

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification: Citral; 3,7- dimethylocta-2,6-dienal

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1 PHYSICAL HAZARDS

Classification for physical hazards is not a part of the CLH proposal for citral.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The information below on toxicokinetics have largely been copied from the public part of the registration dossier (only with minor editorial changes).

2.1.1 STUDY 1

Reference:

Diliberto JJ, Usha G, Birnbaum LS: Disposition of citral in male Fischer rats. Drug Metab. Dispos. 16, 721-727, 1988

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study.

Method: Time course of distribution of ¹⁴C-label in tissues, blood, bile, urine, feces, expired air measured by liquid scintillation counting after single and repeated application; separation of unchanged and metabolized citral in blood and bile by HPLC (metabolites not identified).

Test substance:

Citral and ¹⁴C citral, purity >= 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories, Portage, MI, USA

- Age at study initiation: 2-3 month

- Weight at study initiation: 200-250 g

- Fasting period before study: no data

- Housing: individually

- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Air changes (per hr): no data, air flow rate through the cages 0.3-0.4 L/min
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Acute study: single dose, oral (gavage).

Multiple dosing study: oral pretreatment for 10 days with unlabelled citral at a dose of 5 mg/kg bw/day followed by single oral or i.v. dose of 5 mg/kg ¹⁴C-citral.

Concentrations: oral application: 5, 50, 500 mg/kg/d; i.v. application: 5 mg/kg bw/d

No. of animals per dose: not specified

Sampling:

Tissues and body fluids sampled: urine, faeces, expired air, blood, liver, kidneys, adrenals, thymus, spleen, brain, heart, lungs, testes, skin, adipose tissue, muscle, stomach contents, small intestine contents, large intestine contents, tail site (for i.v. application), bile

Time and frequency of sampling:

- excreta samples at 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs;
- tissue samples at sacrifice at 72 hrs p.a.
- bile samples: at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270 min after dosing by cannulation of the common bile duct
- blood samples: at 1, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min p.a. by cannulation of the jugular vein
- air samples: the total air flow through the metabolism cages was continuously passed through two consecutive traps (charcoal trap and bubbler trap, see below)

Detailed study summary and results:

Citral was rapidly and completely absorbed from the gastrointestinal tract (91 - 95%) after oral exposure. The amount remaining in any tissue was < 2%; the highest concentrations in liver, muscle, blood, adipose tissue. The relative amount in tissue independent of dose or route of administration. The excretion profiles were independent from the dose or route of administration:

Recovery after single 5 mg/kg oral dose:

- 24 hours: 67% with 45% in urine, 16% as exhaled $^{14}\text{CO}_2$, 6% in feces, <1% as exhaled ^{14}C -citral; production of $^{14}\text{CO}_2$ essentially ceased by 12 hrs;
- 72 hours: 83% (+- 10 %) with 51% in urine, 17% as exhaled $^{14}\text{CO}_2$, 12% in feces, 3% in tissues, <1% as exhaled ^{14}C -citral

Recovery after single 5 mg/kg i.v. dose:

- 12 hours: 57% with 47% in urine, 7% as $^{14}\text{CO}_2$, 2% in feces, <1% as exhaled ^{14}C -citral; elimination essentially completed within 24 hrs
- 72 hours: 79% (+- 18 %) with 58% in urine, 8% as $^{14}\text{CO}_2$, 7% via the feces, 6% tissues, <1% as exhaled ^{14}C -citral

Elimination via bile after a single 5 mg/kg i.v. dose: 20% of the dose appeared in bile within 1 hr, with another 7% appearing by 4.5 hrs. The amount excreted in the bile was 4 times higher than that excreted in the feces within 3 days. HPLC of bile, even as early 5 min after treatment, demonstrated the complete absence of any unmetabolized citral.

Effect of multiple dosing: In rats pretreated for 10 days (5 mg/kg bw/d orally), biliary excretion was increased to 34%, while excretion via urine, feces or expired CO_2 was not affected. Therefore, repeated exposure did not alter the overall pattern of disposition.

In summary, most of the citral-derived radioactivity was rapidly eliminated from the body with a whole body half-life of 8 hr after i.v. exposure. However, a small percentage tended to persist with a clearance half-life of 24 hrs.

2.1.2 STUDY 2

Reference:

Diliberto JJ, Srinivas P, Overstreet D, Usha G, Burka LT, Birnbaum LS: Metabolism of citral, an α,β -unsaturated aldehyde, in male F344 rats. Drug Metab Dispos, 18, 866-875, 1990

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (metabolism).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Metabolism

Test guideline: non-guideline study.

Method: Urinary metabolites identified by reverse phase HPLC

Test substance:

Citral and ¹⁴C citral, purity >= 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories
- Age at study initiation: 2-3 months
- Weight at study initiation: 200-250 g
- Housing: individual
- Individual metabolism cages: yes
- Diet: pelleted NIH 31 lab chow ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Single dose, oral (gavage) and i.v. application.

Concentrations: gavage: 5 and 500 mg/kg bw; i.v.: 5 mg/kg bw

No. of animals per dose: N ≥ 3

Sampling - metabolite characterisation studies:

- Tissues and body fluids sampled: urine, bile (only after i.v. application)
- Time and frequency of sampling: 2, 7, 24 hours for urine; 5, 30, 60, 270 min for bile
- From how many animals: no data
- Method type(s) for identification: HPLC, GC, Liquid scintillation counting, UV absorption, 1H-NMR spectra, mass spectrometry

Treatment for cleavage of conjugates:

Enzymatic hydrolysis of pooled samples of urine or bile by treatment with β -glucuronidase or sulfatase, or sequentially with β -glucuronidase followed with sulfatase; subsequently the relative amounts of metabolites and parent citral were analyzed by HPLC and compared to synthetic standards.

Detailed study summary and results:

Seven metabolites could be identified in sufficient purity and quantity, namely:

A: 3-hydroxy-3,7-dimethyl-6-octenedioic acid;

B: 3,8-dihydroxy-3,7-dimethyl-6-octenoic acid;

C: 3,9-dihydroxy-3,7-dimethyl-6-octenoic acid;

D: E-3,7-dimethyl-2,6-octadienedioic acid;

E: 3,7-dimethyl-6-octenedioic acid;

F: Z-3,7-dimethyl-2,6-octadienedioic acid;

G: E-3,7-dimethyl-2,6-octadienoic acid.

Glucuronic acid conjugates only in bile

Pathways of biotransformation:

The biotransformation of citral includes reduction or hydration of the 2,3-double bond, oxidation of the aldehyde function, and allylic oxidation at C-8, and, possibly, C-9. Enzymes involved in the formation of these metabolites can be aldehyde dehydrogenase, β -oxidation by aldehyde oxidase, oxygenation at C-8 or C-9 by cytochrome P-450, and alcohol dehydrogenase for oxidation of intermediate alcohols. The molecules formed by these processes are more hydrophilic, since they contain COOH and other polar groups; conjugation reactions, such as with glucuronic acid, may form even more polar metabolites. Based on the structure of the isolated metabolites, it would appear that nucleophilic 1,4-addition reactions of the α,β -unsaturated aldehyde structure, e.g. with glutathione, are not a major function in the metabolism of citral.

Enzymatic hydrolysis did not appear to affect the chromatographic profile of urinary radioactivity. However, the biliary profile changed after glucuronidase treatment. Sulfatase treatment appeared to have no effect.

2.1.3 STUDY 3

Reference:

Phillips JC, Kingsnorth J, Gangolli SD, Gaunt IF: Studies on the adsorption, distribution and excretion of citral in the rat and mouse. *Fd. Cosmet. Toxicol.* 14, 537-540, 1976.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study

Method: Tissue distribution and time course of excretion in urine, faeces and exhaled $^{14}\text{CO}_2$ measured; metabolites in urine separated by TLC (individual metabolites not identified)

Test substance:

Citral supplemented with [1,2- ^{14}C]-citral, purity 99%.

Radiolabelling, specific activity: 0.305 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Wistar), male

- Source: Scientific Agribusiness Consultants
- Weight at study initiation: 150 g
- Housing: all-glass metabolism cages
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature ($^{\circ}\text{C}$): 20 +/- 1
- Air changes (per hr): air drawn through the system at a constant rate of 250 mL/min

Dosing:

Single dose, oral (gavage)

Concentrations: 5, 770 and 960 mg/kg bw

No. of animals per dose: 3

Sampling:

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids sampled: liver, lung, kidney, heart, spleen, stomach (wall + content), intestine (wall + content), brain
- Excreta: urine, faeces, exhaled $^{14}\text{CO}_2$: trapped in ethanolamine-2-ethoxyethanol (1:4, v/v)
- Time and frequency of sampling: tissues: 96 hours p.a., excreta: 24, 48, 72, 96 hrs, $^{14}\text{CO}_2$: trapping solutions were analyzed after 2, 4, 6, 7, 24, 48, 72, 96 hrs

METABOLITE CHARACTERISATION STUDIES

- Tissues and body fluids sampled: urine

- Time and frequency of sampling: 24, 48, 72, 96 hrs
- From how many animals: 3
- Method type(s) for identification: urine was extracted with hexane; extracts and aqueous residue were subjected to TLC. The distribution of radioactivity along the chromatograms was determined by removing bands 0.5 cm wide and counting (individual metabolites were not identified).

Detailed study summary and results:

Distribution in tissue: at doses of 5 and 960 mg/kg: at 24h [*but tissue sampling reported to be only at 96h*] most ¹⁴C in gastro-intestinal tract (ca. 7 and 12.5%) and the liver (ca. 1.5 and 2%).

Excretion:

5 mg/kg: 24 hours: >95% with 61% in urine, 20% as ¹⁴CO₂ and 17% in faeces; < 0.5% of ¹⁴C in urine as unchanged citral; exhalation of ¹⁴CO₂: constant rate up to 6 h (ca. 16% of the dose applied), substantially completed by 10 h (ca. 20% of the dose applied).

770 mg/kg: 96 hours: >95%; urinary excretion substantially complete by 60 h; faecal excretion slow phase up to 36 hrs, rapid phase between 36 and 72 hrs; excretion of ¹⁴CO₂ complete by 48 hrs.

960 mg/kg: 24 hours: 60 -70% with 47% in urine, 7.3% as ¹⁴CO₂ with constant rate up to 24 h after an initial lag phase of 2 h, 9.5% in faeces.

Metabolites:

Most of ¹⁴C in the urine as polar hexane-insoluble unsaturated compounds (not identified).

2.1.4 STUDY 4

Reference:

Phillips JC, Kingsnorth J, Gangolli SD, Gaunt IF: Studies on the adsorption, distribution and excretion of citral in the rat and mouse. *Fd. Cosmet. Toxicol.* 14, 537-540, 1976.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study

Method: Tissue distribution and time course of excretion in urine, faeces and exhaled $^{14}\text{CO}_2$ measured; metabolites in urine separated by TLC (individual metabolites not identified)

Test substance:

Citral supplemented with [1,2- ^{14}C]-citral, purity 99%.

Radiolabelling, specific activity: 0.305 mCi/mmol (labelled at C1 and C2)

Test animals:

Mouse (LACA strain), male

- Source: Scientific Agribusiness Consultants
- Weight at study initiation: 10-15g
- Housing: SPF conditions
- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS

- Temperature ($^{\circ}\text{C}$): 20 +/- 1

Dosing:

Single dose, oral (gavage)

Concentrations: 100 mg/kg bw

No. of animals per dose: 3

Sampling:

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids: radioactivity present in the body was visualized by autoradiography; data available for muscle, tongue, heart, lung, skin, hair follicles, eye lens, adipose tissue, testes, thymus, spleen, brain, salivary glands, stomach (wall and content), intestine (wall and content), caecum (wall and content), urinary bladder, liver, kidney (cortex and medulla), bone, spinal cord, urine, faeces
- Time and frequency of sampling: 12 and 24 hrs, 2, 3, 5, 7 and 10 d

Detailed study summary and results:

Distribution in tissue: Considerable proportion of ^{14}C appearing throughout the tissues within 12 h but with a declining trend over 72 hours. After 168 h only faint or no distribution of radioactivity could be measured in all tissues except from the liver and kidney cortex. After 240 h the ^{14}C was generally only detected at low levels in all tissues except from the liver.

