_{sens(accid./\ miss\text{-}use)} = F_1 \times C \times T_{der}$$

$$Exp_{sens(accid./\ miss\text{-}use)} = 0.00009 \times 1.0 \times 0.01\ cm = \mathbf{0.9\ \mu g/cm^2}$$

5.1.3.11 Aggregate Systemic Exposure

The overall body burden of consumers to hydroxycitronellal by skin contact through the use of hydroxycitronellal-containing house-hold laundry and cleaning products and by all exposure routes* is calculated to be:

Exp_{sys} = 0.17 μg/kg bw/day

**Considering the contribution of the different routes of exposure, the exposure via the skin represents the major route of exposure (ca. 99.6 % of the total systemic exposure) with the oral route being more prominent (ca. 0.36 % of the total systemic exposure) than the inhalation route (ca. 0.004%).*

The aggregate exposure is an unrealistic, worst-case of the body burden of hydroxycitronellal. It combines several scenarios; each using highly conservative or worst case assumptions and it is virtually impossible that each of these conservative input parameters will apply concurrently in all cases for this overall exposure estimate. It further assumes, again very conservatively, that these unlikely circumstances will be repeated regularly over a substantial period of time.

5.1.3.12 Highest skin exposure for allergic contact sensitisation.

Pretreatment of clothes with a liquid detergent (**0.7 μg/cm^2**) and accidental exposure from splashes of spills (**0.9 μg/cm^2**), represent the highest potential skin exposure doses likely to induce or elicit allergic contact sensitization. Each of these exceeds the exposure doses from all other products by at least two orders of magnitude. In essence, both exposure scenarios are extremely similar and equally improbable. The scenario for pretreatment (hand application with undiluted detergent without any post-application rinsing) is in fact a form of intentional misuse and hence resembles the scenario where accidental exposure to splashed dish washing detergent is also not followed up with any attempt to rinse the product from the skin. Both scenarios are therefore unlikely to occur concurrently. The worse of the two (**0.9 μg/cm^2**) is therefore taken as the scenario that is most likely to lead to the induction or elicitation of allergic contact dermatitis.

5.2. Hazard Assessment

5.2.1. Acute Toxicity

5.2.1.1 Acute Oral Toxicity

On the basis of single dose tests for which few details are available, hydroxycitronellal exhibits low toxicity. No deaths were reported in studies on 10 rats each administered a single dose of 5 g/kg by gavage (RIFM, 1973a).

Conclusion

Hydroxycitronellal shows a low degree of acute oral toxicity.

5.2.1.2 Acute Inhalation Toxicity

No data are available.

5.2.1.3 Acute Dermal Toxicity

No deaths were reported in studies on 2 rabbits each given a dermal exposure of 2 g/kg (RIFM, 1973a).

Conclusion

From limited data, hydroxycitronellal shows a low degree of acute toxicity by the dermal route.

5.2.1.4 Acute Toxicity by intraperitoneal injection

No data were located.

5.2.2. Irritation

5.2.2.1 Skin irritation

a) Skin irritation : animal data

In a study carried out according to the OECD 404 procedure, undiluted hydroxycitronellal (80 mg/ cm^2) gave mean scores for erythema (0.9) and oedema (0.2) (RIFM, 1984) at 24 hours. A second study carried out according to the same procedure gave scores for erythema (0.8) and oedema (0.1) (RIFM, 1985).

The Draize test for dermal irritation in rabbits gave a primary Irritation Index of 0.06 for undiluted hydroxycitronellal (78 mg/ cm^2) (Troy, 1977). However, in another study, open exposure of undiluted hydroxycitronellal (11 mg/cm^2) gave moderate irritation to the skin of rabbits (Motoyoshi *et al.*, 1979). Another Draize test carried out on a 2% solution of hydroxycitronellal in propylene glycol gave a Primary Irritation Index of 0.5 (RIFM, 1972).

Undiluted hydroxycitronellal (5.6 mg/cm^2) was not irritant to the skin of guinea pigs after occlusion for 48 hours in one study but showed moderate irritant effects in another (Motoyoshi *et al.*, 1979). In another study on guinea pigs, daily exposures to undiluted hydroxycitronellal over four days with no occlusion produced a case of marginal erythema in one of ten test animals (Imokawa and Kawai, 1987).

An in vitro study on keratinocytes from male albino rats (Episkin™), the mean cell viability was 81.2% when hydroxycitronellal levels were 20%. This is indicative of mild irritancy (Portes *et al.*, 2002).

Numerous irritancy-screening studies have been carried out preliminary to sensitization testing. In guinea pigs, no irritancy was seen in several studies in which undiluted hydroxycitronellal was applied under occlusion for 6 hours (RIFM, 1987a). The minimum irritant concentration to guinea pig skin in one Open Epicutaneous Test was 10% (12.5 mg/cm^2) while in another carried out under different conditions, it was 25% (6 mg/cm^2) (Klecak *et al.*, 1977).

Conclusion

Undiluted hydroxycitronellal is not irritant to the skin according to the official criteria for classification. However, there is some evidence that high skin loadings, particularly under occlusion can give rise to signs of skin irritancy.

b) Skin irritation: *in vitro* data

Studies on human skin using some incompletely validated *in vitro* systems gave mixed results. In EpiSkin (R) cultured human epidermal cells, mean cell viability was on 81% after exposure to undiluted hydroxycitronellal. Irritancy was judged to occur at concentrations above 50% (Portes *et al.*, 2002). Studies in different laboratories using the EpiDerm (R) reconstituted skin model, showed that undiluted hydroxycitronellal was marginally irritant in two laboratories and non-irritant in a third (Fentem *et al.*, 2001).

Conclusion

Studies on human skin *in vitro* gave mixed results.

c) Skin irritation: human data

In studies on human subjects, the irritation potential of hydroxycitronellal administered under occlusion for 48 hours showed a dose/response effect. A severe response was recorded at concentrations of 100 - 70% in acetone; irritation was moderate at concentrations between 70% and 40% and was mild at concentrations below 40% (Motoyoshi *et al.*, 1979). Undiluted hydroxycitronellal under occlusion for 24 hours gave irritant reactions in 2/22 volunteers (Katz, 1946). In irritancy screens carried out prior to 9 separate Human Maximization Tests on a total of 223 subjects, no irritancy was seen when hydroxycitronallal was applied at 12% in petrolatum (8.4 mg/cm^2) under occlusion for 24 hours. A mild irritant reaction was reported in a preliminary irritancy screen in one of the many Human Repeated Patch Tests on 5% (9 mg/cm^2) hydroxycitronellal in ethanol:diethyl phthalate (3:1) under occlusion for 48 hours (Api and Letizia, 2001).

Conclusion

As with animals, there is some evidence that hydroxycitronellal is irritant to human skin under occlusion, particularly when ethanol is used in the vehicle system and when concentrations are extremely high. Numerous studies on human subjects for skin sensitization effects (see **5.2.3**) were carried out at non-irritant concentrations demonstrating a clear no effect dose at 5% (9 mg/cm^2) under maximised conditions.

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5.2.2.2 Eye irritation

In one study, 0.1 ml of a 2% solution of hydroxycitronellal in propylene glycol produced no irritancy in the eyes of 6 rabbits (RIFM, 1972). In a full study on undiluted hydroxycitronellal, carried out according to the Draize procedure, irritant effects were seen to the iris and conjunctiva but these were reversible after 7 days (RIFM, 1982). Similar results were obtained in another Draize study. Mean scores descended from 35 on day 1 to only 2 on day 7 (Troy, 1977).

Conclusion

Hydroxycitronellal is classified as irritant to the eye (R36) (IFRA, 2004) but produces reversible effects when tested undiluted and shows no irritancy when tested at 2%.

5.2.2.3 Irritation by inhalation

In studies on the respiratory irritation potential of different fragrance raw materials in CF-1 female mice by recording respiratory rate, a 1 minute exposure to hydroxycitronellal aerosol using a nebulizer, there was marked respiratory depression at high doses (ED25 = 183 ug/l). Inhalation of 621 μg/l via a tracheal cannula had a slight depressant effect on lower respiratory tract (Troy, 1977).

Conclusion

Hydroxycitronellal shows signs of respiratory irritation under conditions that are difficult to relate to consumers' use of household products.

5.2.3. Skin sensitization

There are two phases to skin sensitization: induction the initial phase in which an allergy is acquired and elicitation the production of dermal symptoms (such as erythema and oedema) following a subsequent exposure to the substance to which the allergy has been acquired. Many tests have been carried to investigate the potential of hydroxycitronellal to induce an allergic state and to elicit allergic reactions. These are summarized here with tables.

5.2.3.1 Studies on the potential of hydroxycitronellal to induce allergy

5.2.3.1.1 Predictive tests using animals

Tests that use Freund's Complete Adjuvant to potentiate induction of allergenicity are useful for determining if a substance is a significant allergen or not. The skin sensitization potential of hydroxycitronellal has been evaluated in different tests systems. In the guinea pig maximization test according to the Magnusson-Kligman protocol (OECD, 1992), positive results were obtained (Table 11) showing that hydroxycitronellal has a clear potential to induce cell-mediated contact allergy. No clear-cut stereo-specificity was observed, with roughly equivalent reactivity being shown to (R)-(+) hydroxycitronellal and (S)-(-)-hydroxycitronellal and with animals sensitised to one, generally reacting when challenged with the other (Watanabe *et al.*, 1988). Although these results were claimed to show differences between the potency of the two enantiomers, the observed variations were within the variability of this test.

Table 11 : Guinea Pig Maximization Tests on hydroxycitronellal

Induction Conditions*	Challenge Conditions*	Results	Comments	Reference
0.5% i.d. 100% top.	50% top.	6/10	Same test probably reported in both publications	(Basketter and Scholes, 1992) (Basketter and Scholes, 1992)
5% i.d. 20% top.	20% top.	14/50	Results accumulate, results of 5 tests on 10 animals	(Marzulli and Maguire, Jr., 1982)
5% i.d. 25% top.	"sub-irritant concentration"	0/10	Incompletely reported	(Klecak *et al.*, 1977)
10% i.d. topical dose not specified	not specified	"positives"	Only summary report in translated abstract available	(Jimbo *et al.*, 1983)
0.01% i.d. 1% top. 0.03% i.d. 100% top. 0.1% i.d. 1% top. 0.3% i.d. 100% top. 3.0% i.d. 1% top.	3% top. 3% top. 3% top. 3% top. 3% top.	2/8 4/8 3/8 5/8 2/8	Detailed scores at other challenge concentrations not given	(Wahlkvist *et al.*, 1999)
1% i.d. 100% top.	20% top.	4/10	All reactions were questionable	Bush, Boake & Allen Ltd, 1979
10% i.d. top. (R)-(+)-enantiomer	with (R)-(+)-	10/10	Induction with (R)-(+)-hydroxycitronellal	(Watanabe *et al.*, 1988)
	with (S)-(-) -	9/10		
10% i.d. top. (S)-(-)-enantiomer	with (S)-(-) –	9/10	Induction with (S)-(-)-hydroxycitronellal	
	with (R)-(+)-	9/10		
10% i.d. top. (racemate)	with (R)-(+) -	7/10	Induction with racemic hydroxycitronellal	
	with (S)-(-)-	6/10		
5% i.d. top.	10% top.	5-9/10	Tests carried out on three	Avon Products Inc. 1984

	20% top.	8-9/10	different samples	

- *top. : topical, i.d. : intra-dermal*

The sensitisation potential of hydroxycitronellal was further demonstrated in other adjuvant tests such as the Cumulative Contact Enhancement Test (Table 12) and various other assays in guinea pigs and mice (Table 13).

Table 12: Cumulative contact enhancement tests on hydroxycitronellal

Induction Conditions* (topical)	Challenge Conditions* (topical)	Results	Comments	Reference
100%	10%	8/8		(Wahlkvist *et al.*, 1999)
	3%	5/8		
	1%	3/8		
	0.3%	1/8		
	0.1%	1/8		
	0.03%	0/8		
	0.01%	2/8		
20%	10%	7/8		
	3%	7/8		
	1%	4/8		
	0.3%	0/8		
	0.1%	1/8		
	0.03%	0/8		
	0.01%	0/8		
10%	10%	7/8		
	3%	7/8		
	1%	4/8		
	0.3%	2/8		
	0.1%	0/8		
	0.03%	0/8		
	0.01%	1/8		

3%	10%	7/8		
	3%	6/8		
	1%	4/8		
	0.3%	1/8		
	0.1%	0/8		
	0.03%	0/8		
	0.01%	0/8		
1%	10%	5/8		
	3%	3/8		
	1%	4/8		
	0.3%	0/8		
	0.1%	1/8		
	0.03%	1/8		
	0.01%	2/8		
10%	20%	13/30 possible reactions	Inflammation seen in 13 animals, pigmentation in 6	(Imokawa and Kawai, 1987)

** top. : topical, i.d. : intra-dermal*

Table 13: Other adjuvant sensitisation tests on hydroxycitronellal

Induction Conditions*	Challenge Conditions*	Results	Comments	Reference
SPLIT ADJUVANT TEST				
20% i.d. top.	20% top.	5/30		(Marzulli and Maguire, Jr., 1982)
FREUND'S COMPLETE ADJUVANT TEST				
0.01% i.d.	10%	1/8	Detailed scores at other challenge concentrations	(Wahlkvist *et al.*, 1999)
0.03% i.d.	10%	0/8		

0.1% i.d.	10%	1/8	not given	
0.3% i.d.	10%	4/8		
1.0% i.d.	10%	6/8		
50% i.d.	"a sub-irritant concentration"	no reactions	Only summary available	(Klecak *et al.*, 1977)
MOUSE EAR SWELLING TEST				
5% i.d.	50% top.	50% of 10-15 animals sensitized	No details available	(Gad *et al.*, 1986)
20% i.d.	20% top.	positive effects observed	No details available	(Maisey and Miller, 1986)
CYCLOPHOSPHAMIDE CFA BIOASSAY				
20% top.	20% top.	4/30		(Marzulli and Maguire, Jr., 1982)

* *top. : topical, i.d. : intra-dermal*

Tests that do not use Freund's Complete Adjuvant offer a better opportunity of determining non-sensitizing conditions that those that do. The allergenic potential of hydroxycitronellal is also evident from these non-adjuvant tests. In the Buehler Test (Table 14), hydroxycitronellal produced positive results at concentrations of 25% and higher but gave negative results when tested at 5% and 2.5%. Tests carried out using another non-adjuvant method: the modified Draize test. (Table 15), were all negative but were carried out a low concentrations and employ intra-dermal injection to administer the test material. They are therefore of limited value in establishing non-sensitizing doses. On the other hand, Epicutaneous Tests (Table 16), show that hydroxycitronellal produced no sensitisation even at open doses of 100% but when the test material was administered under occlusion.

