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:

Cinnamaldehyde; 3-phenylprop-2-enal; cinnamic aldehyde; cinnamal [1]

(2*E*)-3-phenylprop-2-enal [2]

EC Number:	**203-213-9 [1]**
	604-377-8 [2]
CAS Number:	**104-55-2 [1]**
	14371-10-9 [2]
Index Number:	**Not available**

Contact details for dossier submitter:

Danish Environmental Protection Agency

Tolderlundsvej 5, 5000 Odense, Denmark

e-mail: mst@mst.dk

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CONTENTS

1 PHYSICAL HAZARDS

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

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The information below on toxicokinetics has largely been copied from the public part of the registration dossier.

2.1.1 STUDY 1

Reference:

Adams T.B., Cohen S.M., Doull J., Feron V.J., Goodman J.I., Marnett L.J., Munro I.C., Portoghese P.S., Smith R.L., Waddell W.J., Wagner B.M.: The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. Food and Chem Toxicology 42: 157-185, 2004

Sapienza, P., Ikeda, G.J., Warr, P.I., Plummer, S.L., Dailey, R.E., Lin, C.S.: Tissue distribution and excretion of ^{14}C-labelled cinnamic aldehyde following single and multiple oral administration in male Fischer 344 rats. Food and Chemical Toxicology 31, 253– 261, 1993

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 excretion of [3-^{14}C]-labelled cinnamic aldehyde following single and multiple oral administration in male Fischer 344 rats.

Test substance:

Cinnamaldehyde, Aldrich Chemical Co. and [3-^{14}C]-cinnamaldehyde, Amersham Corporation

Purity of non-radiolabelled Cinnamaldehyde >95% and purity of [3-^{14}C]-cinnamaldehyde 97% (both measured with TLC)

No data available on impurities

Radiolabelling, specific activity: 10.5 mCi/mmol

Trioctanoin, National Centre for Toxicological Research, purity >95% was used as vehicle for oral dosing

Test animals:

Rat (Fischer 344), male (8/group)

- Source: Charles River Breeding Laboratories, Wilmington, MA, USA
- Age at study initiation: No data
- Weight at study initiation: 179±24 g
- Fasting period before study: For the acute study groups of rats were fasted overnight
- Individual metabolism cages: Yes, in both single an multiple dosing study
- Diet: Ad libitum (Rodent Chow Diet No. 5002, Ralston Purina Co., St. Louis, MO, USA)
- Water: Ad libitum
- Acclimation period: No data

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 23 ±3
- Humidity (%): No data
- Air changes (per hr): No data
- Photoperiod (hrs dark / hrs light): 12/12

Dosing:

Acute dosing study: Groups of male rats (8/group) were fasted overnight and given a single dose by gavage at levels of 5, 50, or 500 mg/kg bw of [3-^{14}C]-cinnamaldehyde. After administration of the radioactive dose, the animals were killed at the following time periods for each dose level: 5 mg/kg bw, 0.5, 2.5 or 24 hours; 50 mg/kg bw, 0.5, 3.5, 24 or 72 hours; 500 mg/kg bw 1, 6.5, 24 or 72 hours.

Multiple dosing study: Groups of male rats (8/group) were pre-treated with single daily oral dose levels of 5, 50, or 500 mg/kg bw of cinnamaldehyde by gavage for seven days at 24 hours interval. Twenty-four (24) hours later, animals in each group received a single oral dose of [3-^{14}C]-cinnamaldehyde equivalent to the pre-treatment level. The rats were killed 1, 2.5 or 24 hours after the radioactive dose for the 5- and 50 mg/kg bw dose levels, and at 1, 2.5, 24 or 72 hours after the 500 mg/kg bw dose.

After treatment with [3-^{14}C]-cinnamaldehyde the rats in both the acute and multiple dosing study were placed in individual stainless-steel metabolism cages fitted with a bottom pan which had a screen to separate faeces from urine.

Sampling:

Tissues and body fluids sampled: Urine, faeces, blood, liver, kidneys, spleen, brain, heart, lungs, muscle, gastrointestinal tract, subcutaneous fat and carcass

Time and frequency of sampling:

- Urine and faeces were collected at the end of each experimental period. If the experiment was longer than 24 hours, samples were collected at 24hour intervals

- Tissue samples were collected at the end of each experimental period.

Detailed study summary and results:

Radioactive cinnamaldehyde was distributed primarily to the gastrointestinal tract, kidneys, and liver, after single oral dose and multiple oral administrations.