Excretion: Major route of ¹⁴C-excretion via urine detected up to day 5. Significant proportion of ¹⁴C rapidly excreted with faeces within 12 h, ¹⁴C-excretion via faeces detected up to day 3.

2.1.5 STUDY 5 – dermal absorption (same reference as STUDY 1)

Reference:

Diliberto JJ, Usha G, Birnbaum LS: Disposition of citral in male Fischer rats. Drug Metab. Dispos. 16, 721-727, 1988

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics (dermal absorption)

Test guideline: non-guideline study.

Method: Time course of distribution of ¹⁴C-label in tissues, blood, bile, urine, feces, expired air measured by liquid scintillation counting after single and repeated application; separation of unchanged and metabolized citral in blood and bile by HPLC (metabolites not identified).

Test substance:

Citral and ¹⁴C citral, purity \geq 98%. No data on impurities.

Composition of test material (isomer ratio): 74% geranial, 26% neral

Radiolabelling, specific activity: 10.7 mCi/mmol (labelled at C1 and C2)

Test animals:

Rat (Fischer 344), male

- Source: Charles River Breeding Laboratories, Portage, MI, USA

- Age at study initiation: 2-3 month

- Weight at study initiation: 200-250g

- Housing: individually

- Individual metabolism cages: yes

- Diet: NIH 31 rat chow ad libitum

- Water: ad libitum

- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 +/-2
- Humidity (%): 50 +/- 5
- Air changes (per hr): no data, air flow rate through the cages 0.3-0.4 L/min
- Photoperiod (hrs dark / hrs light): 12/12
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Single dose, dermal (perforated tissue capsule held over the treated area with cyanoacrylate adhesive)

Concentrations: 5, 50 mg/kg

No. of animals per dose: 3

Sampling:

Duration of exposure: 72 h. Initial body burden: immediately after application rats were sacrificed and analyzed for total radioactivity present in the carcass, dosing site and dermal caps at zero time.

- Collection of blood: 72 hrs p.a.
- Collection of urine and faeces: 2, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hrs
- Collection of expired air: the total air flow through the metabolism cages was continuously passed through two consecutive traps (charcoal trap and bubbler trap)

Sacrifice at 72 hrs p.a. Analysis of application site: dermal skin sites and dermal metallic caps. Analysis of organs: liver, kidneys, adrenals, thymus, spleen, brain, heart, lungs, testes, skin, adipose tissue, muscle, stomach contents, small intestine contents, large intestine contents.

Detailed study summary and results:

The total recovery after 72 hrs in tissues, excreta, skin test site and non-occlusive cover at doses of 5 and 50 mg/kg was 61.50% and 68.21% of the Initial body burden (IBB), respectively. About 2/3 of the applied dose was present in the carcass, dosing site and non-occlusive cover at 0h due to evaporation during administration of the dermal dose (loss of about 1/3 of the dose). Less than 50% of the applied dose was available for dermal absorption as the IBB was reduced by adsorption to the dermal caps (24% of IBB).

The distribution of citral (i.e. citral derived radioactivity) in tissues and excreta after 72h was 7-9.5% in total tissues (except dermal skin sites), 8.5-9.9% in dermal skin sites, 8.4-17.3% in urine, 3.5-3.2% in faeces, 3.4-3.8% in expired CO₂ and 2.8-4.5% as expired citral (percentages depending on the dose).

2.1.6 STUDY 6 – dermal absorption

Reference:

Barbier P, Benezra C: The influence of limonene on induced delayed hypersensitivity to citral in Guinea Pigs. II. Label distribution in the skin of ¹⁴C-labelled citral. Acta Dermatovener (Stockholm) 63, 93-96, 1983.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: non-guideline study.

Method: Distribution of ¹⁴C in different layers of skin and in urine and faeces investigated by liquid scintillation counting.

Test substance:

Citral and [1,2-¹⁴C]-citral, no information on purity.

Radiolabelling, specific activity: 83 µCi/mg (labelled at C1 and C2)

Test animals:

Guinea pig (Hartley), female

- Source: Versault, Luisetaines, France

- Weight at study initiation: 300-350 g

PRE-TREATMENT:

As the study was designed to investigate the role of dermal absorption in elicitation of hypersensitivity, groups of guinea pigs were used that had been subjected to different induction procedures by applying the Freund Complete Adjuvant (FCA) Test: Group A - citral treatment: 0.5 mL citral was dissolved in 4.75 mL FCA and then emulsified with 4.75 mL saline, using a syringe. Each animal received intradermally 5 injections of 0.1 mL each, on alternate days. Group B - FCA-treated control: 4.75 mL FCA was emulsified with 4.75 mL saline, using a syringe. Each animal received intradermally 5 injections of 0.1 mL each, on alternate days. Group C - untreated control: no pretreatment procedure. All groups: This induction procedure was followed by a 2-week rest, before elicitation (see below) was performed.

Dosing:

Single dose, dermal

Concentrations: 1.88 mg/animal, ca. 63 µg/cm² skin area. Dose volume: 188 µl of a 0.5% solution per area, 2 areas in total.

No. of animals per dose: 1

Sampling:

- Collection of urine and faeces: during 16 hr via a metabolism cage
- Terminal procedure: 16 hr p.a.
- Analysis of organs: skin

Detailed study summary and results:

The total recovery of radioactivity from the excreta urine and feces, from total skin and from unresorbed citral at the skin surface was 42.1% in a guinea pig without pre-treatment (C) and 47.7% in a guinea pig that had been subjected to an induction treatment with citral (A). Amounts evaporated from the site of application, excreted via exhalation of ¹⁴CO₂, or deposited in body tissues were not recorded in this study. The amounts absorbed into the skin within 16 hrs p.a. were 23.9% (C) and 27.5% (A). The amount of ¹⁴C in the stratum corneum was comparable in all groups (10.0-12.2 %). There was a greater variation in the penetration to deeper skin layers between the single animals (6.4-10.5%). However, the ¹⁴C-recovery after stripping the stratum corneum from the deeper skin layers was incomplete compared to ¹⁴C-activity in total skin. Additionally, the values were recorded from single animals only, so that the values may represent individual variation.

2.1.7 STUDY 7 – dermal absorption

Reference:

Hayes AJ, Markovic B: Toxicity of Australian essential oil Backhousia citriodora (lemon myrtle). Part 2: Absorption and histopathology following application to human skin. Food Chem Toxicol 41: 1409-1416, 2003.

Test type

Non-guideline study, no information on GLP compliance. Basic toxicokinetics (dermal absorption).

Material and methods

Test guideline:

Type of method: In vitro

Objective of study: Toxicokinetics (dermal absorption)

Test guideline: non-guideline study.

Method: In vitro test system with full thickness human skin mounted in a Franz cell diffusion system: absorption into the skin and the subcutaneous fat layer monitored by GC-MS up to 12 hrs post application.

Test substance:

Lemon myrtle oil (*Backhousia citriodora*) from Australia.

Purity: 96.6% citral (no information on impurities)

Composition of isomers: 51.4% geranial, 40.9% neral, 2.6% trans-isocitral, 1.7% cis-isocitral

Radiolabelling: no

Test system:

Human skin (full thickness human skin mounted in a Franz cell diffusion system)

Duration of exposure: 100% essential oil: 1, 4, 8, 12 hrs; 1% essential oil product: 8 hrs

SKIN PREPARATION

- Source of skin: human
- Ethical approval if human skin: yes
- Type of skin: abdominal skin
- Preparative technique: majority of subcutaneous fat layer removed by gross dissection
- Thickness of skin (in mm): 2
- Membrane integrity check: yes
- Storage conditions: immediate use

PRINCIPLES OF ASSAY

- Diffusion cell: Franz cell
- Receptor fluid: DMEM
- Solubility of test substance in receptor fluid: not analyzed in separate pretests, not detectable in the receptor fluid during the skin absorption experiments
- Static system: yes
- Test temperature: 21 +/- 1 °C
- Humidity: not applicable
- Occlusion: watch glass
- Reference substance(s): no data

Dosing:

- 100% lemon myrtle oil: 100 µl or 89.5 mg applied to 4.9 cm², corresponding to 20 µl/cm² or 18 mg/cm²
- 1 % lemon myrtle oil product: 100 mg product (corresponding to 1 mg citral) applied to 4.9 cm², corresponding to 0.18 mg/cm²

Sampling:

4 skin samples per timepoint

Detailed study summary and results:

Citral (neral and geranial) found in all layers of full thickness skin.

Relative recovery of citral (% of administered dose) after 1, 4, 8 and 12 hrs of exposure:

- 100% essential oil: in epidermis/dermis 1.25%, 1.97%, 1.52% and 1.08%, maximum after 4 h; in subcutaneous fat tissue 0.04%, 0.11%, 0.17%, 0.49%, continuous increase up to 12 h; not detectable in receptor fluid; total recovery 1.29%, 2.08%, 1.69%, 1.57%; unresorbed fraction not determined
- 1% essential oil formulation (only data for 8 h exposure): epidermis/dermis 0.22%; in subcutaneous fat tissue 0.061%; not detectable in receptor fluid; total recovery 0.281%

Citral (as main component of lemon myrtle oil) was absorbed in freshly excised full-thickness human skin at all exposure periods tested. Neral and geranial were the only detectable components of the oil in the skin discs (epidermis and dermis) and in subcutaneous fat tissue. As exposure time increased, the recovery in the fat tissue increased also. However, the recovery in epidermis/dermis showed a maximum at 4 hrs p.a.. At all timepoints, the recovery in skin layers was higher than in subcutaneous fat.

Absorption through full-thickness skin (epidermis + dermis + fat) per skin area after application of neat essential oil:

- at 1 hr: 1.16 mg, corresponding to 0.24 mg/cm²
- at 12 hrs: 1.41 mg, corresponding to 0.29 mg/cm²

Receptor media of diffusion cells: no components of lemon myrtle oil detectable, assumed to be caused by low water solubility

3 HEALTH HAZARDS

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 STUDY 1 (LLNA)

Study reference:

Basketter D, Kolle SN, Schrage A, Honarvar N, Gamer AO, van Ravenzwaayb B and Landsiedelb R: Experience with local lymph node assay performance standards using standard radioactivity and nonradioactive cell count measurements. *Journal of Applied Toxicology*; 32 (2012): 590–596.

Detailed study summary and results:

Test type

LLNA (OECD 429), GLP compliant.

Test substance

Citral, purity 96.4% (Sigma–Aldrich, Taufkirchen, Germany)

Test animals

Mice (CBA/CaOlaHsd), female

6 animals per dose

Age: 6-12 weeks old

The animals were kept in fully air-conditioned rooms at a temperature of 20–24 °C and a relative humidity of 30–70%, with a 12 h light-dark cycle (lights on 6 a.m. to 6 p.m.). Tap water and food (Provimi Kliba, Kliba-Labordiät, Maus/Ratte Haltung ‘GLP’, SA, Kaiseraugst, Basel, Switzerland) were given ad libitum.

Administration/exposure

Three groups of mice (n=6 per dose) were treated with 5, 10 and 25% citral. Vehicle: acetone-olive oil (AOO) 4:1. One group was treated with vehicle alone (vehicle control). The test substance or vehicle alone was applied epicutaneously to the dorsal part of both ears (25 µl per ear for three consecutive days at the same site). About 66–72 h after the last application of test substance to the ears, the mice were injected intravenously into a tail vein with 20 µCi of 3H-thymidine in 250 µl of sterile saline. Mice were sacrificed 5 h after the 3H-thymidine injection.

Results and discussion

The responses to test substances exposure were characterized by 3H-thymidine incorporation into the lymph node cells (dpm) and ear weight (EW) and the stimulation index at each dose was calculated as the ratio of the test group mean values divided by those of the vehicle control group. Citral was shown to be sensitising with an EC3 value of 12.6%. Irritation was not observed for citral (determined by whether a greater than 20% increase in ear weight compared to the pre-test value occurred or not). Detailed information of the responses of each animal per test group are not presented in the article.