Table 14: Buehler Tests on hydroxycitronellal

Induction Conditions (topical)	Challenge Conditions (topical)	Results	Comments	Reference
100%	2.5%, 7.5% & 25% with rechallenge	6/20	Reactions only at 25% challenge No reactions on rechallenge at 7.5%	(RIFM, 1987a)
50%		1/20	Reaction at 25% challenge only. 6/20 reactions at rechallenge to 5%	
25%	25%	4/20		(RIFM, 1988a)
2.5%	2.5%	0/20		
30%	10%	25% positive		(Basketter and Gerberick, 1996)
5%	5%	0/10	Protocol similar to Bueher Test	Bush, Boake & Allen Ltd, 1972

Table 15: Modified Draize Tests on hydroxycitronellal

Induction Conditions	Challenge Conditions	Results	Comments	Reference
0.1% i.d.	0.1% i.d.	0/30	Modified draize Test. Ten intra-dermal inductions on alternate days	(Marzulli and Maguire, Jr., 1982)
0.5% i.d.	5% i.d.	0/20		
0.1% i.d.	0.1% i.d.	no reactions	Only summary available	(Klecak *et al.*, 1977)

Table 16: Epicutaneous tests on hydroxycitronellal (open: OET & closed: CET)

Induction Conditions	Challenge Conditions	Results	Comments	Reference
100% open	100% open	no reactions in 6-8 animals	Only summary available	(Klecak *et al.*, 1977)
20% open	20% open	0/20	Non-occlusive topical test	(Marzulli and Maguire, Jr., 1982)
12% open	12% open	no reactions in 6-8 animals	Only summary available	(Klecak *et al.*, 1977)
10% closed	10% closed	2/12		(Ishihara *et al.*, 1986)

In the murine Local Lymph Node Assay (Table 17), positive reactions were observed in numerous tests. Although, strictly speaking a Lowest Observed Effect Level (LOEL), the standard measure used in these studies: the EC3 (Basketter *et al.*, 1999) has been shown to correlate well with non-sensitizing doses in maximized human studies (Gerberick *et al.*, 2001b;Griem *et al.*, 2003;Schneider and Akkan, 2004). EC3 values in eight Local Lymph Node Assays varied between 19% and 33% with a mean around 22.8% (c. 5.7 mg/cm^2).

Table 17 : Local Lymph Node Assays (LLNAs) on hydroxycitronellal

Induction Conditions (topical)	Challenge Conditions (absent in LLNA)	Results	Comments	Reference
100%, 50%, 25%	-	SI > 3 at all doses SI = 3.6 @ 25%	Indicates EC3 of 13.6%	(Basketter and Scholes, 1992)
different topical doses	-	EC3 values 26.4% (6.61 mg/cm^2) 19.3% (4.83 mg/cm^2) 19.7% (4.93 mg/cm^2) 22.2% (5.54 mg/cm^2)	Tests carried out in different solvent systems	(RIFM, 2001) (Isola and Lalko, 2001)
2.5%, 5%, 10%, 25% & 50%	-	EC3 value 33% (8.25 mg/cm^2)		(Basketter *et al.*, 2001)
	-	EC3 value 20% (5.0 mg/cm^2)		(Basketter *et al.*, 2002;Basketter *et al.*, 2003)
1%, 5% & 25%	-	Stimulation index > 3 at 25%		(Montelius *et al.*, 1994)
25%, 50%, 100%	-	EC3 value Estimated as 20.9% (5.2 mg/cm^2)		(Ashby *et al.*, 1995)
1%, 10%, 25%	-	EC3 value		(Smith *et al.*, 2001)

		23% (5.8 mg/cm^2)		
Not given	-	EC3 value 25% (6.3 mg/cm^2)		(Estrada *et al.*, 2003) (Patlewicz *et al.*, 2002)

5.2.3.1.2 Predictive tests on human subjects

In human volunteers, the Human Maximization Test (Kligman, 1966) has been extensively used (Table 18). In 12 of these tests carried out using an induction concentration of 12% in petrolatum, positive reactions were seen in all but four of these tests. At this dose, a total of 26 subjects were sensitized out of 298 tested. At an induction concentration of 10%, reactions were seen to both enantiomers (12/25 to (R)-(+)-hydroxycitronellal and 1/25 to (S)-(-)-hydroxycitronellal) (Watanabe *et al.*, 1988). Studies carried out at 5% and 4% were negative but the number of subjects tested was low.

Table 18: Human maximization tests (HMTs) on hydroxycitronellal

Induction Conditions (pet. : petrolatum)	Challenge Conditions	Results	Comments	Reference
12.0% pet.	12.0% pet.	7/26		(RIFM, 1979b)
12.0% pet.	12.0% pet.	2/22		(Epstein, 1980)
12.0% pet.	12.0% pet.	1/26		(Epstein, 1980)
12.0% pet.	12.0% pet.	2/21		(Epstein, 1980)
12.0% pet.	12.0% pet.	6/26		(RIFM, 1979b)
12.0% pet.	12.0% pet.	1/25		(RIFM, 1979a)
12.0% pet.	12.0% pet.	3/25		(RIFM, 1979a)
12.0% pet.	12.0% pet.	4/27		(RIFM, 1978)
12.0% pet.	12.0% pet.	0/25, 0/25	separate tests	(RIFM, 1979a)
12.0% pet.	12.0% pet.	0/25		(Greif, 1967)
12.0% pet.	12.0% pet.	0/25		(RIFM, 1978)
10.0% pet	10.0% pet	12/25	(R)-(+)- hydroxycitronellal	(Watanabe *et al.*, 1988)
10.0% pet	10.0% pet	1/25	(S)-(-)- hydroxycitronellal	
10.0% pet	10.0% pet	2/25	both reacted also to cinnamic alcohol	(RIFM, 1976b)

10.0% pet	10.0% pet	0/25		(RIFM, 1976b)
5.0% pet	5.0% pet	0/25		(RIFM, 1973b)
5.0% pet	5.0% pet	0/26		(RIFM, 1976a)
4.0% pet	4.0% pet	0/25-30	male volunteers	(Jordan, Jr. and King, 1977)

The Human Repeat Patch Test (HRIP Test) has been extensively used to study the potency and possible induction thresholds of hydroxycitronellal (under 24 hour occlusion). The results of 85 HRIP Tests are shown in Table 19. The standard HRIP Test involves an induction exposure in which the acquisition of allergy is provoked. Acquisition is tested by a single challenge with the test material after a rest period, with re-challenge doses being applied in case of doubtful reactions. In 33 of the HRIP Tests carried out on hydroxycitronellal, a second phase was introduced in which subjects who had completed the first full test and had shown no reactions, were subjected to a new complete HRIP Test. The HRIP Test already maximizes normal consumer exposure significantly and it is not known to what extent this second maximized test produces an unrealistic departure from a realistic simulation of consumer exposure. In any case, this second test gave rise to clear reactions in 32 of the 354 previously negative subjects re-tested this way (i.e. in 9 of the 35 second-phase studies).

Table 19: Human Repeated Insult Patch Tests (HRIPT) on hydroxycitronellal

Induction Conditions (DEP: Diethyl phthalate)	Challenge Conditions	Results	Comments	Reference
2.5% (2.95 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	2.5% in same solvent system	0/65	First phase of two - phase study	(RIFM, 1987b)
5.0% (5.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	5.0% in same solvent system	1/66	Rechallenge six months later at 5% provided no reaction	
7.5% (8.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	7.5% in same solvent system	1/66	Rechallenge six months later at 5% provided no reaction	
2.5% (2.95 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	2.5% in same solvent system 5.0% in same solvent system	4/18 5/18	Second phase of two phase test using same subjects as in Billhimer et al., 1987 Reactor did not participate	(RIFM, 1988b)
	2.5% in same solvent system 5.0% in same solvent system	6/15 7/15		
5.0% (5.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	2.5% in same solvent system	6/20	Second phase of two phase test using same subjects as in Billhimer et al., 1987	
	5.0% in same solvent system	7/20		
1.0% (1.2 mg/cm^2) in DEP	0.1% in DEP 0.3% in DEP 1.0% in DEP	0/62 0/62 0/62	First phase of two - phase study	(RIFM, 1990)
	0.1% in DEP	0/58	Second phase of above study	
	0.3% in DEP	1/58		
	1.0% in DEP	1/58		
	0.1% in DEP	0/57	First phase of two -	

	0.3% in DEP	0/57	phase study	
	1.0% in DEP	0/55		
	0.1% in DEP	0-2/57	Second phase of above study. Questionable reactions in 2 subjects at all doses. Only one reacted to rechallenge at 4 months. No reactions at rechallenge 4 months later.	
	0.3% in DEP	0-2/57		
	1.0% in DEP	0-2/57		

Induction Conditions	Challenge Conditions	Results	Comments	Reference
5.0% (5.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	0.5% in same solvent system	0/42	First phase of two – phase study	(RIFM, 1990)
	1.5% in same solvent system	1/41		
	5.0% in same solvent system	15/42		
	0.5% in same solvent system	0/17	Second phase of above study. Reactors did not participate.	
	1.5% in same solvent system	2/17		
	5.0% in same solvent system	6/17		
5.0% (5.9 mg/cm^2) in DEP	0.5% in DEP	0/37	First phase of two – phase study	
	1.5% in DEP	0/37		
	5.0% in DEP	0/37		
	0.5% in DEP	0/28	Second phase of above study	
	1.5% in DEP	0/29		
	5.0% in DEP	0/28		
	0.5% in DEP	0/35	First phase of two - phase study	
	1.5% in DEP	0/35		
	5.0% in DEP	0/35		
	0.5% in DEP	0/21	Second phase of above study	
	1.5% in DEP	0/21		
	5.0% in DEP	0/21		
5.0% (5.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	0.5% in same solvent system	0/38	First phase of two - phase study.	
	1.5% in same solvent system	2/38		
	5.0% in same solvent system	7/38	One subject failed to react on rechallenge	

	0.5% same solvent system	0/27	Second phase of above study. The reacting subjects did not participate.	
	1.5% same solvent system	0/27		
	5.0% in same solvent system	6/27		

Induction Conditions	Challenge Conditions	Results ? questionable	Comments	Reference
1.0% (1.2 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	0.1% in same solvent system	0/36	First phase of two – phase study.	(RIFM, 1991)
	0.3% in same solvent system	0/37	Questionable reactions disappearing on rechallenge.	
	1.0% in same solvent system	2?/35		
	0.1% in same solvent system	2?/17	Second phase of above study. Reactors did not participate.	
	0.3% in same solvent system	2?/17	Questionable reactions disappearing on rechallenge.	
	1.0% in same solvent system	2?/17		
	0.1% in same solvent system	1?/40	First phase of two – phase study.	
	0.3% in same solvent system	1?/40	Questionable reactions at all three doses.	
	1.0% in same solvent system	1?/40		
	0.1% in same solvent system	1?/24	Second phase of above study. Reactors did not participate.	
	0.3% in same solvent system	2?/24	All reactions questionable disappearing at rechallenge.	
	1.0% in same solvent system	2?/24		
5.0% (5.9 mg/cm^2) in 75.0% Ethanol, 25.0% DEP	0.5% in same solvent system	3?/38	First phase of two - phase study.	
	1.5% in same solvent system	1+3?/39	Three subjects had questionable reactions disappearing at rechallenge.	
	5.0% in same solvent system	1+3?/39		

	0.5% in same solvent system	4?/24	Second phase of above study. Reactors did not participate. Questionable reactions did not appear at rechallenge.	
	1.5% in same solvent system	2+3?/24		
	5.0% in same solvent system	2+5?/25		

Induction Conditions	Challenge Conditions	Results	Comments	Reference
10.0% (12 mg/cm^2) in DEP	1.0% in DEP	0/29	First phase of two – phase study	(RIFM, 1991)
	3.0% in DEP	0/29		
	10.0% in DEP	0/29		
	1.0% in DEP	0/28	Second phase of above study	
	3.0% in DEP	1/28		
	10.0% in DEP	1/28		
Tests on fragrance blends in alcohol. The level of Hydroxycitronellal tested was	Challenge with the same concentration of the fragrance blend in alcohol		Positive reactions may be due to other components of the fragrance blend.	(Steltenkamp *et al.*, 1980)
5.0%	5.0%	1/41		
4.5%	4.5%	0/39		
3.3%	3.3%	3/44		
3.0%	3.0%	0/51		
2.4%	2.4%	0/50		
1.9%	1.9%	0/39		
1.3%	1.3%	1/42		
1.2%	1.2%	0/39		
1.1%	1.1%	0/51		
1.0%	1.0%	2/77		
20.0% petrolatum	20.0% petrolatum	1/99		(Marzulli and Maibach, 1980)
20.0% ethanol	20.0% ethanol	14/73		
1.0% water	1.0% water	0/50		IFF Inc., 1958

4.0% petrolatum	4.0% petrolatum	1/150		(Jordan and King, 1977)
2.0% (2.36 mg/cm^2) (Dimethyl phthalate)	2.0% (Dimethy phthalate)	0/100	Two studies in Dimethyl phthalate	Givaudan Corp., 1965
1.0% (1.2 mg/cm^2) Diethyl phthalate : 75% Ethanol : 25%	1.0% in same solvent	0/110	3 questionable reactions but no reactions on rechallenge	(RIFM, 1992)

In the HRIP Test, the allergenic potency of hydroxycitronellal appears to be vehicle-dependent. The presence of ethanol as a major or sole component of the vehicle, lowers the apparent threshold of induction at both the first and the second phases of testing.