After 24 hours, more than 80% of the radioactivity was recovered in the urine and less than 7% in the feces from all groups of rats, regardless of dose level. At all dose levels, a small amount of the dose was distributed to the fat. At 50 and 500 mg/kg bw, radioactivity could be measured in animals terminated 3 days after dosing. Except for the high dose pre-treatment group, the major urinary metabolite was hippuric acid, accompanied by small amounts of cinnamic and benzoic acid. In the high dose pre-treatment group, benzoic acid was the major 4 metabolite, suggesting that saturation of the glycine conjugation pathway occurs at repeated high dose levels of cinnamaldehyde.

2.1.2 STUDY 2

Reference:

Peters M.M., Caldwell J.: Studies on trans-cinnamaldehyde. 1. The influence of dose size and sex on its disposition in the rat and mouse. Food and Chemical Toxicology 32 (10): 869-76, 1994

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: Metabolism

Test guideline: non-guideline study.

Principles of method: To test the influence of dose size and sex on its disposition in the rat and mouse

Test substance:

trans-[3-^{14}C]Cinnamaldehyde (CAS 14371-10-9; EC 604-377-8); purity 96.8%

No data available on impurities

Radiolabelling, specific activity: 4.1 mCi/mmol

Test animals:

Rat (Fischer 344), male and female (4/group)

Mice (CD1), male and female (6/group)

- Source: Fischer 344 rats, Harlan-OLAC, Bicester Oxon, UK and CD1 mice, Charles River Breeding Laboratories, Manston, Kent, UK
- Age at study initiation: No information available
- Weight at study initiation: Fischer 344 rats 200±10g; CD1 mice 27±2g
- Housing: individual
- Individual metabolism cages: yes
- Diet: ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature (°C): No information available
- Humidity (%): No information available
- Photoperiod (hrs dark / hrs light): No information available

Dosing:
Single dose, oral (gavage) and intraperitoneal injection.
Concentrations: gavage: 250 mg/kg bw; ip.: 2 and 250 mg/kg bw
No. of animals per dose: male and female F344 rats (4/group); male and female CD1 mice (6/group)

Sampling - metabolite characterisation studies:
- Urine and faeces collected on the day before experiment and 3 days after dosing
- From how many animals: No information available
- Method type(s) for identification: *Radio*-HPLC

Detailed study summary and results:
The metabolism of trans-[3-^{14}C]cinnamaldehyde was investigated in male and female Fischer 344 rats and CD1 mice at doses of 2 and 250 mg/kg body weight given by ip injection and in males at 250 mg/kg by oral gavage. Some 94% of the administered dose was recovered in the excreta in 72 hr in both species with most (75-81%) present in the 0-24 hr urine. Less than 2% of the administered dose was found in the carcasses at 72 hr after dosing. Urinary metabolites were identified by their chromatographic characteristics. In both species the major urinary metabolite was hippuric acid (71–75% in mice and 73–87% in rats) accompanied by 3-hydroxy-3-phenylpropionic acid (0.4–4%), benzoic acid (0.4–3%) and benzoyl glucuronide (0.8–7.0%). The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse (4–13%). The oxidative metabolism of cinnamaldehyde essentially follows that of cinnamic acid, by beta-oxidation analogous to that of fatty acids. Apart from the metabolites common to cinnamic acid and cinnamaldehyde, 7% of 0-24-hr urinary ^{14}C was accounted for by two new metabolites in the rat and three in the mouse, which have been shown in other work to arise from a second pathway of cinnamaldehyde metabolism involving

conjugation with glutathione. The excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size and route of administration. The data are discussed in terms of their relevance to the safety evaluation of trans-cinnamaldehyde, particularly the validity or otherwise of extrapolation of toxicity data from high to low dose.

Based upon the metabolism and rapid excretion of the metabolites formed in rats and mice (24 hr), it can be concluded that the chemical trans-Cinnamaldehyde is expected to exhibit low bio-accumulation potential upon entry within the body of animals.

2.1.3 STUDY 3

Reference:

Yuan, J. et al. 1992. Toxicokinetis of Cinnamaldehyde in F344 rats. Fd. Chem. Toxic. 30, 997-1004, 1992.

Yuan, et al. 1993. Application of microencapsulation for toxicology studies. Fundamental and Applied Toxicology 20, 83-87, 1993.

Cited from the publicly available part of REACH registration.

Test type

No information on guideline or GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: No data

Method: Toxicokinetic study by single dose oral (gavage) and intravenous (iv) administration[1]

Test substance:

No details on test substance given by the registrant

Purity of cinnamaldehyde 98%

No data available on impurities

Test animals:

Rat (Fischer 344), male and female (3/group)

No additional data in publicly available part of REACH reg.