Calculated stimulation index, citral

Applied concentration	5%	10%	25%
3H-Thymidine SI	1.29	1.70	9.09

3.1.1.2 STUDY 2 (LLNA)

Study reference:

Jung KM, Jang WH, Lee YK, Yum YN, Sohn S, Kim BH, Chung JH, Park YH, Lim KM: B cell increases and ex vivo IL-2 production as secondary endpoints for the detection of sensitizers in non-radioisotopic local lymph node assay using flow cytometry. Toxicology Letters 209 (2012), 255– 263

Detailed study summary and results:

Test type

LLNA: BrdU-FMC, GLP: not reported.

Test substance

Citral, no information on purity

Test animals

Mice (Balb/c), female

4-6 animals per dose (several substances tested, specific information on number of animals per dose for citral not stated)

Age at study initiation: 8-9 weeks

Weight at purchase: 18-22 (7-8 weeks old)

The animals were kept under controlled conditions of temperature (23±3 °C) and relative humidity (50±10 %) with alternating 12h light and dark. The animals has ad libitum access to tap water and were kept on solid laboratory diet (Purina Co., Korea).

Administration/exposure

Groups of mice (N=4 or 6) were treated daily with 25µl citral in vehicle or vehicle alone on the back of both ears for three consecutive days (day 1-3). The concentrations were 5, 10 and 25% citral and the vehicle was acetone-olive oil (AOO). On day 5 mice were interperitoneally injected with BrdU and were sacrificed after a day. After sacrifice, auricular lymph nodes were isolated, weighed and undergone lymphocyte preparation. After bilateral auricular lymph nodes were pooled on individual basis, lymph node cells were prepared by disaggregation through 70µm mesh in 1 ml PBS. The lymph node cells (LNCs) were counted using a hemacytometer after staining with trypan blue.

Results and discussion

The SI in the LLNA:BrdU-FCM is the ratio of the mean number of LNCs with incorporated BrdU from mic in each of the test substance dose groups to the mean number of LNCs with incorporated BrdU from mice in the vehicle control group. Citral was shown to be sensitising with an EC3 value of 14.1% in the LLNA:BrdU-FCM assay. No information on irritation was reported but it was stated that the test concentrations were selected to include the known LLNA EC3 value for sensitizers that were free from systemic toxicity and/or excess local skin irritation. Detailed information of the responses of each animal per test group are not presented in the article. The obtained EC3 value was compared to the reference EC3 value of 9.2% for citral given in the OECD 429 guideline for the “traditional” LLNA assay (pooled result based on 6 studies). The study as a whole included LLNA:BrdU-FCM assays of the 22 reference substances listed in the OECD TG 429 and EC3 values were compared for these two assays. It was concluded that using BrdU incorporation with flow cytometry can provide a good non-radioisotopic alternative to the traditional radioisotope LLNA.

3.1.1.3 STUDY 3 (LLNA) (cited from REACH registration dossier)

Study reference:

Lalko J and Api AM: Investigation of the dermal sensitization potential of various essential oils in the local lymphnode assay. Food Chem Toxicol 44 (2006): 739-746

Detailed study summary and results:

Test type

LLNA, OECD 429.

Test substance

Citral; 2,6-octadienal, 3,7-dimethyl.

Purity: 99.5%

Test animals

Mice (CBA), female

4 animals per dose

Age at study initiation: 8-12 weeks

Weight at study initiation: 17-21 g

The animals were kept at 19-25 °C and relative humidity 30-70 % with alternating 12h light and dark. The animals has ad libitum access to water and diet.

Administration/exposure

Each test group received one of the five test concentrations in 1:3 EtOH:DEP or the vehicle at a test volume of 25 uL dosed on the dorsum of both ears on three consecutive days. After a two days rest, on the 6th day after the first treatment, all mice were injected i.v. by the tail vein with 20 µCi of [3H]methyl thymidine. Five hours later the mice were euthanized and the draining auricular lymph nodes were excised and pooled for each test group. After separation of the cellular fraction, the incorporation of [3H]TdR in lymph node cells was measured by β-scintillation counting and expressed as dpm (disintegrations per minute) per lymph node for each test group.

Results and discussion

Citral was shown to be sensitising with an EC3 value of 6.3%. No information on irritation is reported in the registration dossier.

Calculated stimulation index, citral

Applied concentration	2.5%	5%	10%	25%	50%
SI	2.8	2.3	5.1	11.4	22.1

3.1.1.4 STUDY 4-13 (LLNA, 10 studies cited in SCCS 2012, only limited information)

Study reference:

Unpublished summary reports by the Research Institute for Fragrance Materials (RIFM), cited in: Scientific Committee on Consumer Safety SCCS OPINION on Fragrance allergens in cosmetic products. June 2012 (SCCS 2012). RIFM references: 2004b, 2003k, 2003l, 2003m, 2003n, 2003o, 2003p, 2003q, 2003r, 2003s.

Detailed study summary and results:

Test type

LLNA with no reported deviations from OECD 429 according to SCCS 2012.

Test substance

Citral, no information on purity.

Test animals

Mice, n= 4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

Citral was tested in concentrations of either 0.4-2-4-8-20%, 0.3-1-3-10-30% or 2.5-5-10-25-50%. Vehicles used were either:

- 1:3 ethanol:diethyl phthalate (EtOH:DEP) (2 studies)
- 0.1% α -tocopherol om 3:1 EtOH:DEP (2 studies)
- 0.3% antioxidant mix (1:1:1 butylated hydroxytoluene [BHT], tocopherol and eugenol) in 3:1 EtOH:DEP (2 studies)
- 0.1% Trolox C in 3:1 EtOH:DEP (2 studies)
- 3:1 EtOH-DEP (2 studies)

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the studies conducted by RIFM the results generally confirm the sensitising properties identified for citral in other LLNA studies. The EC3 values reported by RIFM are in the range 1.2%-6.8%.

3.1.1.5 STUDY 14 (LLNA)

Study reference:

Basketter DA, Wright Z, Gilmour NJ, Ryan CA, Gerberick GF, Robinson MK, Dearman RJ, Kimber I: Prediction of human sensitization potency using local lymph node assay EC3 values. Toxicologist 66 (2002) (1-S), 240, cited in:

Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the study and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

LLNA

Test substance

Citral, no information on purity in cited reference. Vehicle: AOO

Results and discussion

An EC3 value of 13% was reported.

3.1.1.6 STUDY 15 (LLNA) (cited from REACH registration dossier)

Study references:

Basketter DA and Scholes EW: Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 30 (1992): 65-69;

Basketter DA, Scholes EW, Kimber I, Botham PA, Hilton J, Miller K, Robbins MC, Harrison PTC, Waite SJ: Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig data. Toxicol Meth 1 (1991): 30-43

Basketter DA, Scholes EW, Kimber I: The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol 32 (1994): 543-547

Detailed study summary and results:

Test type:

LLNA, equivalent or similar to OECD 429. The study was terminated on day 4 following three days of exposure.

Test substance

Citral; 2,6-octadienal, 3,7-dimethyl.

Purity: > 98%

Test animals

Mice (CBA), male/female

4 animals per dose

Age at study initiation: 8-12 weeks

Administration/exposure

Each test group received one of the 3 test concentrations in acetone:olive oil 4: 1 (v/v) or the vehicle alone at a test volume of 25 uL dosed on the dorsum of both ears on three consecutive days. On the 4th day after the first treatment, all mice were injected i.v. by the tail vein with 20 µCi of [3H]methyl thymidine. Five hours later the mice were euthanized and the draining auricular lymph nodes were excised and pooled for each test group. After separation of the cellular fraction, the incorporation of [3H]TdR in lymph node cells was measured by β-scintillation counting and expressed as dpm (disintegrations per minute) per lymph node for each test group.

Results and discussion

Citral was shown to be sensitising with EC3 values reported from 4 different laboratories ranging from ca. 7-15%. No information on irritation is reported in the registration dossier.

Calculated stimulation index, citral

Applied concentration	Calculated SI			
	Lab. A	Lab. B	Lab. C	Lab. D
2.5%	2.1	0.9	2.2	0.9
5%	5.0	2.2	5.1	2.4
10%	9.3	6.2	20.5	4.7
Data published in	Basketter et al. 1991 Basketter and Scholes 1992	Basketter et al. 1991	Basketter et al. 1991	Basketter et al. 1991

3.1.1.7 STUDY 16 (GPMT) (cited from REACH registration dossier)**Study reference:**

Basketter DA, Allenby CF: Studies of the quenching phenomenon in delayed contact hypersensitivity reactions. *Contact Dermatitis*, 25 (1991), 160-171

Basketter DA, Scholes EW, Kimber I, Botham PA, Hilton J, Miller K, Robbins MC, Harrison PTC, Waite SJ: Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig data. *Toxicol Meth* 1 (1991): 30-43

Basketter DA and Scholes EW: Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. *Food Chem Toxicol* 30 (1992): 65-69;

Detailed study summary and results:**Test type:**

Similar or equivalent to OECD 406 (Guinea pig maximization test) but with less detailed documentation of test conditions and test results.

Test substance

Citral, perfume industry quality. Purity: > 98%. Vehicle: not reported.

Test animals

Guinea pig (Dunkin-Hartley)

10 animals per dose (test group), 4 per control group

Administration/exposure:

10 guinea pigs were treated in the shoulder region with a series of six intradermal injections of test material at a slightly irritant concentration in combination with Freund's complete adjuvant to induce sensitization. 6-8 days later, sensitization was boosted by an occluded 48 h patch of test material at a mildly irritating concentration placed over the injection sites. 12-14 days later, the animals were challenged on one clipped and razored flank by an occluded 24 h patch containing test material at the maximum non-irritant concentration. A group of 4 animals treated as above but without the test material served as controls. Reactions were scored 24 and 48 h after patch removal for oedema and erythema on a scale of 0-3. The intradermal induction dose was 0.2%, the topical induction dose was 5% and the challenge dose 0.5%.

Results and discussion

Sensitisation was observed in 6/10 animals with a mean erythema score of 1.2.

3.1.1.8 STUDY 17 (GPMT)

Study reference:

Ishihara M, Itoh M, Nishimura M, Kinoshita M, Kantoh H, Nogami T, Yamada K: Closed epicutaneous test. Skin Res.28 (Suppl 2)(1986a), 230-240, cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. Regulatory Toxicology and Pharmacology 52 (2008) 62-73.

Detailed study summary and results:

A detailed summary of the study and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximization test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008):

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Doses were 10% citral throughout the induction and challenge period.

Results and discussion

Sensitisation was observed (no further details were provided).

3.1.1.9 STUDY 18 (GPMT)

Study reference:

Goodwin BFJ, Johnson AW: Single injection adjuvant test. Curr. Probl. Dermatol. 14 (1985), 201–207, cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology* 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the study and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximization test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008):

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Induction was conducted with 0.4% for the intradermal injection and 1% for the occluded patch. Challenge application was conducted with 0.25%.

Results and discussion

Sensitization reactions were observed in 40% (4/10) of the animals.

3.1.1.10 STUDY 19 (GPMT) (cited from REACH registration dossier)

Study reference:

Unnamed study report, 1978

Detailed study summary and results:

Test type:

Similar or equivalent to OECD 406 (Guinea pig maximation test).