In petrolatum, one subject only of 110 reacted to 4% hydroxycitronellal (Jordan and King, 1977) and one of 99 reacted to a level of 20% (23.6 mg/cm^2) in this vehicle (Marzulli and Maibach, 1980).

A study carried out in water as vehicle (1% hydroxycitronellal) gave no reactions in 50 subjects (RIFM, 1958).

No reactions were seen in two studies carried out at induction doses of 2% hydroxycitronellal (2.36 mg/cm^2) in dimethyl phthalate (RIFM, 1964), (RIFM, 1965b).

A total of 5 studies using diethyl phthalate as vehicle were repeated in a second-phase. No reactions were seen in any of the first phase HRIP Tests carried out at doses up to 5% (5.9 mg/cm^2) on a total of 220 subjects. In second-phase HRIP Testing on 193 subjects who had not reacted in the first test, one subject reacted at 0.3% (0.35 mg/cm^2) and 1% (1.18 mg/cm^2) (RIFM, 1990) and another subject reacted at 3% (3.5 mg/cm^2) and 10% (11.8 mg/cm^2) (RIFM, 1991). In another second-phase study in diethyl phthalate, 2 subjects reacted to concentrations of 0.1%, 0.3% and 1% but on re-challenge after a period of four months, failed to show any reaction (RIFM, 1990).

In ethanol: diethyl phthalate (3:1) the following results were obtained in 7 separate human repeated patch tests of which 6 involved a second phase of testing. No reactions were seen in the first phase HRIP Tests carried out at a dose of 2.5% (2.95 mg/cm^2) in 65 subjects with one reaction at 5% (5.9 mg/cm^2) and 7.5% (8.85 mg/cm^2) although this disappeared on rechallenge (RIFM, 1987b). No reactions were seen in 42 and 38 subjects tested at 0.5% (0.59 mg/cm^2) in this solvent system although reactions were observed at 1.5% (2.12 mg/cm^2) and 5% (5.9 mg/cm^2) (RIFM, 1990). In two other studies in this vehicle, no reactions were observed in 36 subjects at 0.1% (0.12 mg/cm^2) and 0.3% (0.35 mg/cm^2) with one subject showing a doubtful reaction being seen at 1% (1.18 mg/cm^2) while in the other, a subject showed a doubtful reaction at 0.1%, 0.3% and 1% (RIFM, 1991)). Three subjects also showed doubtful reactions at 0.5% but two other subjects reacted to concentrations of 1.5% and 5% in another study (RIFM, 1991).

Finally, no reactions were seen in 110 subjects tested to a concentration of 1% (1.18 mg/cm^2) in this vehicle (RIFM, 1992). In second phase studies 4/18 and 6/15 subjects who had previously not reacted in the first HRIP Test at concentrations up to 7.5%, reacted to 2.5% hydroxycitronellal in this vehicle (RIFM, 1988b;RIFM, 1990). In another second-phase study on 17 subjects who had previously failed to react to 5% hydroxycitronellal, none reacted to a concentration of 0.5% but 2 reacted to 2.5% (RIFM, 1990). Similarly, 27 subjects who had not reacted to 5% hydroxycitronellal in the first phase, failed to react to 0.5% and 1.5% but six reacted to 5% hydroxycitronellal in the second-phase study (RIFM, 1990). Doubtful reactions were observed in second-phase tests on 17 and 24 subjects who had not reacted to 1% hydroxycitronellal in the first phase (RIFM, 1991). Doubtful reactions were also seen at a level of 0.5% in 4/24 subjects who were negative in first-phase testing at 5% while another two of these 24 subjects showed clear reactions at 1.5% and 5% hydroxycitronellal in this vehicle (RIFM, 1990).

When the solvent was ethanol, an induction dose of 20% gave 14/73 reactions (Marzulli and Maibach, 1980)**,** 10% gave 6/40 reactions (IFF Incorporated, 1964a), 7.5% gave 1/38 reactions (RIFM, 1965a), 5% gave 0/39 reactions (IFF Incorporated, 1964b) and 2.5% gave 3/46 reactions (RIFM, 1980). Other HRIP Tests carried out on hydroxycitronellal in ethanol gave scores of 0/39 at 5%, 1/38 at 7.5% and 6/40 at 10% (Steltenkamp *et al.*, 1980)**.**

When hydroxycitronellal was tested as a component of fragrance blends in the human repeat patch test, these blends (not necessarily hydroxycitronellal) gave positive reactions when the tested concentration of hydroxycitronellal was 5% in ethanol (1/41), 3.3% in ethanol (3/44), 1.3% in ethanol (1/42) and 1.0% in a mixture of dibutyl phthalate and mineral oil (2/77) but gave no reactions when levels were higher (0/39 at 4.5%, 0/51 at 3.0%, 0/50 at 2.4%, 0/39 at 1.9%, 0/39 at 1.2% and 0/51 at 1.1%) (Steltenkamp *et al.*, 1980)**.**

5.2.3.2 Clinical patch testing on patients

There are many published reports of studies in which hydroxycitronellal produces positive reactions in patients in routine diagnostic patch testing. Although there have been numerous reports of patients giving frank allergic responses to hydroxycitronellal in clinical patch testing on dermatological patients, many of these studies do not establish a clear causal relationship according to currently accepted criteria (Lachapelle, 1997;Lachapelle and Maibach HI, 2003;Maibach and Hostynek, 2003). A recent publication (Hostynek and Maibach, 2004b) has pointed out that reactions seen in dermatological clinics, while genuinely allergic in nature, may only occur under the severe conditions use in clinical diagnosis and may not relate to adverse effects from the use of consumer products. In a separate publication, the same authors (Hostynek and Maibach, 2004a) have also defined criteria by which possible causality can be assessed. These criteria have been applied by these authors to a number of other proposed allergens (Hostynek and Maibach, 2003a;Hostynek and Maibach, 2003b).

The same criteria have been used here to assess the strength of a causal link between the observed clinical reaction and everyday exposure to a hydroxycitronellal-containing product. These relatively rare cases are shown in Table 20.

Table 20: Clinical patch testing with hydroxycitronellal establishing possible causative link to presence in a consumer product

Reference	Patch test Conditions	Cases	Products
(Dooms-Goossens *et al.*, 1992)	1% Petrolatum	Several	Shaving, skin-care and deodorants
(Larsen, 1975)	Not given	Single	Eye cream
(Mathias *et al.*, 1978)	4% Petrolatum	Single	Spouse perfume
(de Groot and Liem, 1983)	2% Petrolatum	Single	Hair lotion & after-shave
(Serrano *et al.*, 1989)	1% Petrolatum	Single	Face powder
(Hausen and Kulenkamp, 1990)	2,5% Diethyl phthalate	Single	Lemon oil (Hydroxycitronellal is not found in lemon oil)

Authors reporting on one of the biggest multi-centre studies stated that "we observe what we seek" (Eiermann *et al.*, 1982). Hydroxycitronellal is one of the eight components of the "Fragrance Mix" used by dermatologists to detect possible sensitivity to fragrances. This mix was first proposed (Calnan *et al.*, 1980;Larsen, 1975), on the basis of the components of a fragrance used in a popular Triadcortyl cream (Mycolog®, Squibb Corp.) (Larsen, 1979) and it was concluded that the use of this ointment in treating eczematous and ulcerous skin may have contributed significantly to the cases of clinical dermatitis that had been ascribed to this substance (Larsen, 1979).

Clinical patch testing of patients who have already shown positive reactions to the "Fragrance Mix" frequently gives positive reactions to hydroxycitronellal although in such cases, it is rare

that hydroxycitronellal is the only component of this "Fragrance Mix" to produce positive reactions. In the cases reported in Table 21**,** no clear causal link could be established with the use of consumer products using the criteria of Hostynek and Maibach (Hostynek and Maibach, 2004a). In a large multicentre study covering nearly 60,000 patients tested in German clinics from 1996 to 2002 (Schnuch *et al.*, 2004), the frequency of reactions to hydroxycitronellal and in patients reacting to the fragrance mix has been about 13%. These patients have frequently reacted to other constituents of the fragrance mix (for instance 47.6% and 56.7% of patients reacting to geraniol and amylcinnamic aldehyde respectively, also reacted to hydroxycitronellal).

Table 21: Clinical patch testing of hydroxycitronellal on "Fragrance Mix-sensitive" patients.

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Johansen and Menne, 1995)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	367	27	Not given	A,B
(Santucci *et al.*, 1987)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	54	9	Not given	A,B
(Goh and Yuen, 1994)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	274	12	Not given	A,B
(Sieben *et al.*, 2001)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	32	4	Not given	A,B
(Enders *et al.*, 1989)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	162	10	Not given	A,B
(Katsarma and Gawkrodger, 1999)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	40	1	Not given	A,B
(Buckley *et al.*, 2000a)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	1112	64	Not given	A,B
(Johansen *et al.*, 1996b)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	335	27	Not given	A,B
(Johansen *et al.*, 1997)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	884	78	Not given	A,B
(Temesvari *et al.*, 2002)	1% in Petrolatum Finn Chambers (0.3 mg/cm^2)	104	18	Not given	A,B
(Hendriks and van Ginkel, 1999)	2% in Petrolatum (0.6 mg/cm^2)	757	10	Not given	A,B
(Calnan, 1990)	2% no other details given	172	36	Not given	A,B
(Safford *et al.*, 1990)	2% in Petrolatum (0.6 mg/cm^2)	20	3	Not given	A,B
(de Groot *et al.*, 1993)	5% in Petrolatum Finn Chambers (1.5 mg/cm^2)	677	12	Not given	A,B
(Angelini *et al.*, 1997)	1% in Petrolatum (0.3 mg/cm^2)	144	11	Not given	A,B
(Brites *et al.*, 2000)	1% in Petrolatum (0.3 mg/cm^2)	226	15	Not given	A,B

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

It has been reported that while the proportion of patients reacting to the "Fragrance Mix" has been relatively constant over 17 years, there is a slow decrease (by 5% yearly) in the proportion of patients reacting to hydroxycitronellal (Buckley *et al.*, 2000b). However, the full significance of these findings has been questioned (Wesley and Maibach, 2003). In another study from 1971 to 1980 there was also a reduction in the number of reactions to 10% hydroxycitronellal in each of three successive periods in patients who were sensitive to cosmetics. When tested in a hydrophilic ointment, reactions declined from 21% (1971-1974) to 18% (1975-1977) and to 8% (1978-1980) while in lanoline, reactions declined in these periods from 8% to 6% to 5% (Nakayama *et al.*, 1984).

In patients already classified as "perfume sensitive" (Table 22) or only “cosmetic-sensitive” (Table 23), similar frequencies of positive reactions to hydroxycitronellal have been observed.

Table 22: Clinical patch testing of hydroxycitronellal in “perfume-sensitive” patients

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Meynadier *et al.*, 1986)	5% in Petrolatum (1.5 mg/cm^2)	21	2	Not given	A,B
(Larsen, 1977)	4 or 5% in Petrolatum	20	9	Not given	A,B
(Larsen *et al.*, 1996)	4% in Petrolatum	167	23	Not given	A,B
(Ferguson and Sharma, 1984)	4% in Petrolatum Finn Chambers	241	9	Not given	A,B
(Wohrl *et al.*, 2001)	1% in Petrolatum	747	11	Not given	A,B
(Ishihara *et al.*, 1979)	1% in Petrolatum 2% in Petrolatum 5% in Petrolatum	130 130 130	2 4 9	Not given	A,B

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

Table 23: Clinical patch testing of hydroxycitronellal in "cosmetic-sensitive" patients

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Malten *et al.*, 1984)	10% in Petrolatum	182	19	Not given	A,B
(Nakayama and Kawasaki, 1985)	10% in Petrolatum	119	to d-Hydroxycitronellal 31 to l-Hydroxycitronellal 5	Not given	A,B,C
(de Groot *et al.*, 1985)	8% in Petrolatum	179	36	Not given	A,B
(Nagareda *et al.*, 1992)	5% in Petrolatum	129	7	Not given	A,B,C
(Haba *et al.*, 1993)	5% in Petrolatum	47	3	Not given	A,B,C
(Hayakawa and Japan Patch Test Research Group, 1986)	5% in Petrolatum	376	12	Not given	A,B,C
(Ishihara *et al.*, 1981)	5% in Petrolatum	155	6	Not given	A,B,C
(Nishimura *et al.*, 1984)	5% in Petrolatum	522	15	Not given	A,B
(Adams and Maibach, 1985)	Not specified	713	11	Not given	A,B
(Eiermann *et al.*, 1982)	Not specified	149	9	Not given	A,B
Johansen et al., 1996 c	Not specified	9	6	Not given	B
(Broeckx *et al.*, 1987)	Not specified	156	6	Not given	A,B
(de Groot, 1987)	Not specified	75	6	Not given	A,B
(Isonokami *et al.*, 1990)	Not specified	303	13	Not given	A,B,C
(de Groot *et al.*, 1988)	5% in Petrolatum	119	4	Not given	A,B
(Higashi *et al.*, 1986)	Not specified	227	20	Not given	A,B,C

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

C : Abstract only in English.