[1] Indicated as both intraperitoneal (ip) and iv administration administration in REACH reg. The published article by Yuan et. al., 1992, however states intravenious administration.

Dosing:

Single dose, oral (gavage) and intravenous (iv) administration

Vehicle: oral: corn oil; iv: ethanol-emulphor EL-620-water

Dose: gavage: 50, 150, 500, 1000, and 2000 mg/kg bw; gavage microcapsulated: 50, 250, and 500 mg/kg bw; iv: 5, 15 or 24 mg/ kg bw.

Sampling:

No data in publicly available part of REACH reg.

Detailed study summary and results:

After iv administration a large fraction of cinnamaldehyde was immediately oxidized to cinnamic acid (estimated to be between 37 and 60 % by the authors) within the first 30 minutes. The biological half-life of cinnamaldehyde after iv administration was found to be 1.7 hours in the rat.

After oral administration at 250 or 500 mg/kg bw the maximum blood concentrations were in the order of 1 µg/ml. At 50 mg/kg bw no cinnamaldehyde could be detected in the blood (< 1 µg/ml). The majority of cinnamaldehyde administered orally was excreted in urine as hippuric acid within 24 hours. The maximum excretion rate occurred at 8 hours after gavage.

2.1.4 STUDY 4

Reference:

Zhao H, et al. 2014. Pharmacokinetic study of cinnamaldehyde in rats by GC-MS after oral and intravenous administration. Journal of Pharmaceutical and Biomedical Analysis 89, 150-157, 2014.

Cited from the publicly available part of REACH registration.

Test type

No information on guideline or GLP compliance. Basic toxicokinetics.

Material and methods

Test guideline:

Type of method: In vivo

Objective of study: Toxicokinetics

Test guideline: No data

Method: GC-MS study on toxicokinetics (absorption, metabolism and excretion)

Test substance:

No details on test substance given by the registrant

Purity of cinnamaldehyde 99%
No data available on impurities

Test animals:
Rat (Sprague-Dawley), male (5/group)
- Source: No data
- Age at study initiation: No data in publicly part of REACH reg.
- Weight at study initiation: No data in publicly part of REACH reg.
- Fasting period before study: No data in publicly part of REACH reg.
- Individual metabolism cages: Stainless-steel metabolic cages – no data on indivual cages
- Diet: Free access to food
- Water: Free access to water
- Acclimation period: No data in publicly part of REACH reg.

Dosing:
Single dose, oral (gavage) and intravenous (iv) administration
Vehicle: oral: corn oil
Dose: oral: 500, 250, or 125 mg/kg bw; iv: 20 mg/ kg bw.

Three groups of rats (n = 5) received a single oral dose of 500 mg/kg, 250 mg/kg, or 125 mg/kg cinnamaldehyde (diluted in corn oil). The group of rats (n = 5) used for the urinary and fecal excretion study received a single oral dose of 500 mg/kg cinnamaldehyde. One group of rats (n = 5) were dosed with 20 mg/kg by iv administration.

Sampling:
Blood was collected at 10, 30, 60, 120, 180, 240, 360, 480, 720, 1080, and 1440 min post-administration. For the group with iv administration, blood was collected at 2, 5, 10, 15, 30, 60, 90, 120, and 180 min after iv administration. The blood samples were processed similarly to the blank sample. Urine and feces were collected at 0–4, 4–8, 8–12, 12-18, and 18–24 h post-dosing. The feces were dried at room temperature.

Detailed study summary and results:
The GC–MS technique was used to separate and determine cinnamaldehyde and its metabolites in rat plasma after oral and intraveneous administration. The areas under the plasma concentration–time curve (AUC) from 0 min to terminal time of cinnamaldehyde were 1984 ± 531 and 355 ± 53 ng h/ml for oral (500 mg/kg) and iv (20 mg/kg) administration, respectively. The elimination half-lives of cinnamaldehyde were 6.7 ± 1.5 and 1.7 ± 0.3 h for oral and iv administration, respectively. From dosage 125 to 500 mg, maximum plasma concentration (Cmax) and area under the curve to termination time (AUC0–t) were proportional to the dose;

time at maximum plasma concentration (Tmax) and mean residence time (MRT) did not change following dose escalation. The metabolites in blood were cinnamyl alcohol and methyl cinnamate.

An excretion experiment was also performed. A lower accumulative ratio of cinnamaldehyde was found after 24 hours, with the numbers reaching at 0.3% and 0.8% in feces and urine.

A double peak was observed in the concentration-time profile of 500 mg/kg oral administration; the Cmax was 249 ± 36 ng/ml and the other peak was 130 ± 56 ng/ml. Enterohepatic circulation may be an explanation for this because the double-peak was not observed in the iv concentration-time profile; furthermore, the metabolites of cinnamaldehyde presented the same phenomenon. Half-life was about 6.5 hours independent of oral dose.