Test substance

Citral synthetic; 2,6-Dimethyl-2,6-octadien-8-al; Substance No. 77/711, no further data

Test animals

Guinea pig (Pirbright White)

10 per test group (10 for 1st challenge, 5 for rechallenges), 5 per control group (animals with challenge treatment only)

Weight at study initiation (injection): 507.7 g

Administration/exposure:

1st application = intradermal induction:

- Freund's adjuvant/aqua dest (1:1) without test substance
- 25 % test substance in paraffin oil DAB7
- 25 % test substance in paraffin oil DAB7 and Freund's adjuvant/aqua dest. (1:1)

2nd application = Percutaneous induction:

- with 25 % test substance in paraffin oil DAB7

1st challenge: 10 % test substance in paraffin oil DAB7

1st rechallenge: 5 % test substance in paraffin oil DAB7

2nd rechallenge: 5 % test substance in paraffin oil DAB7

A. INDUCTION EXPOSURE

- 1x6 intradermal injections and one week later, treatment with SLS, followed by one percutaneous induction according to OECD 406
- Readings: 24 hours after the intradermal application and 48 hours after the percutaneous induction
- Control group: not treated during induction phase
- Site: shoulder, respective on the same area

B. CHALLENGE EXPOSURE

- No. of exposures: 3 challenges
- Day(s) of challenge: 1st challenge: 19 days after percutaneous induction, 1st rechallenge: 28 days after percutaneous induction, 2nd rechallenge: 33 days after percutaneous induction
- Site: 1st challenge: right flank, 1st rechallenge: left flank, 2nd rechallenge: right flank
- Concentrations: 1st challenge: 10 % test substance in paraffin oil DAB7, 1st and 2nd rechallenge: 5 % test substance in paraffin oil DAB7
- Evaluation (hr after challenge): 1st challenge: treated and control animals 48 and 72 hours; 1st rechallenge: treated animals only 24 h, control animals 24 and 72 h; 2nd rechallenge: treated and control animals 24, 48 and 72 h.

Results and discussion

Positive reactions were observed in 100% of the animals in the test groups (10/10 for first challenge and 5/5 for rechallenges). The erythema scores were 2 or 3 at all readings except for after the third challenge/third reading, where a score of 1 was observed for 4/5 animals.

3.1.1.11 STUDY 20 (GPMT) (cited from REACH registration dossier)

Study reference:

Unnamed study report, 1978

Detailed study summary and results:

Test type:

Similar or equivalent to OECD 406 (Guinea pig maximation test).

Test substance

Citral synthetic; 2,6-Dimethyl-2,6-octadien-8-al; Substance No. 77/712, no further data

Test animals

Guinea pig (Pirbright White)

10 per treated group (10 for 1st challenge; 5 for rechallenges); 5 per control group without induction treatment

Mean weight at study initiation: 551.3 g

Administration/exposure:

1st application = intradermal induction:

- 25 % test substance in paraffin oil DAB7
- Freund's adjuvant/aqua dest (1:1) without test substance
- 25 % test substance in Freund's adjuvant/aqua dest (1:1)

2nd application = percutaneous induction:

- 25 % test substance in paraffin oil DAB7

1st challenge: 10 % test substance in paraffin oil DAB7;

1st rechallenge: 5 % test substance in paraffin oil DAB7

2nd rechallenge: 5 % test substance in paraffin oil DAB7

A. INDUCTION EXPOSURE

- 1x6 intradermal injections and one week later one percutaneous induction according to OECD 406
- Readings: 24 hours after the intradermal application and 48 hours after the percutaneous induction
- Control group: not treated during induction
- Site: shoulder, respective on the same area

B. CHALLENGE EXPOSURE

- No. of exposures: 3 challenges
- Day(s) of challenge: 1st challenge: 19 days after percutaneous induction, 1st rechallenge: 28 days after percutaneous induction, 2nd rechallenge: 33 days after percutaneous induction
- Site: 1st challenge: right flank, 1st rechallenge: left flank, 2nd rechallenge: right flank
- Concentrations: 1st challenge: 10 % test substance in paraffin oil DAB7, 1st and 2nd rechallenge: 5 % test substance in paraffin oil DAB7
- Evaluation (hr after challenge): 1st challenge: 48 and 72 hours; 1st rechallenge: 24 h and 6 days; 2nd rechallenge: 24, 48 and 72 h.

Results and discussion

Positive reactions were observed in 100% of the animals in the test groups (10/10 for first challenge and 5/5 for rechallenges) except for after 114 hours after a 5% rechallenge where 60% positive reactions were observed. The erythema scores were 2 or 3 at most readings except for after the 2nd challenge/1st reading

(144h) and after the 3rd challenge/3rd reading (72h), where scores of 0 and 1 were observed in some of the animals.

3.1.1.12 STUDY 21 (GPMT)

Study reference:

Klecak G, Geleick H, Frey JR: *Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. J. Soc. Cosmet. Sci. 28 (1977), 53–64, cited in:* Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology 52 (2008) 62–73.*

Detailed study summary and results:

A detailed summary of the study and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Guinea pig maximization test according to the Magnusson and Kligman 1969 method.

Test substance

Citral, no information on purity in cited reference. Vehicle: not reported.

Test animals

Guinea pig

No. of animals per dose not specifically stated

Administration/exposure (cited from Lalko and Api, 2008):

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal.

Induction was conducted with 5% for the intradermal injection and 25% in petrolatum for the occluded patch. Challenge application was conducted with a subirritant concentration in petrolatum.

Results and discussion

Sensitization reactions were observed (No further details were provided).

3.1.1.13 STUDY 22 (Buehler)

Study reference:

Unpublished report by RIFM, 1973: The determination of citral in cosmetic formulations. Unpublished report from Rodia. RIFM Report Number 12471. RIFM, Woodcliff Lake, NJ, USA., cited in:

Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology* 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the study and results are not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type:

Modified Buehler test

Test substance

Citral, no information on purity in cited reference. Vehicle: petrolatum

Test animals

Guinea pigs

No. of animals per dose not specifically stated, but results expressed as reactions in 5/5 animals

Administration/exposure (cited from Lalko and Api, 2008):

“Citral was tested in a Modified Buehler Delayed Hypersensitivity Test in guinea pigs (Buehler, 1965; Ritz and Buehler, 1980). Induction consisted of three 6-h closed patch applications to the same clipped site on the dorsal surface with 20% citral in petrolatum. Induction applications were made once a week for 3 weeks. Following a 10–14 day rest, the guinea pigs were challenged with 20% citral in petrolatum. Challenge application was a 6-h occluded patch at a naive skin site. Control animals were challenged at the same time in an identical manner. Reactions were read 24 and 48 h after patch removal. Sensitization was observed in 5/5 animals (RIFM, 1973). Under the same conditions, samples of citral were tested to determine if changes in sample storage conditions would affect the sensitization potential. Sensitization reactions (5/5 animals per test) were observed to samples of citral that had been stored under nitrogen, stored with the addition of (butylated hydroxyanisole) BHA and after oxygen saturation (RIFM, 1973).”

Results and discussion

Sensitisation was observed in 5/5 animals, but no further information is available from Lalko and Api, 2008.

3.1.2 Human data

3.1.2.1 STUDY 1 (Patch test, selected)

Study reference:

Geier J, Uter W, Lessmann H, Schnuch A: Fragrance mix I and II: results of breakdown tests. *Flavour Fragr. J.* 2015, 30, 264–247.

Detailed study summary and results:

Test type

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data on the standardised fragrance mixtures Fragrance Mix I and II (FMI and FMII) obtained in the period from 1998-2013 and 2005-2013, respectively. Citral is a component of FMII (1% citral). In cases where positive reactions were observed for FMII, testing of the full mix breakdown (and other fragrance allergens) have been done. FMII was patch tested in 84,724 patients in 2005–2013. Of these 4265 patients (5.0%) had a positive reaction. Time trends were analysed by dividing the time span into four 2-year periods and one 1-year period (i.e. 2013). The FM II full mix breakdown was tested in 1058 patients with a positive reaction to FM II. The results obtained with citral alone are based on patch tests with 2% citral in petrolatum.

Description of test method as cited from Geier et al. 2015: *“Diagnosing contact sensitization is done by patch testing. Briefly, during this procedure, the incriminated allergen, incorporated in a vehicle (usually petrolatum or water) in a standardized concentration, is filled into a test chamber which is applied occlusively on the patient’s upper back for 1 or 2 days. After removal of the patches, reactions in the test areas are observed at least until 3 days after the application. In case of an allergen-specific sensitization, a positive reaction with erythema, infiltration and possibly papules (+), additionally vesicles (++), or even coalescing vesicles (+++) occurs, depending on the degree of sensitization. Patients, who are not sensitized, usually show no reaction at all; however, in some cases, irritant or doubtful reactions can occur, which are coded as ‘ir’ and ‘?’, respectively. Within the IVDK, patch tests are performed according to international and DKG guidelines [ref]. All patch test preparations were obtained from Almirall Hermal, Reinbek, Germany.”*

Patch test results at day three were evaluated (except in a few cases where no reading could be done at day 3, a day 4 reading was chosen instead). Statistical analysis and data management were done using SAS software (SAS 9.3, SAS Institute, Cary, NC, USA).

The results for citral showed that during the period 2005-2013 16.2% of the 1058 selected (FMII positive) patients were tested positive for citral. The results divided into time spans are listed in the table below (note

that the patient counts of the single time periods do not sum up to 1058 as FMII and its single components were tested in different time periods in 26 patients):

IVDK results of retrospective analysis of patch tests with citral 2% in petrolatum:

Year, patient count	2005-2006 n = 170	2007-2008 n = 250	2009-2010 n = 300	2011-2012 n = 222	2013 n = 90	2005-2013 n = 1058
Percent positive reactions (95% conf. intervals)	18.2% (12.7-24.9)	14.4% (10.3-19.4)	14.7% (10.9-19.2)	18.0% (13.2-23.7)	18.9% (11.4-28.5)	16.2% (14.0-18.5)

3.1.2.2 STUDY 2 (Patch test, selected)

Study reference:

Pónyai G, Németh I, Altmayer A, Nagy G, Irinyi B, Battyáni Z, Temesvári E: Patch Tests With Fragrance Mix II and Its Components. *Dermatitis*, Vol 23 no. 2 (2012), 71-74.

Detailed study summary and results:

Test type

A prospective study of data from 6 centres participating in a multicentre study in Hungary from 2009-2010 has been performed on behalf of the Hungarian Contact Dermatitis Group. A total of 565 patients with a history of skin symptoms provoked by scented products were included in the study. Clinical diagnoses of the patients were: contact dermatitis, 388 (allergic 208, irritative 180); atopic dermatitis, 44; dyshidrosis, 22; seborrhoeic dermatitis, 23; rosacea, 9; perioral dermatitis, 3; nummular eczema, 4; stasis dermatitis, 17; psoriasis, 16; and others, 39 (urticaria, 24; prurigo nodularis, 4; Morbus Hailey-Hailey, 4; discoid lupus erythematoses, 2; alopecia areata, 2; lichen simplex, 1; systemic lupus erythematoses, 1; and purpura pigmentosa, 1).

Description of test method as cited from Pónyai et al.: “*All the tests were performed with Brial GmbH D-Greven allergens; the skin reactions were evaluated (with 48-hour occlusion) at 48, 72, and 96 hours and also on the seventh day. The following fragrances were tested in the environmental contact patch test series: FM II (14% in vaseline [vas]), FM I (8% in vas), Myroxylon pereirae (balsam of Peru; 25% in vas), colophonium (20% in vas), wood-tar mix (12% in vas), propolis (10% in vas), and sesquiterpene lactone mix (0.1% in vas.). Apart from the environmental contact patch test series, tests with the FM II components were also carried out - citral 2%, farnesol 5%, coumarin 5%, citronellol 1%, >-hexyl-cinnamaldehyde (AHCA) 10%, and hydroxy-isohexyl-3-cyclohexene-carboxaldehyde (HICC; Lyrál) 5% (all of them in vaseline). The test results were analyzed by using items of the MOAHLFA index.*”

The results showed that 3.4% (19/565) of the selected patients had positive reactions (contact hypersensitivity) for citral when tested in 2% vaseline.

3.1.2.3 STUDY 3 (Patch test, selected)

Study reference:

Nardelli A, Carbonez A, Drieghe J, Goossens A: Results of patch testing with fragrance mix 1, fragrance mix 2, and their ingredients, and Myroxylon pereirae and colophonium, over a 21-year period. Contact Dermatitis, 68 (2013), 307–313.

Detailed study summary and results:

Test type

The Department of Dermatology at University Hospital St Rafael, Belgium, has performed a retrospective study of patch test data for 13332 patients who had been patch tested in the period from 1990-2011. A total of 3416 patients were tested with FMII (starting from 2005). The number of patients reacting to FMII (which includes 1% citral) was 205. Subsequent patch testing was in done with the individual ingredients of the fragrance mixture.