Patients suffering from special dermatological conditions and patients with no known sensitivities also reacted to hydroxycitronellal (Tables24 & 25)

Table 23: Clinical patch testing of hydroxycitronellal in on patients with special conditions (see comments)

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Hayakawa *et al.*, 1983)	5% vehicle not specified	181	11	Not given	A,B,C,D
(Addo *et al.*, 1982)	4% in Petrolatum	50	8	Not given	A,B,E
(Katsarou, 1999)	1% in Petrolatum (0.3 mg/cm^2)	38	7	Not given	A,B,F
(van Joost *et al.*, 1984)	10% in Petrolatum	28	3	Not given	A,B,G
(Ducombs *et al.*, 1986)	not given	3	1	Not given	B,H
(Van Joost *et al.*, 1985)	10% in Petrolatum (3.0 mg/cm^2)	242	11	Not given	A,B,I
(Fransway and Schmitz, 1991)	Dose not given Finn Chambers	300	25	Not given	A,B,J
(Keil, 1947)	1% in Petrolatum	2	2	Yes	A,B,K

Comments :
- *A : Not a primary study. Review of several studies or multicentre study.*
- *B : Patients probably reacted to other test materials in the same study.*
- *C : Abstract only in English.*
- *D : Melanosis patients.*
- *E : Patients with Photosensitivity dermatitis/Actinic Reticuloid Syndrome.*
- *F : Peru Balsam-sensitive patients.*
- *G : Wood tar-sensitive patients.*
- *H : Musk Ambrette-sensitive patients.*
- *I : Cinnamic aldehyde sensitive patients.*
- *J : Formaldehyde-sensitive patients.*
- *K : Citronella-sensitive patients.*

Table 24: Clinical patch testing of hydroxycitronellal in patients with no identified sensitivities

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Sugai, 1986)	2% in a perfume mix	Not specified	11% of those tested	Not given	A,B,C
(Itoh *et al.*, 1988)	5% in Petrolatum	680	17	Not given	A,B,C
(Schauder and Ippen, 1997)	1% in Petrolatum (0.3 mg/cm^2)	41	2	Not given	A,B
(Goossens and Merckx, 1997)	10% in Petrolatum (3.0 mg/cm^2)	7	2	Not given	B
(Mid Japan Contact Dermatitis Research Group, 1984)	10% in Petrolatum 5% in Petrolatum 2% in Petrolatum	571 571 571	24 14 9	Not given	A,B,C
(Hashimoto *et al.*, 1990)	5% in Petrolatum	254	4	Not given	A,B,C
(Santucci *et al.*, 1987)	5% in Petrolatum (1.5 mg/cm^2)	1200	13	Not given	A,B
(Suzuki *et al.*, 1997)	5% in Petrolatum	74	1	Not given	A,B,C
(Sugai, 1982)	5% in Petrolatum	1401	71	Not given	A,B,C
(Hirose *et al.*, 1987)	5% in Petrolatum	569	21	Not given	A,B
(Heydorn *et al.*, 2002)	5% in Petrolatum (1.5 mg/cm^2)	315	6	Not given	A,B
(Frosch *et al.*, 1995a)	5% in Petrolatum Finn Chambers (1.5 mg/cm^2)	1072	8	+ to +++ another 5 were questionnable	A,B
(Rudner, 1978)	4% vehicule not specified	900-2000	2.8%	Not given	A,B
(Remaut, 1992)	4% vehicule not specified	115	3	Not given	A,B
(Takase *et al.*, 1984)	4% vehicule not specified	45	1	Not given	A,B,C
(Hirano and Yoshikawa, 1982)	4% vehicule not specified	178	8	Not given	A,B,C
(Van Joost *et al.*, 1985)	4% in Petrolatum	242	11	Not given	A,D
(Mitchell *et al.*, 1982)	4% in Petrolatum	441	12	Not given	A,B
(Ohela and Saramies, 1983)	3% in Petrolatum Finn Chambers (0.9 mg/cm^2)	1377	48	Not given	A,B

Reference	Patch test conditions	Number tested	Number reacting	Scores	Comments (see below)
(Malanin and Ohela, 1989)	1-3% in Petrolatum	1967	68	Not given	A,B
(Ishihara, 1977)	2% in Petrolatum	81	1	Not given	A,B,C
(Cronin, 1985)	2% in Petrolatum	1836	20	Not given	A,B
(Storrs *et al.*, 1989)	1,5% in Petrolatum	1049	16 + 4 doubtful	Not given	A,B
(Frosch *et al.*, 1995b)	1% in Petrolatum (0.3 mg/cm^2)	709	1 clear positive 1 questionnable reaction	Yes	A,B
(Storrs, 1975)	1% in Petrolatum	200	8	Not given	A,B
(Nethercott *et al.*, 1989)	1% in Petrolatum	70	1	Not given	A,B
(Wennersten *et al.*, 1984)	1% in Petrolatum Finn Chambers	283	10	Not given	A,B
(Asoh and Sugai, 1986)	no conditions specified	370	16	Not given	A,B
(Rudzki and Grzywa, 1986)	no conditions specified	5315	299	Not given	A,B
(Becker *et al.*, 1994)	no conditions specified	50	5	Not given	A,B
(Asoh and Sugai, 1987)	no conditions specified	318	21	Not given	A,B,C
(Fujimoto *et al.*, 1997)	no conditions specified	95	2	Not give	A,B,C
(Mitchell, 1977)	no conditions specified	35	4	Not given	A,B
(Lynde and Mitchell, 1982)	no conditions specified	66	2	Not given	A,B
(Ruhnek *et al.*, 1989)	no conditions specified	245	14	9 strong 5 weak 6 doubtful	A,B

Comments : *A : Not a primary study. Review of several studies or multicentre study.*

B : Patients probably reacted to other test materials in the same study.

C : Only abstract available in English.

Differences in patch test reactivity between the two enantiomers of hydroxycitronellal have been observed (Watanabe *et al.*, 1988). In 119 cosmetic sensitive patients, patch testing with 10% (R)-(+)-hydroxycitronellal in a cream base gave 37 strong and 19 weak reactions. Similar testing of (S)-(-)-hydroxycitronellal gave only 7 strong and 7 weak reactions. In 82 other dermatologigical patients, the same testing gave 5 strong and 5 weak reactions to the (R)-(+)- enantiomer but only 1 strong and 5 weak reactions to its (S)-(-)- antipode.
Not all clinical patch testing has lead to positive reactions in patients. Numerous publications report studies in which none of the tested patients reacted to hydroxycitronellal (Emmons and Marks, Jr., 1985) , (Itoh *et al.*, 1988) (Malten *et al.*, 1984), (Armstrong *et al.*, 1997) , (Weston *et al.*, 1983).

Conclusions

Skin sensitization to hydroxycitronellal is clearly a significant hazard. Hydroxycitronellal shows a definite skin sensitization potential in a wide variety of predictive test systems and is classified as a skin sensitizer (R43) (IFRA, 2004). Non-adjuvant tests in animals and maximized tests carried out on human subjects offer a sound basis for a "weight of evidence" judgment on what doses are unlikely to induce allergy in naïve individuals during use of household products.

Numerous patch tests carried out on dermatitic patients have indicated that acquired allergy to hydroxycitronellal is wide-spread even though most of these clinical studies were not carried out under conditions that enable establishment of an unambiguous causal role of hydroxycitronellal in the patients' dermatitis.

5.2.4. Phototoxicity and photo-allergenicity

5.2.4.1 *In* vitro Phototoxicity

Photohaemolysis of human erythrocytes did not occur in the presence of 0.1% hydroxycitronellal in ethanol under 1.2.mW/cm^2 UV-A irradiation. However, when UV-B (15 minutes at 1.5 mW/cm^2), some pohototoxic effects were seen (Addo *et al.*, 1982). A concentration of 5% hydroxycitronellal (in paraffin) produced no phototoxic effects in studies where the yeast Candida utilis was exposed 1.5 mW/cm^2 UV-A. As this was negative, the light source was changed to a normally non-toxic 15 minute flux of approximately 1350 mJ/cm^2 of UV-B, minimal phototoxic effects were seen (Addo *et al.*, 1982). No phototoxic effects were seen in a study that measured the enhancement of photo-oxidation of Histidine by hydroxycitronellal (Addo *et al.*, 1982).

Conclusions

There is some evidence to show that hydroxycitronellal is potentially (but minimally) phototoxic when irradiated with UV-B but not UV-A.

5.2.4.2 Phototoxicity in humans

No studies were located.

5.2.4.3 Photoallergy in humans

In clinical studies on 745 suspected photoallergic patients, photopatch testing of 1% hydroxycitronellal gave 2 reactions (Wennersten *et al.*, 1984). Two patients with actinic reticuloid photodermatitis also reacted after photopatch testing with an unspecified concentration of hydroxycitronellal (Addo *et al.*, 1982). However, in other studies, photopatch testing of 1% hydroxycitronellal in petrolatum gave no effects (Galosi and Plewig, 1982), (Nakayama, 1998), (Schauder and Ippen, 1997), (Hashimoto *et al.*, 1990); (Nagareda *et al.*, 1992); (Lan *et al.*, 1994).

Conclusions

No predictive tests have been undertaken to demonstrate a potential for hydroxycitronellal to cause photoallergies. Clinical photopatch tests produced a low rate of response of uncertain linkage to the causality of hydroxycitronellal. Some clinical studies have given positive photo-patch results. However, this may not indicate a photoallergenic potential, but rather that increased dermal penetration permits elicitation of weak allergies to hydroxycitronellal.

5.2.5. Repeated Dose Toxicity

5.2.5.1 Oral route

The only repeated dose study on hydroxycitronellal is old and incompletely reported. This 24-month feeding study demonstrates none-the-less a high tolerance of rats to quite high doses of hydroxycitronellal. In 60 male and female rats dosed at 0.5% in the diet, 31 survived until termination of the study but none showed effects on growth or haematology or any macroscopic and microscopic tissue changes. Of 20 rats dosed at 0.1% in the diet, 5 reached termination but none of these showed adverse effects (Bar and Griepentrog, 1967).

Conclusions

Data from a single 24-month feeding study are inadequate. The available data are from an old feeding study that was only summarily reported and that lacks the rigor, diversity and numbers of animals, multiplicity of dose levels and width of observation of modern studies. None-the-less, it shows that dietary levels of 0.5 % (approximately 400 mg/kg/day) are well tolerated in rats for the duration of their life-span. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recently taken 250 mg/kg as conservative NOEL from this limited study (IPCS (International Programme on Chemical Safety), 2004).

5.2.5.2 Other routes

No data are available.

5.2.6. Genetic Toxicity

As part of a large study of the genotoxicity of 76 flavouring substances, hydroxycitronellal was tested in three different test systems.

5.2.6.1 Bacterial tests

In *Salmonella typhimurium* (strains TA 98, 100, 1535, 1537 & 1538, with and without Aroclor 1254-induced S9) duplicate studies in DMSO at doses up to the limit of toxicity or 3.6 mg/plate gave no effects whereas some of the other tested flavouring substances (as well as the positive controls: sodium azide and benzo[a]pyrene) gave positive results (Wild *et al.*, 1983).

5.2.6.2 Studies *in vivo*

In a Basc study in Drosophila melanogaster, a dose level of 37 mmol/l (approximately 6.3 g/l) fed to the flies in a 5% saccharose solution, was negative giving 0.33%, 0.15% and 0% sex-linked recessive lethal mutations per chromosome tested in three broods (Wild *et al.*, 1983).

A micronucleus test was carried out on 10-14 week old male and female NMRI mice by single intraperitoneal injection in olive oil with four animals per dose group (Wild *et al.*, 1983). Animals dosed at 861 mg/kg showed an average rate in bone marrow smears of 2.0 per thousand micronucleated polychromatic erythrocytes. This was not considered to be statistically different from the control rate of 1.85 per thousand cells. At 603 mg/kg, the rate observed was one per thousand and at 354 mg/kg it was 1.5 per thousand. No information was provided regarding the choice of positive and negative controls. However, some of the other flavouring substances tested were positive and others were negative in this test system.

Conclusions

From this limited data set, hydroxycitronellal is not considered to be genotoxic. It is negative in bacterial and in vivo screens including one carried out in a mammalian system.

5.2.7. Carcinogenicity

No tumours were identified in a limited reported above (5.2.5.1.) (Bar and Griepentrog, 1967).

5.2.8. Developmental Toxicity/Teratogenicity

5.2.8.1 Studies in chick embryos

Abnormal development and lethal effects in chick embryos were studied by administering different doses of hydroxycitronellal in olive oil by suprablastodermic injection on the third day of development. These studies showed a clear dose/response relationship. A dose of 2150 μg/embryo gave 92.3% mortality. An equivalent level (90.3%) was obtained at 860 μg/embryo but this diminished to 50% at 86 μg/embryo, 18.7% at 43 μg/embryo while the solvent control gave 17.8% mortality. Observed abnormalities rose from 23% at 2150 μg/embryo to 32.2% at 860 μg/embryo and 37.5% at 86 μg/embryo but then diminished to 12.5% at 43 μg/embryo. In an earlier study by the same group, a dose of 8.6 μg/embryo which had shown 7.9% abnormalities (Forschmidt *et al.*, 1979). The solvent control also gave 7.9% abnormalities.

Conclusions

These studies are of uncertain relevance to the assessment of risks to humans exposed to low doses. However, they show clear dose/response relationships with rates of teratogenic effects being lower than those of mortality.

5.2.8.2 Other routes

No data are available.

5.2.9. Toxicokinetics

5.2.9.1 Dermal route in rat skin (*in vitro*)

A series of studies were carried out on excised dorsal skin from male Fischer F344 rats in a flow-through diffusion cell system (Tonge, 1995). In these 4 µl (3.72 mg) of a 20% w/v solution of [14C]-hydroxycitronellal (184 mg/cm3) was applied to a 2.27 cm2 disk of skin (dose 1.64 mg/cm2) that was either full thickness, or tape-stripped to remove the stratum corneum or dermatomed to remove the epidermis. The vehicle and receptor fluid were either ethanol or ethanol/diethyl phthalate (3/1) and the test material was applied to the skin with or without occlusion by a Teflon cap.

Two separate studies were run in this system to assess the rates of absorption through full skin under occlusion, the maximum rate of absorption was observed at 30 hours being 25.35 µg/h in one study and 26.9 µg/h in another. This declined to 14.4 µg/h at 72 hours. After 72 hours, accumulated quantities absorbed into the receptor fluid were 1.15 mg (31 % of the applied dose) with between 1.10 mg and 1.29 mg (30-35%) being located in the skin and between 1.28 mg and 1.46 mg (34-39%) remaining in the donor fluid.