Description of test method as cited from Nardelli et al.: *All subjects had been tested with the European baseline series (Trolab, Hermal, Reinbeck, Germany) containing FM 1, M. pereirae (balsam of Peru), and colophonium. Since 2002, 3927 have been tested with HICC 5% pet., and from 2005, 3416 have been tested with FM 2. The patients reacting to FM 1 and FM 2 were, in most cases, tested with the individual ingredients, and some of the subjects were occasionally also tested with other fragrance components. The patch tests were administered with Van der Bend patch test chambers (Van der Bend, Brielle, The Netherlands) applied on the back with Micropore™ (3M Health Care, Borken, Germany), and fixed with Fixomull (Beiersdorf, Hamburg, Germany), and later with Mefix (Mölnlycke Health Care, Göteborg, Sweden). The patch test readings were performed according to the international guidelines of the International Contact Dermatitis Research Group (12) after 2 days, 3 days (exceptionally), and 4 days, and sometimes later.*

Statistical analysis of the patch data were performed with SAS™ version 9.2 (SAS Institute, Cary, NC, USA).

The results showed that 11.2% of the selected patients (23/ 205) had positive reactions for citral when tested at 2% in petrolatum.

3.1.2.4 STUDY 4 (Patch test, selected)

Study reference:

Bakker CV, Blömeke B, Coenraads P-J, Schuttelaar M-L: Ascaridole, a sensitizing component of tea tree oil, patch tested at 1% and 5% in two series of patients. *Contact Dermatitis*, 65 (2011), 239–248

Detailed study summary and results:

Test type

The Department of Dermatology, University Medical Centre Groningen, The Netherlands has performed a study investigating the sensitising properties of ascaridole, a component of tea tree oil. In this study patch tests were performed with the European baseline series, a cosmetic and/or perfume series and ascaridole (1% or 5%) in two series of consecutive patients (602 and 144 patients, respectively). In the patients with positive reactions to ascaridole concomitant positive patch reactions were registered.

Description of test method as cited from Bakker et al., 2011: *“From March 2008 until August 2010, 602 consecutive patients who were suspected of having allergy related to cosmetic or perfume use underwent patch tests with the European baseline series, a cosmetic and/or perfume series, and ascaridole 1% pet. From August 2010 to February 2011, we consecutively tested a similar series of 144 patients with ascaridole 5% pet. instead of 1% pet. Patch tests were applied and read according to the International Contact Dermatitis Research Group guidelines. Ascaridole was provided by the Institute of Pharmacology, University of Bonn, Germany.”*

The results showed that among the 30 selected patients with positive reactions to ascaridole (1 or 5%), 7% (2 of 30 patients) had concomitant positive reactions to citral.

3.1.2.5 STUDY 5 (Patch test, selected)

Study reference:

Cuesta L, Silvestre JF, Toledo F, Lucas A, Pérez-Crespo M, Ballester I: Fragrance contact allergy: a 4-year retrospective study. *Contact Dermatitis* 63 (2010): 77–84.

Detailed study summary and results:

Test type

The Department of Dermatology, Hospital General Universitario in Alicante, Spain performed a 4-year retrospective study of patients tested with the Spanish baseline series and/or fragrance series. A total of 1253 patients were patch tested with the baseline Spanish Group series. Positive reactions to a fragrance marker in were observed in 9.3% (n = 117) of these patients. A total of 86 patients were further tested with the Chemotechnique® fragrance series either because they were positive to the baseline series or because of a clinical suspicion. The objective of the study was to define the characteristics of the population allergic to

perfumes, to determine the usefulness of markers of fragrance allergy in the baseline GEIDAC series, and to describe the contribution made by the fragrance series to the data obtained with the baseline series.

Description of test method as cited from Cuesta et al., 2010:

“The allergens used both in the standard series and in the fragrance series were supplied by Chemotechnique Diagnostics®. The markers of the baseline Spanish Group series used in our study to detect fragrance allergic contact dermatitis were: the ‘traditional’ markers (M. pereirae and FM I), hydroxyisohexyl 3-cyclohexene carboxaldehyde (included as of October 2005), and FM II (included as of January 2007).”

“The patches were prepared using Finn Chambers® fixed with Scanpor® adhesive and removed after 2D in contact with the skin. Readings were taken at D2 and D4, with the evaluation criteria (+, ++, and +++) recommended by the ICDRG. If the result was doubtful, a late reading was taken at D7. The relevance was considered current if the clinical picture could be attributed totally or partially to the fragrance obtained, past if this positivity explained only previous dermatitis, and unknown if the clinical picture could not be attributed to the use of these fragrances. Patients who were positive to any fragrance marker in the GEIDAC baseline series (M. pereirae, FM I, hydroxyisohexyl 3-cyclohexene carboxaldehyde, or FM II) were identified, and the percentage of patients positive to each of the markers was determined.”

The results showed that among the patients tested with the Chemotechnique® fragrance series 2.3% of the selected patients (2/86) had positive reactions to citral when tested at 2% in petrolatum. It was concluded that the fragrance markers detect the majority of cases of fragrance contact allergy. Furthermore it was recommended to include FM II in the Spanish baseline series, as in the European baseline series, and to use a specific fragrance series to study patients allergic to a fragrance marker.

3.1.2.6 STUDY 6 (Patch test, selected)

Study reference:

Heydorn S, Johansen JD, Andersen KE, Bruze M, Svedman C, White IR, Basketter DA, Menné T: Fragrance allergy in patients with hand eczema – a clinical study. *Contact Dermatitis* 2003; 48: 317–323.

Detailed study summary and results:

Test type

A study of fragrance allergy in hand eczema patients from three dermatological departments in Denmark and Sweden (Gentofte, Odense, Malmö) was done in 2001-2002. 658 consecutive patients presenting with hand eczema were patch tested with the European standard series and the developed selection of fragrances. The aim of the study was to investigate patients referred with hand eczema concerning their frequency of positive patch tests to allergens in a selection of fragrances and to the European standard series. Citral (95%) was obtained from Dr. D. Basketter, Unilever Research (Sharnbrook, UK).

Description of patch test as cited from Heydorn et al., 2003: “*The patch tests were applied to the skin of the upper back for 2 D, using Finn Chambers I (Epitest, Helsinki, Finland) on Scanpor I tape (Norgesplaster A/S, Vennesla, Norway). Readings were taken on D2 and/or D3–4 and on D7. ICDRG recommendations were followed (10). A patch test was considered positive when the reading was +, ++ or +++. A + patch-test reaction was defined as homogeneous erythema and infiltration, whereas only erythema was not. The standard series used in Gentofte was from Hermall (Reinbek, Germany) apart from sesquiterpene lactone mix, which became unavailable from Hermall and was therefore obtained from Chemotechnique I (Malmö, Sweden). In Odense, the standard series was TRUE Test™ (Chemotechnique I), supplemented by test substances from Hermall. In Malmö, the standard series was from Chemotechnique I. In Odense, they tested 229, in Gentofte 220 and in Malmö 209 patients with hand eczema. As seen in tables 2 and 3, patch-test results from Hermall, Chemotechnique I and TRUE Test™ were combined for each allergen in the standard series.*

Statistical analysis of the data was performed using the SAS® system for Windows® release 8.02 TS level 02MO© 1999–2001 by SAS Institute Inc. (Cary, NC, USA)

The results showed that 4.3% (28/658) of the patients were tested positive for citral at 2% (in petrolatum). Although the patients are described as consecutive patients in the publication they are considered to represent selected patients in this context (selected based on hand eczema).

3.1.2.7 STUDY 7 (Patch test, selected)

Study reference:

Wilkinson JD, Andersen KE, Camarasa JG, Ducombs G, Frosch P, Lahti A, Menné T, Rycroft RJG and White I: Preliminary results of the effectiveness of two forms of fragrance mix as screening agents for fragrance sensitivity. In Frosch PJ et al. (eds): Current Topics in contact dermatitis. Heidelberg: Springer-Verlag, 1989:127-131.

Detailed study summary and results:

Test type

A total of 2455 consecutive patients attending dermatological clinics in England, Denmark, Spain, France, Germany and Finland were tested with two fragrance mixes: the Hermal (“Larsen”) standard fragrance mix and a new experimental fragrance mix (“Hausen mix”) containing citral. 78 selected patients positive to either of the mixes were patch tested with the individual ingredients.

Description of patch test as cited from Frosch et al., 1989: “*The two fragrance mixes studied were (a) the Hermal (Larsen) 8% fragrance mix and (b) a 9.5% (Hausen) fragrance mix. The Hermal 8% Mix consists of cinnamyl alcohol 1%, cinnamaldehyde 1%, eugenol 1%, amyl cinnamaldehyde 1%, hydroxycitronellal 1%,*

geraniol 1%, isoeugenol 1% and oak moss absolute 1% with sorbitan sesquileate as emulsifier. The Hausen 9.5% Mix contained cinnamyl alcohol 0.5%, cinnamaldehyde 0.5%, isoeugenol 1%, eugenol 1%, dihydrocoumarin 2.5%, hydroxycitronellal 2.5%, geraniol 0.5% and citral 1%. 2455 consecutive patients attending patch test clinics in England, Denmark, Spain, France, Federal Republic of Germany and Finland were, wherever possible, tested to both fragrance mixes and, when one or other of these was positive, to all the individual fragrance compounds contained in both mixes. Patch test technique and readings were as recommended by the International Contact Dermatitis Research group and, for positive results, an assessment of clinical relevance was also made.

The results of the study showed that 16.7% of the selected patients (13/78) were tested positive for citral at 2% in petrolatum.

3.1.2.8 STUDY 8-10 (Patch test, selected, 3 studies)

Study reference:

Ishihara et al. (1981), Itoh et al. (1986, 1988), Nishimura et al. (1984) cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology* 52 (2008) 62–73.

Detailed study summary and results:

A detailed summary of the study and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Test type

Diagnostic patch tests with citral

Description of studies as cited from Lalko and Api 2008: “*Ishihara et al. (1981) reported on the results of patch tests with 5% citral. Reactions were observed in cosmetic dermatitis patients (4/155) and eczema/dermatitis patients (5/159). No reactions were observed in control subjects (0/48). Patch tests were conducted between the years 1978–1986 in dermatologic patients (Itoh et al., 1986, 1988; Nishimura et al., 1984). When citral was tested at 5% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (8/310), non-cosmetic dermatitis patients (9/408) and in one control subject (1/122). When citral was tested at 2% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (1/240) and non-cosmetic dermatitis patients (2/584). No reactions were observed in control subjects (0/105).*”

The results of the studies showed that

- when patch tested with 5% citral: 2.6% of cosmetic dermatitis patients (8/310), 2.2% non-cosmetic dermatitis patients and 0.8% control subjects (1/122) were tested positive for citral
- when patch tested with 2% citral: 0.4% of cosmetic dermatitis patients (1/240), 0.3% non-cosmetic dermatitis patients (2/584) and 0 control subjects (0/105) were tested positive for citral
- when patch tested with 5% citral: 2.6% cosmetic dermatitis patients (4/155) and 3.1% eczema/dermatitis patients (5/159) were tested positive for citral.

Higher frequencies of positive reactions were thus observed with increasing dose of citral.

3.1.2.9 STUDY 11 (Patch test, selected)

Study reference:

Malten KE, van Ketel WG, Nater JP, Liem DH: Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 1984;11:1-10.

Detailed study summary and results:

Test type

The working group on Occupational Dermatoses of the Dutch Society for Dermatology and Venereology and the (Dutch) Governmental Food Inspection Service carried out an investigation of allergic reactions to fragrance raw materials with the aim of composing a fragrance patch test screening series for patients with suspected cosmetic dermatitis. 182 patients suspected of suffering from contact sensitization to cosmetics were patch tested with a series of 22 fragrance and flavour raw materials including citral. The patients were suspected of suffering from cosmetic dermatitis based on either 1) history between severity of reaction and contact with cosmetics, 2) dermatitis localised on body regions where cosmetics are commonly applied, 3) generalised pruritus, redness and slight scaling without any other apparent causes, 4) patients with frequent occupational contact with cosmetics and related materials, 5) positive patch test reactions to specific indicator substances such as woodtars, colophony, oil of turpentine and/or balsam of Peru and 6) positive reactions after tests with dilutions of patients own cosmetics.

Description of the patch test as cited from Malten et al., 1984: *“The following investigations were performed. (i) The history and clinical patterns were recorded, guided by a questionnaire which also contained data about atopy. The patients were asked whether they had suffered from asthma (chronic bronchitis), hay fever, allergic rhinitis, or atopic dermatitis, and whether such atopic conditions were or had been present in members of their family: parents, brothers, sisters or children. (ii) Primary site and distribution of the eruption. (iii) Patch tests with: (a) 20 standard ICDRG allergens (Table 1); (b) 22 fragrance raw materials selected as possible contact allergens (Tables 2 and 3). The patch test reactions were read at 48 and 72 h; the last reading was recorded as definitive. (iv) Analysis was performed on the presence of the 22 substances in 79 cosmetics which were sent in by the patients or their physicians because they were suspected of*

causing actual complaints (Table 3). 1 year after their manufacture, the stability of the 22 room-stored solutions was controlled (Table 3)''.

Citral was tested in a concentration of 2.0% in pet. The results showed that 2.6% of the selected patients (n = 182) were tested positive for citral. Citral was found to be present in 4 out of 79 cosmetics used (i.e. citral appeared in approx. 5% of the products analysed).

3.1.2.10 STUDY 12 (Patch test, consecutive)

Study reference:

Mann J, McFadden JP, White JML, White IR, Banerjee P: Baseline series fragrance markers fail to predict contact allergy. *Contact Dermatitis*, 70 (2014), 276–281.

Detailed study summary and results:

Test type

The St Johns' Institute of Dermatology at St Thomas' Hospital, UK has performed a retrospective study of patch test data by reviewing the records of 1951 eczema patients, routinely tested with the 26 fragrance substances requiring labelling and with an extended European baseline series (FMI and FMII) in 2011 and 2012. The objective was to determine the frequencies of positive test reactions to the 26 fragrance substances for which labelling is mandatory in the EU, and how effectively reactions to fragrance markers in the baseline series (FMI and FMII) predict positive reactions to the fragrance substances that are labelled. The study thus explored whether routine patch testing with all individual fragrance substances that are labelled above a threshold identified cases of fragrance contact allergy that would have remained undetected when using the baseline series.

Description of test method as cited from Mann et al.: *The patch test records of all eczema patients who underwent routine testing with the fragrance series and the European baseline series during 2011 and 2012 were retrieved from the database at St John's Institute of Dermatology at St Thomas' Hospital, London. The data recorded at the time of consultation included the age, sex and occupation of patients, the primary site affected by eczema, and the duration of eczema. Positive reactions, on or after day 4 of testing, to fragrance markers in the European baseline series (FM I, FMII, Myroxylon pereirae, and HICC) or allergens from the fragrance series (the 26 labelled fragrances and trimethylbenzenepropanol, but excluding HICC) were tabulated with spss™ version 12. Data were also collected for patients who reacted to colophonium and epoxyresin. The concentrations and constituents of the fragrance markers are shown in Table 1, and those of the allergens used in the fragrance series are shown in Table 2. Limonene and linalool were used in their unoxidized forms throughout the study. Patch testing was performed with aluminium Finn Chambers® provided by Bio-Diagnostics® (Upton-Upon-Severn, United Kingdom) and allergens provided by Bio-Diagnostics®, Trolab® (Hermal Almirall, Reinbeck, Germany) and Chemotechnique®*

(Vellinge, Sweden). Allergens were in petrolatum. Reactions were read on days 2 and 4, according to the recommendations of the International Contact Dermatitis Research Group. Reactions documented as questionable or irritant were considered to be negative.

The results showed that 1.03% (20/ 1951) (95% CI: 0.6-1.4%) of the consecutive eczema patients had positive reactions for citral when tested at 2% in petrolatum.

Overall the study showed that >40% of those patients reacting to a substance in the fragrance series would have been missed if evidence of fragrance allergy had been investigated exclusively with the European baseline series, and that a similar proportion of those reacting to FM I or FM II constituents did not react to the mixes themselves. In general the study indicates a very high rate of fragrance allergy as >14% of the patients reacted to either a fragrance marker or a substance in the fragrance series.

3.1.2.11 STUDY 13 (Patch test, consecutive)

Study reference:

Hagvall L, Christensson LB: Cross-reactivity between citral and geraniol – can it be attributed to oxidized geraniol? *Contact Dermatitis* 71 (2014), 280–288.

Detailed study summary and results:

Test type

The Department of Dermatology at Sahlgrenska University Hospital, Gothenburg, Sweden has performed a prospective study of patch test data for 655 patients who were patch tested with citral and its constituents neral and geranial as well as pure and oxidised geraniol. Data were obtained in the period from 2010-2011. Citral (66% geranial, purity: 98% and 34% neral, purity >99%) was obtained from Bedoukian Research Inc. (Danbury, CT, USA) and was distilled prior to use.

Prior to the patch test study an irritancy study was conducted. 22 patients were thus treated with 2.5 and 5% citral in petrolatum. The test substances were applied together with the ordinary patch test, and irritant reactions were evaluated on D3–D4. The reactions were evaluated visually (with a scale from 0-9 described by Basketter et al.). For citral the irritancy was low at 2.5% (mean score: 0.09) and increased at 5.0% (mean score: 0.91). A concentration of 3.5% pet. was chosen for further separate testing (of citral) on the basis of the results from the irritancy study.

Description of patch test as cited from Hagvall and Christensson 2014: “*Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period September 2010 up to and including December 2011, were included in the study. Six hundred and fifty-five patients participated in the study (200 men, 455 women, mean age*

45.2 years, SD ±17.8). Patch test preparations of ~20mg (30) were applied in small Finn Chambers® (diameter 8 mm, inner area of 0.5 cm²; Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway) to the back of the patient, left under occlusion for 2 days, and then removed by the patient. Readings were performed according to the ICDRG recommendations (31) on D3–D4 and D7.”

Statistical analysis was carried out with R version 3.0.3: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). Wilcoxon's signed rank test ($p < 0.05$) was used to compare the visual readings. McNemar's test was used to evaluate differences in frequencies of positive patch test reactions to the study materials.

The results showed that 0.92% of the consecutive patients ($n = 655$) were tested positive for citral.

The results further suggest that geraniol is the main sensitizer in the mixture citral, and that there is little cross-reactivity between pure geraniol and citral (results regarding cross-reactivity not described in detail in this annex).

3.1.2.12 STUDY 14 (Patch test, consecutive)

Study reference:

Hagvall L, Karlberg A-T, Christensson JB: Contact allergy to air-exposed geraniol: clinical observations and report of 14 cases. *Contact Dermatitis*, 67 (2012), 20–27.

Detailed study summary and results:

Test type

The Department of Dermatology at Sahlgrenska University Hospital, Gothenburg, Sweden has performed a prospective study of patch test data for 1055 patients who were patch tested with citral and its constituents neral and geraniol as well as pure and oxidised geraniol. Data for citral were obtained in the period from 2006-2008. Citral (66% geraniol, purity: 98% and 34% neral, purity >99%) was obtained from Bedoukian Research Inc. (Danbury, CT, USA) and was distilled prior to use.

Description of patch test as cited from Hagvall et al., 2012: *“Consecutive patients patch tested with the Swedish baseline series at the Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, during the period January 2006 to August 2010, were included in the study. Patch test preparations of approximately 20 mg were applied in small Finn Chambers® (diameter 8 mm, inner area of 0.5 cm²; Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway) to the back of the patient, left under occlusion for 2 days, and then removed by the patient. Readings were performed according to the International Contact Dermatitis Research Group recommendations (28) on D3–4 and D7.”*

The results showed that 0.66% of the consecutive patients (n = 1055) were tested positive for citral. (In addition, further 0.28% of the patients showed doubtful reactions to citral).

3.1.2.13 STUDY 15 (Patch test, consecutive)

Study reference:

Heisterberg MV, Menné T, Johansen JD: Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis*, 65 (2011), 266–275 and corrigendum in: *Contact Dermatitis*, 67 (2012), 58.

Detailed study summary and results:

Test type

The Department of Dermato-Allergology, Copenhagen University Hospital, Gentofte has performed a retrospective study on consecutive eczema patients patch tested with citral. The objective of the study was to investigate frequencies of sensitization to the 26 individual fragrances and evaluate the sensitivity of the standard fragrance screening markers (FMI and FMII), i.e. would testing with the individual substances reveal fragrance allergy that is not detected when using the standard fragrance markers. Patients (n = 1508) were patch tested with at least one of the 26 fragrance ingredients in the period from January 2008 to July 2010 were included in the study. 1502 patients were patch tested with citral.

Description of patch test as cited from Heisterberg et al., 2011: *“The patch tests were performed according to international guidelines (9), with Finn Chambers applied on the back with Scanpor tape (Vitalfo Scandinavia, AB, Allerød, Denmark) for a period of 2 days. Readings were performed on days 2, 3 or 4, and 7, according to the recommendations of the International Contact Dermatitis Research Group (10). Not all subjects were patch tested with limonene and linalool, as the patch test material during the study period changed from being the pure compounds (Hermal) to oxidized materials (Göteborg), because several studies have shown that it is the oxidized products that cause allergy (11–17). In this study, we report the results of patch testing with the pure compounds. Methyl 2-octyonate 1% was not patch tested in all of the subjects routinely patch tested, because active sensitization was observed in two patients, and we then stopped patch testing with it; thus only 211 patients were tested (18). Data management and statistical analysis were performed using SPSS™ version 15. Percentages of positive patch test reactions and confidence intervals were calculated with www.openepi.com. Chi-square tests and Fisher’s exact tests for characteristic differences were performed, and $p < 0.05$ was considered to be significant.”*

The results showed that 0.3% of the consecutive patients (5/ 1502) were tested positive for citral. It was furthermore concluded that 11.7% of fragrance allergy subjects would be undetected with a fragrance allergy if they had not been patch tested with the fragrance series, which underlines the value of patch testing all subjects with a fragrance series.

3.1.2.14 STUDY 16 (Patch test, consecutive)

Study reference:

Van Oosten EJ, Schuttelaar M-L A, Coenraads PJ: Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis* 2009; 61: 217–223.

Detailed study summary and results:

Test type

The Department of Dermatology, University of Groningen, the Netherlands performed a retrospective study of patients with eczema of which a minor part of the patient group were suspected of being contact allergy to fragrances or cosmetics. In the study 320 patients were patch tested with the 26 EU-declared fragrance chemicals, FM I and FM II. The objective of the study was to describe frequencies of contact allergy to these 26 fragrance substances, and to evaluate clinical relevance of these positive reactions.

Description of test method as cited from Van Oosten et al., 2009: “*All 320 patients were tested with the series of 26 EU fragrance ingredients that are labelled. Additionally, the European baseline series (TRUE® test, Mekos laboratories, Denmark), which includes FM I, was tested in 295 patients, and the FM II (Hermal/Trolab, Reinbek, Germany) was tested in 227 patients. The fragrance compounds were obtained from Hermal/Trolab and from other international suppliers (International Flavors & Fragrances, USA; Robertet, France; Givaudan, Switzerland, Millennium Speciality Chemicals Inc., USA; Bedoukian Research Inc., USA; Rhodia, France; Symrise, Germany and Firmenich, Switzerland). All fragrances were dissolved in petrolatum, except for Evernia furfuracea which was dissolved in di-ethyl phthalate (Table 1). Patch tests were performed and read according to the guidelines of the International Contact Dermatitis Research Group (ICDRG) (12). The patches were applied for 2D. Final reading was done on D3. (7, 13). Reading of doubtful reactions was done up to D7 after the application of the patch test material. The relevance of the positive reactions (1+ through 3+) was determined and categorized as certain, probable, possible or not relevant. Contact allergy was defined as clinically relevant according to the following criteria: (i) certain exposure to the sensitizer and (ii) the patients dermatitis can be explained by the exposure (8, 11, 14, 15)*”.