After one hour, 13.4% of the applied dose was located in the skin and this increased to 16.43% after six hours with no noticeable transfer into the receptor fluid. Measurement of levels of [14C]-hydroxycitronellal in stratum corneum removed by tape stripping revealed that after one hour, 5.82% of the applied dose was in the stratum corneum; reducing slightly to 5.63% after 6 hours. In the same experiment, 7.8% of the applied dose was located in lower layers of the skin after one hour, increasing to 10.8% after six. This indicates that over 40% of the total skin-bound hydroxycitronellal was in the stratum corneum after one hour but this reduced to about 34% after six hours as hydroxycitronellal moved from the stratum corneum into the lower strata of the skin (Tonge, 1995).

In order to assess the effectiveness of the stratum corneal barrier, it was found that tape-stripped skin permitted much faster absorption (52.2 µg/h at 72 hours) than full skin (15.0 µg/h). This difference is probably greater at shorter times than 72 hours because at this stage, most of the test material (2.29 mg, 62%) had already passed through the stripped skin into the receptor fluid compared with 0.79 mg (21%) for full skin. Removal of both the stratum corneum and the epidermis resulted in a 4-fold increase in the proportion of hydroxycitronellal passing through the skin. At 72 hours, 2.87 mg (77%) had passed through dermatomed skin compared with 0.76 mg (20%) for full skin. The maximum rate of absorption was 169.6 g/h for dermatomed skin compared with 0.76 g/h for full skin (Tonge, 1995).

From these data the dermal penetration coefficient can be calculated from the dermal flux (0.22 mg/cm2) according to the following algorithm: Kp = dermal flux/(exposure time x concentration of test solution):

$Kp = (0.22\ mg/cm^2)/(1h \times 184\ mg/cm^3) = 1.2 \times 10^{-3}\ cm/h.$

On the basis of the finding that the rate of penetration was maximal at 30 hours and that after 72 hours, 61-66 % of the applied dose had either passed the skin at 24 hours.

Conclusions

When maintained in the continuous presence of a solution of hydroxycitronellal, excised skin was still absorbing this material after 72 hours. Such contact conditions are unlikely to be encountered in the use of household cleaning and laundry products. However, these studies have permitted to obtain conservative estimates of the dermal penetration coefficient and the degree of absorption arising from the use of these products after 24 hours. These have been applied to estimates of systemic exposure detailed in sections **5.1.3.1** to **5.1.3.6**. It is also assumed that all skin-bound hydroxycitronellal is eventually absorbed. Recent studies into the receptor fluid or was bound to the skin, it is conservatively assumed that only 50% had not been absorbed into or through (Yourick *et al.*, 2004) have shown that chemicals absorbed into the so-called “skin reservoir” are not necessarily available for systemic absorption. None-the-less, the estimates of the dermal penetration coefficient and the extent of absorption after 24 hours have both counted skin-bound hydroxycitronellal as being completely systemically available.

5.2.9.2 Other studies

Like other aliphatic aldehydes, hydroxycitronellal would be expected to undergo rapid oxidation or conjugation with glucuronic acid preliminary to excretion in the urine. However, products of incomplete oxidation have also been observed. In a study on rabbits, 6 prior-fasted males were given a single orally administered dose of 2 g hydroxycitronellal in aqueous Tween 80. Analysis of uring collected after 3 days showed the presence of 7-hydroxycitronellol and 7-hydroxycitronellic acid (Ishida *et al.*, 1989).

5.2.10. Neurotoxicity

No data are available.

5.3. Risk Characterisation

5.3.1. Hazard Summary

Hydroxycitronellal shows a low order of acute toxicity by the oral and dermal routes and is not classified as harmful according to the criteria outlined in the European Dangerous Substances Directive.

Undiluted hydroxycitronellal is not irritating to the skin of man or animals according to the official criteria for classification. However, there is some evidence that high skin loadings, particularly under occlusion and particularly when the vehicle is ethanol, can give rise to signs

of skin irritancy. Hydroxycitronellal is classified as an ocular irritant on the basis of anecdotal human experience.

Studies *in vitro* show that about 50% of the administered dose of hydroxycitronellal is absorbed into or passed through the skin in 24 hours. A conservative estimate of the dermal penetration constant is 1.2×10^{-3} cm/h.

The Key hazard shown by hydroxycitronellal shows is its skin sensitization potential. This is manifested in a wide variety of predictive test systems. Patch tests carried out on dermatitic patients have indicated that acquired allergy to hydroxycitronellal is widespread. However, these clinical studies were carried out under conditions that are predisposed to detecting allergies that may not manifest themselves in the normal use of consumer products (Hostynek and Maibach, 2004b). With a few exceptions, these clinical studies did not establish an unambiguous role of hydroxycitronellal as the cause of the patients' dermatitis (Hostynek and Maibach, 2004a).

Although hydroxycitronellal shows some marginal photoxic effects in unvalidated *in vitro* systems using UV-B, hydroxycitronellal is not phototoxic to human skin in the presence of UV-A. Some clinical studies have given positive photo-patch results. However, this may not indicate a photoallergenic potential, but rather that increased dermal penetration permits elicitation of weak allergies to hydroxycitronellal.

Although hydroxycitronellal contains some structural alerts for mutagenicity (Ashby and Tennant, 1991), it failed to show genotoxic effects in a number of studies. It gave negative results in a standard bacterial assay, in a study in fruit flies and in the mouse micronucleus test. No tumors are reported in the above-mentioned chronic feeding study.

In sufficient data are available to allow the determination of a clear no observed adverse effect level for the systemic toxicity of hydroxycitronellal. A chronic feeding study carried out over 30 years ago shows that levels of 400 mg/kg/day in the diet are well tolerated but when considering this for the risk assessment of hydroxycitronellal when used as a flavouring agent, WHO has used a dose of 250 mg/kg bw/day as the NOEL. In view of the limitations of this study and bearing in mind recent considerations of structure-based thresholds of toxicological concern (Kroes *et al.*, 2004), to assume with confidence that the true NOEL for systemic exposure to hydroxycitronellal by the oral route falls above a certain conservative value. Hydroxycitronellal has a chemical structure corresponding to Class I in the "Decision Tree" procedure of (Cramer *et al.*, 1978). The 5^{th} percentile of NOELs of a large number of similarly classified chemicals gives such a threshold, which with a 300 fold safety factor in relation to limitations of these animal tests in relation to human exposure, gives a threshold of toxicological concern (TTC) for hydroxycitronellal of 1800 μg/capita/day (30 μg/kg bw/day).

High-dose studies carried out on chick embryos show that the rates of malformations at different doses are lower than concomitant mortality rates and are reduced to background control levels at lower doses.

5.3.2. Exposure summary

Based on information from the Habits and Practices tables, it can be concluded that skin exposure for topical effects and for systemic toxicity resulting from the use of hydroxycitronellal in household laundry and detergent products is the major route of exposure to hydroxycitronellal. Using the algorithms recommended in the HERA methodology document it has been estimated that *ca*. 99.6% of systemic body burden from the use of these products, results from dermal absorption, resulting almost entirely from direct skin contact of concentrated or diluted detergent products. Highly conservative estimates of oral intake of hydroxycitronellal in food and drinking water or from residues present on eating utensils and crockery give a value of 7.25×10^{-4} μg hydroxycitronellal/kg bw/day. Inhalation of hydroxycitronellal from detergent powder dusts or to aerosol sprays will give rise to only 7×10^{-7} μg hydroxycitronellal/kg bw/day. This represents an extremely minor fraction of overall systemic exposure. A highly conservative estimate of aggregate systemic exposure has been calculated as 0.17 μg/kg bw/day.

For topical effects, the highest anticipated exposures will be 0.9 $\mu g/cm^2$ arising from accidental or unintentional exposure. A slightly lower value of 0.7 $\mu g/cm^2$ is estimated to arise from the use of liquid detergents in laundry pretreatment.

5.3.3. Rational for identification of critical endpoints

Dermal exposure is the main exposure route for consumers and consequently it is necessary for human risk assessment to consider direct dermal effects such as skin irritation and sensitization as well as systemic toxicity due to dermally absorbed hydroxycitronellal. There is a substantial amount of data available for assessing the skin irritation and skin sensitization potential of hydroxycitronellal and for assessing the risks associated with these effects due to the use of consumer product formulations containing hydroxycitronellal. Exposure levels are too low in household cleaning products for hydroxycitronellal to contribute significantly to irritant effects. However, the possibility that allergic contact sensitization might be produced by low-level exposures to hydroxycitronellal coupled with a background of numerous reports of clinical allergy to this substance justify attribution of this effect as a critical endpoint.

Dermal penetration studies with excised skin have shown that hydroxycitronellal have shown that hydroxycitronellal has the potential to penetrate the skin and become systemically available. There are no long-term, systemic toxicity studies using the dermal route. Adequate repeat dose studies by the oral route are also lacking at this time. However, on the basis of an old chronic feeding study in a limited number of rats, systemic effects after dermal exposure can also be assessed using some conservative assumptions. A lower limit for systemic toxicological concern can be obtained from recent data-based theoretical approaches to dealing with substances having inadequate empirical NOAELs.

No other critical endpoints were identified. Hydroxycitronellal was not considered to be mutagenic or genotoxic. High dose studies on the teratogenic and embryotoxic effects of hydroxycitronellal in a model system show that rates of malformations are lower than the mortality rates. These studies cannot be extended to human health risk assessment.

5.3.4. Quantitative evaluation of data – No effect levels

Skin sensitisation: The No Expected Sensitising Level (NESL) for hydroxycitronallal has been are estimated from a large number of studies carried out in animals and human volunteers using a "weight-of-evidence" approach to be **2.95 mg/cm^2 (2.95 x 10^3 µg/cm^2)** (see Appendix 1). Attempts have been made to determine elicitation thresholds in subjects who have already been sensitized to hydroxycitronellal (see Appendix 2). However, there is convincing evidence that these levels are themselves subject to a number of variable factors that are more artefacts of their measurement than true no-effect-levels that can be used in risk assessment (see Appendix 3).

Systemic effects: An old chronic feeding study in rats shows that levels around 400 mg/kg/day in the diet are well tolerated. However, this study is inadequate. Two measures are used in this risk assessment:

(a) the No Observed Effect Level (NOEL) from this study used by JECFA of **250 mg/kg/day (2.5 x 10^5 µg/kg bw/day)** (IPCS (International Programme on Chemical Safety), 2004);
(b) the highly conservative threshold of toxicological concern (TTC) of **30 µg/kg bw/day** (Kroes *et al.*, 2004;Munro *et al.*, 1996).

5.4. Risk Assessment

5.4.1. Margin of Exposure calculations

5.4.1.1 Margin of exposure: for contact allergy (skin sensitization)

Taking the No Expected Sensitizing Level (NESL) for hydroxycitronallal as **2.95 x 10^3 µg/cm^2** (Section 5.3.4.), it is possible to determine Margins of Exposure (MOE_{sens}) using the dermal exposure estimates from Section 5.1.3. These exposure estimates are all based on exceptional "worst-case" scenarios. Direct exposure from product use assumes that the consumer does not take normal precautions to rinse or wipe hands after use. Hydroxycitronellal is assumed to remain on the skin after use and hands have dried. Indirect and accidental exposures are also assumed to be the result of highly unlikely scenarios. For this reason, it is reasonable to neglect the additional effects of multiple uses of the same or different products over this period.

The Margins of Exposure are as follows:

5.4.1.1.1. Exposure scenario: Direct skin contact

A. Hand-washed laundry. The MOE was calculated by dividing No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from hand washing detergents of **7 x 10^{-3} µg/cm^2**.

MOE_{sens} = **2.95 x 10^3 µg/cm^2 /7 x 10^{-3} µg/cm^2. = > 400,000**

B. Pre-treatment of clothes (liquid detergent). The MOE was calculated by dividing the No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from pre-treatment of clothes using a liquid detergent of **7 x 10^{-1} µg/cm^2**..

MOE_{sens} = **2.95 x 10^3 µg/cm^2 /7 x 10^{-1} µg/cm^2.. = > 4000**

C. Hand dish-washing. The MOE was calculated by dividing the No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from hand dish-washing of **9 x 10^{-4} µg/cm^2**.

MOE_{sens} = **2.95 x 10^3 µg/cm^2 /9 x 10^{-4} µg/cm^2 = > 3,000,000**

D. Hard surface cleaning. The MOE was calculated by dividing the No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from hard surface cleaning of **1.2 x 10^{-3} µg/cm^2**.

MOE_{sens} = **2.95 x 10^3 µg/cm^2 /1.2 x 10^{-3} µg/cm^2 = > 2,000,000**

5.4.1.1.2. Exposure scenario: Indirect skin contact

From wearing clothes. The MOE was calculated by dividing the No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from wearing clothes of **5.6 x 10^{-11} µg/cm^2**.

MOE_{sens} = **2.95 x 10^3 µg/cm^2 / 5.6 x 10^{-11} µg/cm^2 = > 5 x 10^{13}**

5.4.1.2 Accidental or intentional over-exposure.

The MOE was calculated by dividing the No Expected Sensitizing Level (NESL) of **2.95 x 10^3 µg/cm^2** by the estimated exposure from accidental over-exposure of **9 x 10^{-1} µg/cm^2**.