The results of the study showed that 0.6% of the consecutive eczema patients (2/320) had positive reactions to citral when tested at 2% in petrolatum.

3.1.2.15 STUDY 17 (Patch test, consecutive) (also cited in REACH registration dossier)

Study reference:

Schnuch A, Uter W, Geier J, Lessmann H, Frosch, PJ: Sensitization to 26 fragrances to be labelled according to current European regulation. *Contact Dermatitis* 2007; 57: 1–10.

Detailed study summary and results:**Test type**

The IVDK (a network of departments of Dermatology in Germany, Austria and Switzerland) has performed a retrospective study of patch test data from a multicentre project. During 2003-2004, 26 fragrances were patch tested additionally to the standard series in a total of 21325 patients; the number of (consecutive, unselected) patients tested with each of the fragrances ranged from 1658 to 4238.

Description of patch test as cited from Schuch et al., 2007: *“Patch tests are performed in accordance with the recommendations of the International Contact Dermatitis Research Group (12) and the German Contact Dermatitis Research Group (DKG) (13). Patch test material is obtained from Hermal/Trolab, Reinbek, Germany. Patch test preparations are applied for 24 or 48 hr. Readings are done until at least 72 hr using the following grading based on international standards (14), further refined by the German Contact Dermatitis Group (13): neg, ?, +, ++, +++, irritant, follicular. The patch test results of every reading, a standardized history (including age, sex, atopic diseases, current and former occupation(s), presumptive causal exposures), along with final diagnoses and site(s) of dermatitis are assessed and documented. All data are transferred to the data centre in Göttingen in an anonymized format every 6 months. During 4 periods of 6 months each, from 1 January 2003 to 31 December 2004, 25 fragrances (Table 1) were successively patch tested additionally to the standard series, i.e. in unselected patients, by departments of the IVDK. In the first period 8, in the second 6, in the third 3, and in the last period 8 compounds were added to the standard series, the number of patients tested with each preparation ranging from 1658 (tree moss) to 4238 (farnesol; tested during 2 periods).”*

Statistical analysis of the data was performed using the statistical software package SAS (version 9.1, SAS Institute, Cary, NC, USA).

The results showed that 0.6% (95% CI: 0.3-1.0%) of the consecutive patients (13/2021) were tested positive for citral.

3.1.2.16 STUDY 18 (Patch test, consecutive) (also cited in REACH registration dossier)**Study reference:**

An S, Lee A-Y, Lee CH, Kim D-W, Hahm JH, Kim K-J, Moon K-C, Won YH, Ro Y-S, Eun HC: Fragrance contact dermatitis in Korea: a joint study. *Contact Dermatitis* 2005; 53: 320–323.

Detailed study summary and results:**Test type**

A multicentre study was performed by the Korean Society for Contact Dermatitis and Skin Allergy. Nine dermatology departments at university hospitals in Korea took part in this prospective analysis of allergic

responses to fragrances where 422 patients (some of which with suspected contact allergy) were patch tested. In addition to the Korean (fragrance) standard and a commercial fragrance series, 18 additional fragrances were patch tested.

Description of patch test as cited from An et al., 2005: *“Test substances: The Korean standard series, which is a variant of the European standard series, and a fragrance series (Table 1) were purchased from Chemotechnique Diagnostics, Malmö, Sweden. We selected additional allergens based on past relevant references and information as to usage frequency. Chemical names, suppliers and test concentrations are summarized in Table 2. The additionally selected 18 fragrances were prepared in batches by the Korean cosmetic company and distributed to researchers at the different hospitals. Patch test method: Finn Chambers on Scanpor tape (Epitest, Tuusula, Finland) tape was used for patch testing, and the results were evaluated according to the recommendation of the International Contact Dermatitis Research Group (15).”*

The results of the study showed that 1.2% of the consecutive patients (5/422) were tested positive for citral at 2% in petrolatum.

3.1.2.17 STUDY 19-20 (Patch test, consecutive)

Study reference:

Frosch PJ, Pirker C, Rastogi SC, Andersen KE, Bruze M, Svedman C, Goossens A, White IR, Uter W, Arnau EG, Lepoittevin J-P, Menné T, Johansen JD: Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005; 52: 207–215. (Frosch et al., 2005a)

Frosch PJ, Rastogi SC, Pirker C, Brinkmeier T, Andersen KE, Bruze M, Svedman C, Goossens A, White IR, Uter W, Arnau EG, Lepoittevin J-P, Johansen JD, Menne T: Patch testing with a new fragrance mix – reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005; 52: 2016-225 (Frosch et al., 2005b)

Detailed study summary and results:

Test type

Six dermatological departments from Dortmund, Copenhagen, Malmö, Odense, London and Leuven have performed a prospective study of 1701 consecutive patients patch tested with FMI and FMII and their single constituents (SC), including citral, during 2002-2003. The aim of the study was to evaluate the new fragrance mix (FMII) and assess whether FMII can identify additional patients with a positive fragrance history that are not identified with FMI and to evaluate whether FMII should be added to the European standard series.

Citral was obtained from Dragoco/Symrise (Holzminden, Germany). FMII was prepared in 3 test concentrations: 28%, 14% and 2.8% (containing 2.0%, 1.0% and 0.2% citral, respectively).

Description of patch test as cited from Frosch et al., 2005a: *“Consecutive patients attending contact dermatitis clinics at 6 dermatology departments were tested between October 2002 and June 2003 (Dortmund, Copenhagen, Malmö, Odense, London and Leuven). In addition to the standard series, all 3-concentrations of FM II and the SC of 28%FM II and 14% FM II were applied to the skin of the back for 2 days. In all centres, Finn Chambers™ on Scanpor1 tape (Epitest, Tuusula, Finland) were used. Readings were taken at most centres on day 2 and 4. The second reading, usually at day 3 or 4, was used for the overall evaluation of positive test results. The reactions were categorized according to published guidelines (7).”*. Citral was thus tested in individual concentrations of 2.0% and 1.0%.

Further description of patch test of the single constituents as cited from Frosch et al., 2005b: *“The individual constituents of 14% FMII and of 28% FMII were applied simultaneously with the mix. The single constituents of 2.8% FMII were tested only if there was a positive or doubtful (+ or ?) reaction to this concentration of the new mix.*

Statistical analysis of the data was performed using the SAS™ software package (version 8.2, SAS Institute, Cary, NC, USA).

The results showed that 0.35% (6/1701) and 0.7% (12/1701) of the consecutive patients were tested positive for citral at concentrations of 1% and 2% (in petrolatum), respectively.

Higher frequencies of positive reactions were thus observed with increasing dose of citral.

3.1.2.18 STUDY 21 (Patch test, consecutive)

Study reference:

Frosch PJ, Johansen JD, Menné T, Pirker C, Rastogi SC, Andersen KE, Bruze M, Goossens A, Lepoittevin J-P, White IR: Further important sensitizers in patients sensitive to fragrances*. I. Reactivity to 14 frequently used chemicals. *Contact Dermatitis* 2002, 47, 78–85.

Detailed study summary and results:

Test type

Six dermatological departments from Dortmund, Copenhagen, Malmö, Odense, London and Leuven have performed a prospective study of 1855 consecutive patients patch tested with FMI and FMII and their single constituents (SC), including citral, during October 1997- October 1998. The aim of the study was to

determine the frequency of responses to selected fragrance materials in consecutive patients patch tested in 6 dermatological centres in Europe.

Description of patch test as cited from Frosch et al., 2002: “*Consecutive patients of contact dermatitis clinics at 6 dermatology departments were tested (Dortmund, Copenhagen, Malmo, Odense, London and Leuven) in the time period between October 1997 and October 1998. In addition to the standard series, the 8% FM from the same source and batch (Hermal, Reinbek, Germany) was applied to the back skin for 2 days. Finn Chambers on Scanpor tape were used in all centres except Leuven (van der Bend chambers). Readings were taken at most centres on days (D) 2 and 4. The reading at D3 or D4 was used for overall evaluation of positive test results. Test reactions were categorized according to published guidelines (6)*”.

The results showed that 1.1% (21/1855) of the consecutive patients were tested positive for citral at 2% (in petrolatum).

3.1.2.19 STUDY 22 (Patch test, consecutive)

Study reference:

De Groot AC, Coenraads JP, Bruynzeel DP, Jagtman BA, van Ginkel CJW, Noz K, van der Valk PGM, Pavel S, Vink J, Weyland JW: Routine patch testing with fragrance chemicals in The Netherlands. *Contact Dermatitis* 2000; 42: 162-185

Detailed study summary and results:

Test type

A prospective study of 1825 consecutive patients from different dermatological departments in the Netherlands has been performed, data were obtained in the period from September 1998 to April 1999. In this multicentre study 9 fragrance allergens including citral (2%, in petrolatum) were tested in all patients routinely patch tested. The 9 fragrances were selected either because of their widespread use in cosmetics or because they had been identified as relatively frequent allergens.

Description of patch test as cited from de Groot et al., 2000: “*Test procedures were carried out according to internationally accepted criteria. Hydroabietyl alcohol was purchased from Chemotechnique, the other fragrances from the Regional Health Inspectorate, Enschede. Test concentrations were chosen on the basis of published data (1) and potential irritancy was excluded in a pilot study involving 200 patients.*

The results showed that 1.0% (19/1825) of the consecutive patients were tested positive for citral at 2% (in petrolatum).

3.1.2.20 STUDY 23-24 (Patch test, consecutive)

Study reference:

Frosch PJ, Pilz B, Andersen KE, Burrows D, Camarasa JG, Dooms-Goossens A, Ducombs G, Fuchs T, Hannuksela M, Lachapelle JM, Lahti A, Maibach HI, Menné T, Rycroft RJG, Shaw S, Wahlberg JE, White IR, Wilkinson JD: Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Con/ac/ Dermalilis*, 1995, 33, 333-342.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 1323 patients tested in 11 centres was performed. The study involved testing of 48 frequently used constituents of perfumes, including citral, as well as patch testing with a standard series fragrance mix (FM) (not containing citral). 192 patients were patch tested with citral in the Copenhagen center.

Description of patch test as cited from de Frosch et al., 1995: *“In each centre, a minimum of 100 consecutive patients were tested with the allocated FF (Fenn fragrance) materials and the 8% FM with its constituents. For each patients positive to any 1 of the FF materials, a questionnaire was filled out regarding clinical relevance and other sensitizations. Patch testing was performed with Finn Chambers on Scanpor tape applied for 2 days to the back. Readings were made following the guidelines of the ICDRG (16) on days 2 and 3, or in some centres on days 2 and 4”.*

The results showed that 0% (0/192) of the consecutive patients were tested positive for citral at 0.1% or 1% citral (in petrolatum).

3.1.2.21 STUDY 25 (Patch test, consecutive)

Study reference:

Michell JC, Adams RM, Glendenning WE et al.: Results of standard patch tests with substances abandoned. *Contact Dermatitis* 1982;8:336-337.

Detailed study summary and results:

Test type

The North American Contact Dermatitis Research Group have tested various fragrance substances on eczema patients as part of the routine testing series. Data obtained in the period 1973-1980 are presented in this publication. No information is provided on the patients nor the methods used and the publication solely gives an overview of the results obtained for the fragrances tested.

The results show that 1.7% of the patients (n = 228) were tested positive for citral tested at a concentration of 1% in pet. Data for citral were obtained in 1973/1974.

3.1.2.22 STUDY 26-30 (HRIPT, 5 studies) (also cited in REACH registration dossier)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology* 52 (2008) 62–73.

Detailed study summary and results:

Test type

A detailed summary of the study and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. Only the following data are presented:

Description of HRIPT tests and results, cited from Lalko and Api, 2008: “*The HRIPT is generally performed utilizing a total of nine 24-h occluded applications over 3-weeks with test material and appropriate controls followed by a 2-week rest period. A single 24-h challenge application is then made to a naïve site with the same materials. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Marzulli and Maibach, 1977; McNamee et al., 2008). Citral has been tested in the HRIPT over a range of concentrations. The following results were obtained. An HRIPT was conducted on 8 female volunteers with 5% citral in alcohol SDA39C. The patches consisted of a 1 in2 webril pad with 0.5 ml of test material; which resulted in a dose of 3876 µg/cm². Sensitization reactions were observed in 5/8 subjects. Four subjects, who reacted during the initial study, were rechallenged approximately 7 months later with both a patch and a single open application behind one ear. Two subjects (2/4) reacted to the patch at rechallenge, no reactions (0/4) were observed following open application (RIFM, 1964a). No reactions were observed to 1400 µg/cm² citral in an HRIPT conducted on 101 subjects (30 male and 71 female). The patches consisted of a 25 mm Hill Top Chamber , corresponding to a dosing area of 2.54 cm², with 0.3 ml of 1.2% citral in 3:1 DEP:EtOH (RIFM, 2004b). When 1240 µg/cm² citral was tested in an HRIPT, no reactions were observed in 50 subjects. The patches consisted of a 1 in2 webril pad with 0.2 ml of 4% citral in petrolatum (RIFM, 1971a). No reactions were observed to 755 µg/cm² citral in an HRIPT conducted on 40 subjects (11 males and 29 females). The patches consisted of a 1 in2 webril pad with 0.5 ml of 1% citral in alcohol SDA39C (RIFM, 1965). An HRIPT was conducted on 12 male and 29 female volunteers with 0.5% citral in alcohol SDA39C. The patches consisted of a 1 in2 webril pad with 0.5 ml of test material; which resulted in a dose of 388 µg/cm². No sensitization reactions (0/41) were observed (RIFM, 1964b).”*

3.1.2.23 STUDY 31 (HRIPT)

Study reference:

Opdyke DLJ. Citral. Fd. Cosmet Toxicol 1979;17:259-266 cited in: Opinion concerning fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. The Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers. SCCFNP 1999.

Detailed study summary and results:

Test type

A detailed summary of the study and results is not available in the SCCFNP opinion from 1999 which presents a review of the available data on sensitisation for citral. Only the following data are presented:

“Citral was also studied in the repeated insult patch procedure at 4-8% and sensitized 48% of a panel of 40 human volunteers (33)”.

3.1.2.24 STUDY 32-45 (HMT, 14 studies) (also cited in REACH registration dossier)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), cited in: Lalko J and Api AM: Citral: Identifying a threshold for induction of dermal sensitization. *Regulatory Toxicology and Pharmacology* 52 (2008) 62–73.

Detailed study summary and results:

Test type

A detailed summary of the study and results is not available in the article by Lalko and Api which presents a review of the available data on sensitisation for citral. The following data are presented:

Description of HMT tests and results, cited from Lalko and Api, 2008: “*The HMT is typically conducted on 25 human subjects by utilizing 5 alternate day 48-h occluded induction applications of test material and appropriate controls. Following a 10 to 14-day rest period 48-hour challenge applications are made to naïve sites. Patches may be made with and without pretreatment of sodium lauryl sulfate depending upon the inherent irritancy of the test material. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Kligman and Epstein, 1975). Citral has been tested in the HMT over a range of concentrations. The patches utilized for each of the reported studies consisted of a 14.5 cm² webril pad with 0.5 ml of test material.*”

The results of the HMT studies showed that positive reactions generally occurred at concentrations exceeding 500 µg/cm². A high percentage of sensitisation reactions were seen in most of the HMT studies except for the one study where no sensitisation reactions occurred (the one study using butylene glycol as a

vehicle). The doses tested were in the range 2-8%. Citral was stored and treated under differing conditions in the different studies. A dose-dependant trend can be seen from these studies with sensitisation frequencies generally increasing with the dose as seen from the table below:

Results of 14 HMT studies, unpublished reports from RIFM cited in Lalko and Api, 2008:

Citral, dose	Vehicle	Response in % (no of positive reactions)	RIFM reference in Lalko and Api, 2008
2% (1379 µg/cm ²)	Petrolatum	8.3% (2/24)	1972d
4% (2759 µg/cm ²)	Petrolatum	12% (3/25)	1972b
4% (2759 µg/cm ²)	Petrolatum	12% (3/25)	1972c
4% (2759 µg/cm ²)	Petrolatum	20% (5/25)	1972c
4% (2759 µg/cm ²)	Petrolatum	36%(9/25)	1971c
4% (2759 µg/cm ²)	Petrolatum	16% (4/25)	1971c
4% (2759 µg/cm ²)	Petrolatum	20% (5/25)	1971c
5% (3448 µg/cm ²)	Petrolatum	64% (16/25)	1974a
5% (3448 µg/cm ²)	Petrolatum	56% (14/25)	1974c
5% (3448 µg/cm ²)	Petrolatum	48% (12/25)	1974c
5% (3448 µg/cm ²)	Petrolatum	32% (8/25)	1974c
5% (3448 µg/cm ²)	Petrolatum	45.8% (11/24)	1974d
5% (3448 µg/cm ²)	Butylene glycol	0% (0/25)	1974e
8% (5517 µg/cm ²)	Petrolatum	33.3% (8/24)	1971b

3.1.2.25 STUDY 46 (Case study)

Study reference:

De Mozzi P, Johnston GA: An outbreak of allergic contact dermatitis caused by citral in beauticians working in a health spa. *Contact Dermatitis* 2014, 70, 376–388.

Detailed study summary and results:

Test type

Due to onset of bilateral hand dermatitis, 9 female beauticians working in the same high-end luxury health spa in the UK were separately and independently referred to the Dermatology Department, University Hospitals of Leicester by their general medical practitioners. The dermatitis was reported to improve with work avoidance. In their job all 9 patients were applying a wide variety of beauty treatment products, including essential oils.

Description of patch test as cited from De Mozzi and Johnston 2014: *“Patch testing was performed with the British baseline series in all patients, with an additional fragrance series being applied to 7 patients, and a cosmetic series to 4 patients. Allergen series were supplied by Chemotechnique Diagnostics (Vellinge, Sweden), and applied with Finn Chambers® at D0; readings were performed at D2 and D4, according to International Contact Dermatitis Research Group guidelines”.*

The results of the patch test showed that 5 of the 9 patients had positive reactions to both FMII as well as to citral 2.0% in pet. A site visit to the health spa revealed that the predominant brand of products used consisted of a large range of essential oils and spa products all of which contained citral.

3.1.2.26 STUDY 47 (Case study)

Study reference:

Hindle E, Ashworth J, Beck M H: Chelitis from contact allergy to citral in lip salve. Contact Dermatitis 2007; 57: 125-126.

Detailed study summary and results:

Test type

At the Contact Dermatitis Unit, Hope Hospital in Salford, UK, a 30-year-old female with a 5-year history of recurrent chelitis was patch tested with the standard series including FMII, as well as with a range of different other series and products. No further information of the patch test is provided in the reference.

The result of the patch test showed positive reactions to FMII as well as to citral, 2% (in pet.). Based on the testing and information on the products frequently used by this patient the chelitis was attributed to the use of a lip salve containing citral (a Vaseline lip balm with citral listed as an ingredient on the packaging). Changing to plain Vaseline for lip care and avoidance of perfume and nail varnish resulted in symptomatic improvement.

3.1.2.27 STUDY 48 (Case study)

Study reference:

Malten KE. Four Bakers showing positive patch-tests to a number of fragrance materials, which can also be used as flavors. Acta Dermato-venereologica 1979;suppl 85:117-121.

Detailed study summary and results:

Test type

The Department of Dermatology in Nijmegen, Holland have described four cases of bakers showing positive patch tests to a number of fragrance materials which can also be used as flavours. All four bakers had developed contact dermatitis on their fingers/hands and one of the also in the face. The development of the contact dermatitis seemed to have a clear time relation with their professional activities although one of the

bakers (case no. 4) also had a history of contact dermatitis that could possibly be attributed to non-occupational exposure. One of the four bakers (case no. 3) had a positive reaction to citral in 0.5% pet. He also showed clear positive reactions to certain flavours/spices used for different kinds of sweet biscuits. Following the patch test the person was not seen again at the clinic. In the SCCNFP opinion from 1999 where the study is also cited, the relevance of the study is described as “unknown”.

3.1.2.28 STUDY 49 (Patch test, experimental study)

Study reference:

Nagtegaal MJC, Pentinga SE, Kuik J, Kezic S, Rustemeyer T: The role of the skin irritation response in polysensitization to fragrances. *Contact Dermatitis*, 67 (2012), 28–35.

Detailed study summary and results:

Test type

The Department of Dermatology of the VU University Medical Centre, The Netherlands, has performed a prospective study of 100 selected patients with contact allergy who were patch tested with 25 individual fragrance chemicals and fragrance mixes I and II in the period from 2005-2010. The objective of the study was to investigate whether enhanced skin irritability is a risk factor for the development of polysensitization to fragrance chemicals.

Description of test method as cited from Nagtegaal et al., 2012:

Patch tests: *“Patch tests were performed in accordance with the recommendations of the ICDRG (12). Preparations of test materials in petrolatum were obtained from Trolab® (Almirall-Hermal, Reinbeck, Germany) or Chemotechnique Diagnostics® (Vellinge, Sweden). Van der Bend® patch test chambers (Van der Bend BV, Brielle, The Netherlands) on Fixomull® tape were used. Test chambers were manually filled by a specially trained investigator. The test substances consisted of 27 commercial patch test materials of fragrance chemicals, including FM I (8%) and FM II (14%), and were coded to ensure that the study could be performed in a double-blind fashion. The materials were supplied in polypropylene syringes, and stored in a refrigerator at 5°C. The patches were applied for 2 days on the upper back, and readings were performed on day 2 (48 hr), day 3 (72 hr), and day 7 (144 hr). Methodological and observer errors were minimized, as preparation and reading of the test were performed by only one specially trained person. Polysensitization was defined as three or more allergic reactions to non-cross-reacting fragrance allergens.”*

Skin irritation tests: *“This test consisted of the application of SLS at five sites in a row on the non-dominant upper arm for 1 day (24 hr). Van der Bend® patch test chambers on Fixomull® tape were filled with 20 µl of test solution. The SLS test concentrations were 0.0%, 0.45%, 0.67%, 1% and 1.5% in water. New test solutions were prepared every 3 weeks. The participants removed the patches themselves 24 hr after*

application, after which the test was assessed at day 2, day 3 and day 7 by bioengineering techniques. This included a non-invasive measurement of TEWL by means of a TEWAmeter® (TM300; Courage & Khazaka, Cologne, Germany) and of redness of the skin (erythema index) by means of a DermaSpectrometer® (Cortex Technology, Hadsund, Denmark). The increase in TEWL and erythema index reflects the sensitivity of the skin to SLS irritation. As baseline values of erythema index and TEWL are known to vary day to day, these values were measured every visit. The existing guidelines for assessment of these parameters were followed (13, 14), meaning that the volunteers rested for at least 15 min with uncovered arms before measurement, in a room with a temperature of 20–22°C, a relative humidity of 35–45%, and no direct incursion of sunlight.”

Statistical analysis: *“All data were analysed for significance by paired samples t-test or Mann–Whitney U-test with SPSS™ statistical software (version 17). The distribution of data was tested by the Shapiro–Wilk normality test. For non-normally distributed data, we applied the Mann–Whitney test. For testing the differences in TEWL between different SLS concentrations and the control site, we used a non-parametric Friedman test followed by Dunn’s multicomparison test ($p < 0.001$).”*

Although not a clinical diagnostic patch test study, patch tests were nevertheless performed according to the guidelines of the International Contact Dermatitis Research Group. The results showed that specifically for citral 9.0% (9/ 100) (95% CI: 4.2-16.4%) of the selected patients had positive reactions when tested at 2% in petrolatum.

Individuals with polysensitization (defined as multiple patch test reactions to > 3 non-related allergens) showed significantly higher irritation responses to SLS 1% and 1.5% (as assessed by transepidermal water loss). It was concluded that an enhanced skin irritation response is associated with polysensitization, and that it could be a phenotype for susceptibility to contact allergy.

4 ENVIRONMENTAL HAZARDS

Classification for environmental hazards is not a part of the CLH proposal for citral.