MOE_{sens} = **2.95 x 10^3 µg/cm^2 /9 x 10^{-1} µg/cm^2 = > 3,000**

5.4.1.3 Margin of exposure: Systemic effects

For systemic effects from exposure to hydroxycitronellal, two measures are used in this risk assessment:

(a) the JECFA No Observed Effect Level (NOEL) of **2.5 x 10^5 µg/kg bw/day;**
(b) the Threshold of Toxicological Concern (TTC) of **30 µg/kg bw/day***

***** Threshold of Toxicological Concern already incorporates a 300-fold safety factor.

The Margins of Exposure (MOE) are as follows:

5.4.1.3.1 Exposure scenario: Direct skin contact from hand-washed laundry.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^5 µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the

systemic dose of **1.4 x 10^{-2} µg/kg bw/day** estimated as exposure from hand washing detergents.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.4 x 10^{-2}= 1.7 x 10^{7}**
MOE$_{\text{from TTC}}$ = **30/1.4 x 10^{-2}= 2.1 x 10^{3*}**

5.4.1.3.2 Exposure scenario: Direct skin contact from pre-treatment of clothes

The MOE was calculated by dividing the JECFA No Observed Effect Level of 2.5 x 10^{5} µg/kg bw/day and the Threshold of Toxicological Concern of 30 µg/kg bw/day by the systemic dose of 1.39 x 10^{-1} µg/kg bw/day estimated as exposure from pre-treatment of clothes using a paste detergent.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.39 x 10^{-1} = 1.7 x 10^{6}**
MOE$_{\text{from TTC}}$ = **30 /1.39 x 10^{-1} = 2.1 x 10^{2*}**

5.4.1.3.3 Exposure scenario: Direct skin contact from hand dish-washing.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the systemic dose of **1.6 x 10^{-2} µg/kg bw/day** estimated as exposure from hand dish-washing.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.6 x 10^{-2} = 1.5 x 10^{7}**
MOE$_{\text{from TTC}}$ = **30 /1.6 x 10^{-2} = 1.8 x 10^{3*}**

5.4.1.3.4 Exposure scenario: Direct skin contact from hard surface cleaning.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the systemic dose of **6.7 x 10^{-4} µg/kg bw/day** estimated as exposure from hard surface cleaning.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /6.7 x 10^{-4} = 1.4 x 10^{8}**
MOE$_{\text{from TTC}}$ = **30 /6.7 x 10^{-4} = 4.4 x 10^{3*}**

5.4.1.3.5 Exposure scenario: Indirect skin contact from wearing clothes.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the systemic dose of **1.5 x 10^{-7} µg/kg bw/day** estimated as exposure from wearing clothes.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.5 x 10^{-7} = 1.6 x 10^{13}**
MOE$_{\text{from TTC}}$ = **30 /1.5 x 10^{-7} = 2 x 10^{8*}**

5.4.1.3.6 Exposure scenario: Aggregate Direct & Indirect skin contact

In a worst-case scenario, the aggregate consumer exposure from dermal penetration after all of the above scenarios does not exceed **1.697 x 10^{-1} µg/kg bw/day.** The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by this aggregate dose.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.7 x 10^{-1} = 1.4 x 10^{6}**
MOE$_{\text{from TTC}}$ = **30 /1.7 x 10^{-1} = 1.7 x 10^{2*}**

5.4.1.3.7 Exposure scenario: Indirect exposure by oral route from food and drinking water.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the systemic dose of **2.75 x 10^{-4} µg/kg bw/day** estimated as exposure from drinking water.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /2.75 x 10^{-4} = 9 x 10^{8}**
MOE$_{\text{from TTC}}$ = **30 /2.75 x 10^{-4} = 1 x 10^{5*}**

5.4.1.3.8 Exposure scenario: Indirect exposure by oral route from dishwashing residues.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the daily systemic dose of **4.5 x 10^{-4} µg/kg bw/day** estimated as exposure from dishwashing residues.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /4.5 x 10^{-4} = 5.5 x 10^{8}**
MOE$_{\text{from TTC}}$ = **30 /4.5 x 10^{-4} = 6.6 x 10^{4*}**

5.4.1.3.9 Aggregate of exposure by the oral route.

The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5 x 10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the daily aggregate oral exposure (from 5.4.1.3.7 and 5.4.1.3.8) of **7.25 x 10^{-4} µg/kg bw/day** estimated as exposure from dishwashing residues.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /7.25 x 10^{-4} = 7.5 x 10^{8}**
MOE$_{\text{from TTC}}$ = **30 /7.25 x 10^{-4} = 4 x 10^{4*}**

5.4.1.3.10 Exposure scenario: Indirect inhalation

The exposure estimates were **9.5 x 10^{-7} µg/ kg bw/day** from the inhalation of detergent dust and **6 x 10^{-7} µg/kg bw/day** from the inhalation of aerosols giving an aggregate inhalation exposure of **1.55 x 10^{-6} µg/kg bw/day**.

MOE$_{\text{from NOEL}}$ = **2.5 x 10^{5} /1.55 x 10^{-6} = 1.6 x 10^{11}**
MOE$_{\text{from TTC}}$ = **30 /1.55 x 10^{-6} = 4 x 10^{7*}**

5.4.1.3.11 Exposure scenario: dermal route from accidental or intentional over-exposure.

As this type of exposure would not be repeated for a significant number of times, the systemic MOE is meaningless.

5.4.1.3.12 Total Consumer Exposure

In a worst-case scenario, the aggregate consumer exposure from all of the above scenarios would be unlikely to exceed **1.7×10^{-1} µg/kg bw/day.** The MOE was calculated by dividing the JECFA No Observed Effect Level of **2.5×10^{5} µg/kg bw/day** and the Threshold of Toxicological Concern of **30 µg/kg bw/day** by the daily systemic dose of **1.7×10^{-1} µg/kg bw/day** estimated as exposure from all sources.

$MOE_{\text{from NOEL}} = 2.5 \times 10^{5} / 1.7 \times 10^{-1}$ µg/kg = 1.47×10^{6}
$MOE_{\text{from TTC}} = 30 / 1.7 \times 10^{-1}$ µg/kg = 176*

***** *Threshold of Toxicological Concern already incorporates a 300-fold safety factor.*

5.4.2. Risk characterization

5.4.2.1 Contact allergy

Cell-mediated (Type IV) contact allergy results from dermal exposure. It may be induced after a single exposure episode, although the likelihood of its acquisition is increased by multiple exposures and is dependent on the concentration of the allergen in the different products to which the skin is exposed (Basketter *et al.*, 1997;Marzulli and Maibach, 1974) although the true measure of "dose" for this effect is the quantity applied per unit area (Boukhman and Maibach, 2001;Roggeband *et al.*, 2001). For this reason, it is necessary to consider each individual exposure scenario as a separate occasion for inducing allergy to hydroxycitronellal.

The skin of consumers will be exposed to hydroxycitronellal in a repetitive fashion due to its presence in household laundry and cleaning products. All potential dermal exposure scenarios arising from the use and accidental miss-use of these products have been identified, quantified and assessed by comparing the estimated dermal exposures with the non-induction threshold doses determined from studies in human subjects and reinforced by studies carried out on animals. The Margin of Exposure (MOE) for this induction dose resulting from the worst case of potential allergy-inducting exposure (accidental or intentional over-exposure) is still over 3,000.

This MOE is certainly large enough to account for the inherent uncertainty and variability of the hazard data on which it is based. The MOE is based on worst-case exposure assumptions and a value for the No Expected Sensitizing Level is taken from studies that may be inappropriately severe due to the use and duration of occlusion. The true maximum dermal exposure is probably significantly lower in real life than is presented here for a number of reasons. The material has significant solubility in water and will be even more soluble in aqueous solutions of cleaning products containing surfactants. It will largely be rinsed off the

skin under normal conditions of anticipated use thereby reducing exposure to levels considerably below those used in the exposure calculations in section 5.1.

There are experimentally observable threshold doses below which the allergic state is not induced and also, once subjects have been sensitised, there are certainly threshold doses below which an allergic response is not elicited to the degree of producing clinically recognisable symptoms. As explained in **Appendix 3** however, the exposure level is only one a multitude of factors that predispose prior-sensitised subjects to producing the clinical manifestations of allergic reactions. Furthermore, methods used to determine this critical exposure level can be criticised (**Appendix 3**). For this reason, empirically observed non-elicitation levels are not reliable indicators for risk assessment.

Although, numerous cases of positive patch test reactions to hydroxycitronellal in dermatitic patients are recorded, none have been specifically linked to the use of laundry or cleaning products. In a multi-centre study involving 738 patients suffering from contact dermatitis, little evidence that aqueous solutions of 0.1% granular or liquid laundry detergents were able (even after occlusion for 48 hours in special occlusive chambers) were able to elicit the patients' contact allergies (Belsito *et al.*, 2002). None-the-less, allergy to hydroxycitronellal is manifestly quite widespread (**Section 5.2.3.2: Tables 20-24**) but is probably at a “sub-clinically” low level with the result that allergenic reactions will only be manifested at exposure levels that are higher than those that result from ordinary daily exposure to consumer products (Hostynek and Maibach, 2004). A significant number of consumers using these types of products can therefore be expected to be already sensitized to hydroxycitronellal due to other causes. It has been shown that some of these may react to doses of hydroxycitronellal as low as 1 $\mu g/cm^2$ in open non-occluded exposure situations as found with the use of these products (**Appendix 2**). Even if this was to be a reasonable no-effect level for elicitation, there would still be a MOE of greater than one between this and the exposure dose resulting from direct skin exposure resulting from accidental or intentional over-exposure (hardly a regular twice-daily event). However, for various reasons (see **Appendix 3**), there is still insufficient knowledge relating to thresholds for elicitation, to be able to make proper risk assessments for this effect. It is also clear that if the risk of induction is adequately managed, the assessment of the risk of elicitation becomes unnecessary.

In summary, the use of hydroxycitronellal in consumer products such as laundry and other household cleaning products does not raise any safety concerns with regard to the induction of contact allergy. Although it is not possible in theory to exclude the likelihood that pre-existing allergies to hydroxycitronellal may be elicited in exquisitely sensitive subjects as a result of the use of some of these laundry and cleaning products, these cases should be extremely rare and would be obviated by adequate risk management of induction.

5.4.2.2 Systemic effects

Inadequate data exist for establishing a reliable No Observed Adverse Effect Level (NOAEL) for long-term systemic exposure to hydroxycitronellal. Two measures have been employed as substitutes for this. One is a conservative No Effect Level used by the Joint FAO/WHO Expert Committee on Food Additives when it assessed the safety in use of hydroxycitronellal as an artificial flavouring agent. The other is a base-line Toxicological Threshold of Concern

(TTC) based on the classification of the chemical structure of hydroxycitronellal with regard to a large body of NOAELs for similarly-classified chemicals.

Consumers are exposed to hydroxycitronellal through its use in laundry and cleaning products. All significant potential exposure scenarios were identified and quantified and assessed by comparing theses estimated maximum exposures with these two measures (NOEL and TTC). The Margin of Exposure (MOE) for an aggregate of all possible routes of consumer exposure is above one million for the NOEL of JECFA and over one hundred for the TTC (which incorporates a safety factor of 300). This MOE calculation represents the total of all possible exposure scenarios using worst-case assumptions, an exposure situation that is very unlikely to occur in real life.

The determined MOEs using both measures are certainly large enough to account for the inherent uncertainty and variability of the hazard data on which it is based. The MOE derived from the NOEL from an old gavage study which itself may have produced effects due to its manner of exposing sensitive rodent gastric tissues to a bolus of hydroxycitronellal; a material with known irritant properties. The true consumer exposure is probably significantly lower than presented here particularly as hydroxycitronellal has significant solubility in water and will be even more soluble in cleaning products containing surfactants. It will generally be rinsed off the skin under normal conditions of anticipated use thereby reducing exposure to levels considerably below those used here in the calculating the MOEs. Even under reasonably foreseeable conditions of miss-use, it is unlikely that these conditions will be repeated with the same daily rhythm as in the referenced repeated dose studies in rodents.

The available toxicological information indicates that hydroxycitronellal is not mutagenic or genotoxic. There was no evidence of reproductive toxicity, developmental or teratogenic effects at doses that did not already cause lethal embryotoxicity.

An overwhelmingly large proportion of the total systemic hydroxycitronellal exposure results from the percutaneous absorption of hydroxycitronellal in applications involving transient skin contact. The percutaneous absorption of hydroxycitronellal was measured in studies carried out *in vitro* on excised rat skin. This system is known to over-estimate absorption into and through human skin. The measures used to estimate probable penetration of hydroxycitronellal assume conservatively but incorrectly that all material bound to or within the skin will eventually become bioavailable.

In summary, the use of hydroxycitronellal in consumer products such as laundry and cleaning products does not raise any safety concerns with regard to systemic toxicity.

5.4.2.3 Other local effects

The irritation potential (and possible phototoxic potential) of hydroxycitronellal are concentration dependent. Under normal use conditions of all of these products, these effects are not likely to be manifested. For this reason, these endpoints were not identified as being critical. The same argument applies for acute effects resulting from the accidental ingestion of a hydroxycitronellal containing detergent products.

5.4.3. Summary and Conclusion

Exposure to hydroxycitronellal due to its presence in laundry and cleaning products occurs overwhelmingly by the dermal route. Skin exposure occurs mainly in hand-washed laundry, laundry pre-treatment and hand dishwashing. Some dermal exposure will result from the use of other products or from indirect exposures such as through contact with hydroxycitronellal residues in fabrics after the washing cycle and skin contact during hard surface cleaning. Oral exposure occurs from the possible environmental presence of hydroxycitronellal resulting in residues being consumed in drinking water and in food. Oral exposure can also arise from residues on eating utensils and dishes after hand washing. Hydroxycitronellal is also used in spray cleaners that may give rise to inhalation exposure via the aerosols generated during spraying. Inhalation of hydroxycitronellal will also arise from detergent dusts. However, these routes give rise to extremely minor exposure levels compared to direct dermal exposure from the use of a few specific laundry products. The consumer aggregate exposure (body burden) has been estimated to be less than 0.17 μg/kg/day. Maximum dermal exposure expressed as the dose that is critical to the induction and elicitation of contact allergy is 0.9 $\mu g/cm^2$ from accidental or intentional over-exposure.

From the available toxicological data and information *in vivo* and *in vitro*, only two end-points: contact allergy and systemic toxicity were identified as being critical.

There is a large body of data in man and animals to show that hydroxycitronellal is a skin sensitizer. A No Expected Sensitization Level of 2.95 mg/cm^2 was determined on a “weight of evidence” approach. Less certain data exist for the threshold dose for elicitation of a previously acquired allergy to hydroxycitronellal. Available evidence shows that “threshold” is only one of many factors that determine if an allergy will be elicited. This complicates the determination of these “thresholds”, a determination that is rendered more complicated by the fact that the method used to measure this threshold is known to affect the measured threshold. None-the-less, from limited tests carried out under maximized conditions, it appears that exquisitely sensitive individuals may react to doses down to 1 $\mu g/cm^2$. This is still higher than the highest estimate of likely exposure.

On the basis of the worst-case exposure scenarios resulting from the use and miss-use of these laundry and cleaning products, a MOE of more than 3,000 was obtained for the induction of contact allergy to hydroxycitronellal.

For systemic toxicity, an old multiple dose study on hydroxycitronellal was judged to be sub-optimal for the determination of a reliable NOAEL. Instead, two conservative measure were taken: a NOEL determined on the basis of this study and previously used by JECFA and the Threshold of Toxicological Concern based on a large data set of NOAELs for substances having a similar structure classification as that of hydroxycitronellal. Comparison with aggregate exposure results in a MOE of over one million for the NOEL and about 176 for the TTC. These large margins of exposure are large enough to account for the inherent uncertainty and variability of the available hazard data and also for inter- and intra-species extrapolations.

Human experience has shown that neat hydroxycitronellal may be irritating to the eye. The irritation potential of this substance depends on concentration. Local dermal and ocular effects due to direct or indirect contact with hydroxycitronellal containing solutions in hand-washed laundry or hand dishwashing are not of concern because hydroxycitronellal is not expected to be irritating at the extremely low concentrations of use in these products.

In summary, this human health risk assessment has demonstrated that the use of hydroxycitronellal in household laundry and cleaning products is safe and does not cause concern with regard to consumer use.

APPENDIX 1.

NO EXPECTED SENSITISATION LEVELS (NESLs)

At Induction

On the basis of a weight of evidence approach, a No Expected Sensitization Level (NESL) of 2.95 mg/cm^2 has been chosen for hydroxycitronellal.

Non-induction levels from animal tests.

Studies using adjuvants and/or intradermal injection are not particularly appropriate for determining no effect levels for induction. None-the-less, these indicate that hydroxycitronellal has a clear sensitization potential. Several of these studies have shown also that there are clear dose/response relationships and thresholds below which animals are not sensitized (Wahlkvist *et al.*, 1999).

Studies in non-adjuvant predictive tests in animals gives a better opportunity for estimating induction threshold doses of hydroxycitronellal. In 7 Buehler Guinea pig tests, reactions occurred at induction doses of 25% but not at 30% and 20% giving a non-induction threshold around 20%. In the Open Epicutaneous Test, reactions were seen down to induction doses to 10%. EC3 values in eight Local Lymph Node Assays varied between 19% and 33% with a mean around 22.8% (c. 5.7 mg/cm^2). The EC3 dose has been shown to correlate well with the No Expected Sensitization Levels in predictive tests on humans (Gerberick *et al.*, 2001b;Griem *et al.*, 2003;Schneider and Akkan, 2004).

Non-induction levels in human tests.

In human studies 11 maximization tests gave an aggregate of 26/298 reactions at an induction dose of 12% (c. 6 mg/cm^2) in petrolatum. Positive reactions were seen to the separate enantiomers at 10% (but not the racemate). Negative results were seen in human maximization tests at 5% and 4% (c.2 –2.5 mg/cm^2).

Non-induction levels of hydroxycitronellal in Human Repeat Insult Patch Tests appear to depend on the vehicle. Water, the vehicle that is most relevant to the exposure scenarios described in section 5.1, was used in only one study in which no reactions were observed at a dose of 1% (1.18 mg/cm^2). No studies were performed at higher levels in water. In dimethyl phthalate, no reactions were observed in 110 subjects at 2% (2.4 mg/cm^2). Only one reaction

was observed in 150 subjects at 4% (4.8 mg/cm^2) hydroxycitronellal in petrolatum. In diethyl phthalate, no reactions were seen at 1% (1.18 mg/cm^2 – in a total of 117 subjects), 5% (5.9 mg/cm^2 – in 72 subjects) and 10% % (11.8 mg/cm^2 – in 29 subjects). In this vehicle, further maximized "second phase" HRIP Tests produced one clear reaction at 1% (in 115 subjects) but no reactions at 5% (49 subjects) and only one at 10% (28 subjects).

In a total of 7 "first-phase" human repeat insult patch tests on hydroxycitronellal in ethanol:diethyl phthalate (3:1), the aggregate scores (disregarding reactions that did not reappear on re-challenge) were 0/65 at 2.5% (2.95 mg/cm^2), 23/185 at 5% (5.9 mg/cm^2) and 1/66 at 7.5% (8.85 mg/cm^2). Second-phase HRIP Tests produced more reactions (10/33 at 2.5% and 21/83 at 5%).

In ethanol this test gave no reactions in limited numbers of subjects (39) at 5% (c. 2.5 mg/cm^2) but gave reactions at levels of 7.5% (c. 3.75 mg/cm^2). In other human repeat patch tests carried out on fragrance blends containing hydroxycitronellal, a total of 268 subjects did not react to blends containing between 4.5% (c. 4 mg/cm^2) and 1.1% hydroxycitronellal (Steltenkamp *et al.*, 1980).

Weight-of-Evidence No Expected Sensitization Levels (NESL)

In view of the particularities of allergic contact dermatitis, it is not appropriate to use terms like No Effect Levels. The No Expected Sensitization Levels (NESLs) are doses (expressed as quantities retained on unit areas of skin) that are not expected to give rise to sensitization of subjects under exaggerated test conditions. The non-inducing levels seen in the different test systems are:

- in animal tests (LLNA EC3): 5.7 mg/cm^2
- in human maximization tests (HMT): c. 2.5 mg/cm^2
- in HRIPTs with non-ethanolic vehicles: up to 11.8 mg/cm^2
- in HRIPTs with ethanol-containing vehicles: 2.95 mg/cm^2
- in HRIPTs (second-phase/ethanol-containing vehicles): 1.18 mg/cm^2

On the basis of a weight of evidence approach, a NESL of 2.95 mg/cm^2 has recently been chosen (RIFM/COLIPA, 2004).

At Elicitation

See Appendix 2 for studies carried out on human subjects and Appendix 3 for considerations concerning non-elicitation levels.

APPENDIX 2.

ELICITATION STUDIES ON SUBJECTS ALREADY SENSITIZED TO HYDROXYCITRONELLAL

1. Elicitation studies on subjects who had been sensitized to hydroxycitronellal in Repeat Insult Patch Testing.

In three subjects sensitized in human repeat insult patch tests on hydroxycitronellal in diethylphahthalate, the maximum non-eliciting doses were 0.1% (0.12 mg/cm^2) in two subjects and 1% (1.18 mg/cm^2) in the other (RIFM, 1990). Further data come from apparent elicitation thresholds in subjects sensitized in the course of a series of human repeat insult patch tests on hydroxycitronellal in ethanol:diethyl phthalate (3:1). Where this could be determined, the maximum non-eliciting doses in subjects sensitized in these studies were 5% (5.9 mg/cm^2) in one subject, 2.5% (2.95 mg/cm^2) in 3 subjects, 1.5% (1.77 mg/cm^2) in 30 subjects, 0.5% (0.59 mg/cm^2) in 8 subjects and 0.3% (0.35 mg/cm^2) in 3 subjects. In 2 subjects, questionable reactions were still observed at the lowest challenge dose of 0.1% (0.12 mg/cm^2) (RIFM, 1992), (RIFM, 1987b) (RIFM, 1988b) (RIFM, 1990).

In a use test carried out on 10 subjects sensitized in 3 weeks previously in human repeat insult patch testing, hydroxycitronellal was applied 21 times over 8 days as a 1% solution in ethanol:diethyl phthalate (1:3). 3/10 of these subjects reacted in this test (RIFM, 1988b). In another identical use test carried out on another 10 prior-sensitized subjects to the same concentration of hydroxycitronallal in the same vehicle over 21 days, no reactions were observed (RIFM, 1988b). Another use test involving 31 subjects who had been sensitized to hydroxycitronellal in human repeat patch testing, open application of hydroxycitronellal in a cologne format three times a day over one month, produced one positive reaction when the concentration of hydroxycitronellal was 0.05% and three positives when it was 1% (RIFM, 1991). In another use test under exactly the same conditions, no reactions were seen in 8 prior-sensitized subjects (RIFM, 1988b). In 59 prior-sensitized individuals, occlusive patch testing of hydroxycitronellal at 5% (5.9 mg/cm^2) in ethanol:diethyl phthalate (1:3) gave positive reactions in 10 (negative in the other 49) (RIFM, 1991).

In a study involving 41 subjects who had been sensitized in human repeat patch tests, 18 who had participated in these but who had not been sensitized, and 26 naive subjects, elicitation studies on hydroxycitronellal in ethanol:diethyl phthalate using 48 hour occlusion in 19 mm Hill Top chambers gave the following results:

21/41 pre-sensitized subjects reacted to 5% (9 mg/cm^2) of racemic hydroxycitronellal;

23/41 reacted to 5% (9 mg/cm^2) (R)-(+)-hydroxycitronellal;

13/41 reacted to 5% (9 mg/cm^2) (S)-(-)-hydroxycitronellal;

none of the other two groups of test subjects reacted to any of these (Api and Letizia, 2001).

In another study involving 29 subjects who had been sensitized in human repeat patch tests, 13 who had participated in these but who had not been sensitized, and 17 naive subjects,

elicitation studies on hydroxycitronellal in ethanol:diethyl phthalate using 48 hour occlusion in 19 mm Hill Top chambers gave the following results:

- 10/19 pre-sensitized subjects reacted to 5% (9 mg/cm^2) of racemic hydroxycitronellal;

- 11/29 reacted to 5% (9 mg/cm^2) (R)-(+)-hydroxycitronellal;

- 8/29 reacted to 5% (9 mg/cm^2) (S)-(-)-hydroxycitronellal.

None of the other two groups of test subjects reacted to any of these (Api and Letizia, 2001).

In yet another study involving 34 subjects who had been sensitized in human repeat patch tests, 15 who had participated in these but who had not been sensitized, and 22 naive subjects, elicitation studies on hydroxycitronellal in ethanol:diethyl phthalate using 48 hour occlusion in 19 mm Hill Top chambers gave the following results:

- 15/34 pre-sensitized subjects reacted to 5% (9 mg/cm^2) of racemic hydroxycitronellal;

- 20/34 reacted to 5% (9 mg/cm^2) (R)-(+)-hydroxycitronellal;

- 10/34 reacted to 5% (9 mg/cm^2) (S)-(-)-hydroxycitronellal.

None of the other two groups of test subjects reacted to any of these (Api and Letizia, 2001).

When the first and third studies were repeated using racemic hydroxycitronellal in aqueous soap solution using 48 hour occlusion in 19 mm Hill Top chambers, the following results were obtained (Api and Letizia, 2001):

- 4/41 pre-sensitized subjects reacted to 0.5% (0.9 mg/cm^2) of racemic hydroxycitronellal;

- 0/18 HRIPT-negative subjects reacted to 0.5% (0.9 mg/cm^2) racemic hydroxycitronellal;

- 5/26 naive subjects reacted to 0.5% (0.9 mg/cm^2) racemic hydroxycitronellal;

and

- 3/34 pre-sensitized subjects reacted to 0.05% (0.09 mg/cm^2) of racemic hydroxycitronellal but 1/34 reacted to the vehicle alone;

1/15 HRIPT-negative subjects reacted to 0.05% (0.09 mg/cm^2) racemic hydroxycitronellal but 2/15 reacted to the vehicle alone;

- 1/22 naive subjects reacted to 0.05% (0.09 mg/cm^2) racemic hydroxycitronellal but - 4/22 reacted to the vehicle alone.

Use tests on 75 prior-sensitized subjects with a bar soap (containing 0.05% hydroxycitronellal) that was used once a day for 4 weeks on hands and fore-arms and then for 2 months over the whole body gave no reactions (Api and Letizia, 2001). Another use test was carried out on the same subjects who had completed six months whole-body use of the bar soap. In this case they were given a moisturising lotion containing 0.03% hydroxycitronellal for daily use over 3 months. No reactions were observed (Api and Letizia, 2001). As a second phase to these use tests, 31 subjects who had participated in the first phase, participated in another a use test involving three daily applications of a cologne-type product made up of increasing concentrations of hydroxycitronellal gave reactions only in the group of prior-sensitized subjects with one (possibly false) reaction at 0.05%, none at 0.1% or 0.3% and 3 subjects reaction at 1%. (Api and Letizia, 2001).

2. Elicitation studies on hydroxycitronellal-sensitive dermatological patients.

Although there have been numerous reports of patients giving frank allergic responses to hydroxycitronellal in clinical patch testing on dermatological patients, only a few of these studies provide any information on dose/response relationships and elicitation threshold doses.

Serial dilution studies on 3 patients who were sensitive to hydroxycitronellal and isoeugenol, 2 who were sensitive to both oakmoss and hydroxycitronellal and one who was sensitive to both geraniol and hydroxycitronellal were used to obtain dose/response relationships where the response was recorded as the intensity of the reaction measured by clinical grading and Doppler flowmetry. When these were compared with dose response relationships obtained from a limited number (5) patients who were sensitive to only one of these, it appears that more intense reactions are observed in general for patients who are simultaneously sensitive to two allergens (Johansen *et al.*, 1998). This study does not yield any specific information on thresholds of elicitation but it does warn us that information obtained from substances tested alone may underestimate the intensity of reactions that would be experienced in a multi-allergen environment.

Patch testing on 12 hydroxycitronellal-sensitive patients, starting from low concentrations and moving up, thereby minimising "boosting" (see Appendix 3), but still using 48 hour occlusive Scanpor patches (0.05 ml on 0.5 inch diameter disks), gave reactions in 9 of these with "thresholds" at 4% (400 $\mu g/cm^2$) in two, 3% (300 $\mu g/cm^2$) in another two, with the others showing individual "thresholds" of 1.5% (150 $\mu g/cm^2$), 1% (100 $\mu g/cm^2$), 0.5% (50 $\mu g/cm^2$), 0.1% (10 $\mu g/cm^2$) and 0.025% (2.5 $\mu g/cm^2$) (Benke and Larsen, 1984).

In a use test of shampoos perfumed with an mixture of hydroxycitronellal, geraniol and hydroxyisohexyl-3-cyclohexene carboxaldehyde, 12 patients who were sensitive to hydroxycitronellal (see above) were given shampoo to use daily for three two weekly periods, moving at the end of each of these periods to a shampoo containing three times more of this mixture (the actual levels being based on the thresholds of elicitation determined in serial dilution patch testing). No reactions were seen to shampoos containing 0.01%, 0.03%, 0.1%, 0.3%, 1% and 3% hydroxycitronellal, with one patient reacting to a shampoo containing 5% hydroxycitronellal (Benke and Larsen, 1984). A 3% threshold dose would correspond to 2.4 $\mu g/cm^2$ (Gerberick *et al.*, 2001b).

Repeated Open Application Tests (ROATs) with a roll-on deodorant were carried out on seven hydroxycitronellal-sensitive patients (Svedman *et al.*, 2002), (Svedman *et al.*, 2003). Paired deodorants (one with hydroxycitronellal and the other without) were applied twice daily over with the hydroxycitronellal concentration increasing in step-wise manner from 0.032% to 0.1% to 0.32% over three successive 2 weekly periods. Four of the seven patients reacted to the lowest concentration 0.032% within the first two weeks; three reacting only in the second week. A further patient reacted in the fourth week when using the deodorant containing 0.1% hydroxycitronellal and the remaining two patients reacted in the fifth week when using the deodorant containing 0.32% hydroxycitronellal (this was during concomitant

patch testing of hydroxycitronellal in ethanol and in the deodorant base (see below). The quantities of deodorant applied per application were between 172 and 591 mg. The female patient who reacted in the first week to the deodorant containing 0.032% was reported to have used 238 mg deodorant/application. On the basis of standard total axilliary areas in females of 122 cm^2 (Felter *et al.*, 2003), the applied dose would have been 625 ng/cm^2. On the basis of the amount of deodorant used by the other three female patients reacting in the second week of usage of this deodorant containing this low level of hydroxycitronellal, the eliciting doses would have been 645 ng/cm^2, 947 ng/cm^2 and 493 ng/cm^2.

Closed patch tests were carried out (during weeks 5 & 6 of a the above ROAT testing) on the same 7 patients. These were carried out by serial dilution at 15 different concentrations in ethanol. These gave Minimum Eliciting Concentrations (MECs) [and Maximum Non-Eliciting Concentrations (MNECs)] of MEC = 4% [MNEC = 2% (0.58 mg/cm^2)] in one patient, MEC = 1% [MNEC = 0.5% (0.145 mg/cm^2)] in another patient, MEC = 0.5% [MNEC = 0.25% (72.5 ug/cm^2] in two patients, MEC = 0.25% [MNEC = 0.125% (36 $\mu g/cm^2$)] in one patient, MEC = 0.125% [MNEC = 0.063% (18 $\mu g/cm^2$)] in another patient and an extremely low MEC = 0.00012% [MNEC = 0.00006 % (17 ng/cm^2) in another patient. Seven dermatological patients who were not previously sensitive to hydroxycitronellal or to the Fragrance Mix gave no reactions in ROATs at any concentrations up to 4% (1.16 mg/cm^2) (Svedman *et al.*, 2003). Additional serial dilution 48-hour closed patch tests were carried on the same 7 patients using scented and unscented deodorant base (aluminium chlorohydrate, PPG-15, stearyl ether, Steareth-2, Steareth-21, dichlorobenzyl alcohol, phenoxyethanol) as vehicle in Finn chambers. Hydroxycitronellal was present at three concentrations: 0.32%, 1.0% and 0.032%. Only three of the patients reacted to these. One of the patients who had shown a MEC of 0.5% hydroxycitronellal in ethanol, showed an MEC 0f 0.32% [MNEC = 0.1% (0.029 mg/cm^2)], the patient who had an MEC of 0.00012% in ethanol, failed to react to the lowest concentration in this vehicle and had an MEC of 1% [MNEC = 0.032% (9 ug/cm^2)] and a patient who had shown an MNEC of 0.125% in ethanol, reacted to the lowest concentration in this vehicle [0.032% (9 ug/cm^2)] and hence failed to show a non-eliciting concentration under the conditions of 48 hour closed patch testing in a deodorant base (Svedman *et al.*, 2003).

APPENDIX 3.

CONSIDERATIONS REGARDING NON-ELICITATION LEVELS

A number of observations point to complications that prevent us from simply taking the lowest figures from the studies detailed in Appendix 2 as the "thresholds of elicitation". These relate to

(a) doubts over the "realism" of occlusive patch testing;

(b) lack of correlation between serial dilution patch tests and repeated open application testing;

(c) the influence of the severity of the induction regime on these thresholds;

(d) the influence of additional challenges on these thresholds.

(a) IS 48 HOUR OCCLUSIVE PATCH TESTING RELEVANT TO TRANSIENT OPEN EXPOSURE?

The potentiating effects of occlusion on dermal penetration of fragrance ingredients both *in vitro* (Ryatt *et al.*, 1988), (Bronaugh *et al.*, 1985), (Roper *et al.*, 1997) and *in vivo* (Bronaugh *et al.*, 1985), (Bronaugh *et al.*, 1990), are well documented and appear to be without exception in chemicals spanning the range of molecular weights, volatility and lipophilicity ofhydroxycitronellal. Indeed, the only substances for which occlusion does not appear to enhance penetration would seem to be amphiphillic substances like caffeine (Ryatt et al., 1998) and some high molecular weight steroids (Bucks *et al.*, 1988). The potentiating effects of occlusion on the intensity and frequency of allergic contact dermatitis have also been reported (numerous publications including (Kraus *et al.*, 1990), (Ale and Maibach, 1995), (Funk and Maibach, 1994), (Zhai and Maibach, 2001). Furthermore, the duration of exposure (48 hours in patch testing compared to shorter periods to consumer products even when these are not immediately rinsed or wiped from the skin) also has a similar enhancing effect (McFadden *et al.*, 1998). As a result, it is extremely difficult to extrapolate to real-life scenarios from apparent thresholds obtained from studies using closed patches.

(b) BAD CORRELATION BETWEEN SERIAL DILUTION PATCH TESTING AND REPEATED OPEN APPLICATION TESTING

Studies that identify the performance of individual patients in both of these studies have revealed that often those that appear to be most sensitive in serial-dilution patch testing are found to be among the least sensitive in repeat open application tests. This has been demonstrated for studies on isoeugenol, formaldehyde and chromium (Villarama and Maibach, 2004). This lack of correlation between individual performances in these two test systems is also seen in the study on hydroxycitronellal (Svedman *et al.*, 2003) with for example, the patient who was the least sensitive in patch testing being among the most sensitive in the open test. As pointed out by Villarama and Maibach, there are many factors leading of elicitation that are not understood.

(c) THRESHOLDS OF ELICITATION VARY ACCORDING TO THE SEVERITY OF THE INDUCTION REGIME.

Unlike most other toxicological thresholds, there is an increasing body of evidence to show that this *elicitation threshold* is not simply an intrinsic property of the allergenic substance. Indeed, there is now good evidence to show that it depends on a number of factors that are dependent on extraneous conditions. Recently published data show that the severity of the induction regime (i.e. the severity of the conditions under which allergy has been acquired) has an important influence over the no effect dose for elicitation products (Hostynek and Maibach, 2004b).

There has for some time been evidence to show that reactions observed at challenge are more intense following more severe induction exposures. Early studies (Marzulli and Maibach, 1974) showed that dose response relationships exist for both induction and elicitation of sensitization in humans to a number of substances. Subsequently, this was taken a step further by demonstrating that the elicitation concentrations necessary to sensitize any given proportion of animals to the chloromethylisothiazolinone/ methylisothiazolinone biocide in a Buehler Guinea Pig Test, was inversely proportional to the induction concentration. (Chan *et al.*, 1983). Subsequently, (Friedmann and Moss, 1985) (although they did not go as far as determining thresholds), demonstrated that the induction dose determines not only the proportion of subjects sensitized but also the intensity of the allergic response at challenge. In studies on three groups of volunteers who were experimentally sensitized by exposure to three different doses of Dinitrochlorobenzene, the increase in skin-fold thickness at challenge to three increasing doses gave three parallel dose response curves. When all three groups were challenged to the same three doses, the curve for subjects sensitized to 62.5 $\mu g/cm^2$ was lower in terms of skin-fold thickness, than that for the subjects sensitized by induction to 500 $\mu g /cm^2$ that in turn was lower than (and parallel to) that for those sensitized to 1000 $\mu g /cm^2$.

Subsequent studies have shown how thresholds of elicitation vary with the severity of the induction regime. One of the earliest studies in this area (Jayjock and Lewis, 1992) was carried out on the chloromethylisothiazolinone/ methylisothiazolinone biocide. Although criticised for the low number of animals used (Basketter *et al.*, 1997) these Buehler studies are reinforced by the more detailed studies carried out after this. The work of (Nakamura *et al.*, 1999) gives a rare insight into the relationship between the induction dose and the observed threshold of elicitation. Although this study was primarily aimed at comparing the Guinea Pig Maximization Test, the Adjuvant and Patch Test and the Buehler Test, it provides valuable data on this relationship. The results of these three tests on four different substances (2,4-dinitrochlorobenzene, maleic anhydride, hexylcinnamic aldehyde and 2-Dodecen-1-yl succinic anhydride) show that as the induction dose increases, the threshold of elicitation decreases. (van Och *et al.*, 2001) also carried out similar studies on three chemicals (diethylamine, Tetramethyl thiuram disulfide & Zinc Dimethyl dithiocarbamate) using the Guinea Pig Maximization Test. Here too the same trend was seen in each case. (Scott *et al.*, 2002) have carried out studies on mice on two substances (2,4-dinitrochlorobenene and squaric acid dibutyl ester). Challenge was made on the flanks of the animals and despite the moderate degree of biological variation one would expect in this type of study, the threshold of elicitation (measured as the challenge dose which produced significant increase in flank fold thickness) also showed the same dependence on the induction concentration. Other studies using a modified Guinea Pig Maximization Test have also shown the same trend. Most

notably these were the studies carried out on PTBS (p-t-butylphenylsalicylate) (Yamano *et al.*, 1995), on TPN (2,4,5,6-tetrachloroisophtalonitrile) and BIT (1,2-benzisothiazolin-3-one) (Noda *et al.*, 1998) and on IPBC (3-iodo-2-propynylbutylcarbamate) and CPIP (p-chlorophenyl-3-iodopropargylformyl) (Shimizu *et al.*, 2000).

Hydroxycitronellal is no exception. In the Cumulative Contact Enhancement Test (see Table 12, Section 5.2.3.1.1.), the apparent elicitation threshold decreases from 0.1% (induction at 3% and 10%) to 0.03% (induction at 20%) and then to below 0.01% (induction at 100%) (Wahlkvist *et al.*, 1999).

Hence we see that studies using different protocols in guinea pigs, mice and even in human volunteers using 15 different test materials provide the necessary robustness to conclude that this trend is general: the threshold for elicitation decreases according to the severity of the induction regime.

(d) THRESHOLDS OF ELICITATION DECREASE PROGRESSIVELY WITH EACH ELICITATION EXPOSURE.

Thresholds of elicitation are lowered by sequential exposures. The rarity of reactions in the first days of the Repeat Open Application Test is empirical testimony to this “boosting” effect (Friedmann, 1990). This is also true for the elicitation studies on hydroxycitronallal (Epstein, 1982), (Johansen *et al.*, 1996b), (Andersen *et al.*, 2001) (Svedman et al., 2003) that all clearly show that in sensitized patients, the threshold of elicitation diminishes with successive exposures. In the ROATesting reported in the papers of Johansen et al., and Andersen et al., sensitized subjects failed to react to the test material until at least 14 applications. In the study by (Epstein, 1982), 2 patients only reacted on the 11 and 14 days of repeated exposure. In another study on cinnamic aldehyde sensitive patients (Johansen *et al.*, 1996a), nearly half of these patients reacted in the same ROATesting as described here, after day 7 and some went up to day 14. It is difficult to quantify this "boosting" effect but this seems to be a general effect (Villarama and Maibach, 2004).

Exposure to hydroxycitronellal present in a multitude of different consumer products will also be expected to "boost" the existing allergic sensitivity to this substance. However, the degree of exposure from consumer products is of a different order than from closed patch testing (which preceded open use testing in the work described above - (Epstein, 1982), (Johansen *et al.*, 1996b), (Andersen *et al.*, 2001), (Svedman *et al.*, 2003). The relative severity of patch testing can be seen by comparing doses. As we have seen in Section 5.1.3, exposure to hydroxycitronellal in household products will lead to levels below 0.001 mg/cm^2. If hydroxycitronellal was used at 1% in the product-type that produces the highest on-skin level of fragrance: a perfume spray, it would give rise to a dermal loading of 0.026 mg/cm^2 (Gerberick *et al.*, 2001a). For shampoos the loading would be even lower (0.00008 mg/cm^2 according to (Robinson *et al.*, 2000), (Gerberick *et al.*, 2001a). Yet the use of diagnostic patch tests with 1% of the same ingredient in 8 mm Finn Chambers, will deliver a skin loading of 0.3 mg/cm^2 (Robinson *et al.*, 2000), a large increase over levels found in consumer products.

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7. CONTRIBUTORS TO THIS REPORT

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