2.1.5 STUDY 5

Reference:

D. Bickers, P. Calow, H. Greim, J.M. Hanifin, A.E. Rogers, J.H. Saurat, I.G. Sipes, R.L. Smith, H. Tagami, 2005. A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43 (2005) 799–836.

Hotchkiss SAM, 1998. Absorption of fragrance ingredients using in vitro models with human skin. In: Frosch, P.J., Johansen, J.D., White, I.R. (Eds.), Fragrances: Beneficial and Adverse Effects. Springer-Verlag, Berlin, pp. 125–135, 1998. Cited in Bickers (original literature not available).

Cited from the publicly available part of REACH reg.

Test type

Skin absorption model with human skin or diffusion cell technique with excised human abdominal skin and rat skin. Dermal absorption.

Material and methods

Test guideline:

Type of method: In vitro/ex vivo

Objective of study: Dermal absorption

Test guideline: No data

Method: Skin absorption model

Test substance:

No details on test substance given by the regristrant

Dosing:

Type of coverage: open and occlusive

Duration: 72 hours

Detailed study summary and results:

Using a skin absorption model system with human skin for cinnamaldehyde it was reported that 34% and 66% cinnamyl alcohol, 24% and 52% cinnamaldehyde and 18%, and 61% cinnamic acid (non-occluded and occluded, respectively) were absorbed by 72h.

Using a skin absorption model system with excised rat skin, 34% and 42% cinnamaldehyde (non-occluded and occluded, respectively) have been reported to be absorbed within 48–72h (Hotchkiss, 1998).

3 HEALTH HAZARDS

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 STUDY 1 and 2 (LLNA)

Study reference:

Williams W.C., Copeland C., Boykin E., Quell S.J., Lehmann D.M.: Development and utilization of an *ex vivo* bromodeoxyuridine local lymph node assay protocol for assessing potential chemical sensitizers. Journal of Applied Toxicology; 35: 29-40, 2015.

Detailed study summary and results:

Test type

ex vivo LLNA: BrdU-ELISA – No OECD guideline exists

LLNA:BrdU-ELISA (*in vivo)* according to the ICCVAM, 2010 protocol which is comparable to OECD guideline 442B

GLP: Not stated

Test substance

Cinnamaldehyde (Sigma–Aldrich)

Purity: No information on purity available

Test animals

Mice (BALB/c), female

6 animals per dose

Age: 8-9 weeks old

All mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility that provided constant environmental conditions with an ambient temperature of 21.5 ± 1.5 °C, relative humidity of 55 ± 5%, a 12 h light/dark cycle. Mice were housed (six per cage) in polycarbonate cages with hardwood chip bedding (NEPCO, Warrensburg, NY, USA) and were provided a balanced diet of mouse chow (5POO Prolab RMH3000, PMI Nutrition International, Richmond, IN, USA) and water ad libitum. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of NHEERL, US EPA.

Administration/exposure

Three groups of mice (n=6 per dose) were treated with 1, 5 and 10% cinnamaldehyde. Vehicle: acetone-olive oil (AOO) 4:1. One group was treated with vehicle alone (vehicle control). The test substance or vehicle alone was applied 25 µl to the dorsum of each ear on experimental day 1, 2 and 3. On experimental day 6, mice for *in vivo* LLNA:BrdU-ELISA was injected i.p. with 0.5 ml of pyrogen-free saline containing 5mg BrdU. Twenty-four (24) hours later, the mice were killed. Immediately following killing, the lymph nodes draining the ears were harvested and placed in PBS at room temperature. Lymph nodes were mechanically disaggregated using a disposable plastic pestle and passed through a 100 µm Celltrics filter into a sterile 15 ml collection tube. Lymph node cells were pelleted by centrifugation and re-suspended in 1ml PBS. Cells were counted using a Coulter Counter, and viability was determined by trypan blue dye exclusion. Cell suspensions were diluted to a final volume of 15 ml, and 100 µl aliquots were then plated into duplicate wells of a 96-well plate. Cells were adhered to the plate by centrifugation and then dried to the plate at 60 °C for 1 h. After drying, the plates were stored at 4 °C until assessment of BrdU incorporation by ELISA.

On experimental day 6, mice for *ex vivo* LLNA:BrdU labelling was killed. Immediately following killing, the lymph nodes draining the ears were harvested and placed in room temperature RPMI 1640 with 25mM HEPES and 2.05mML-glutamine supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin. Lymph nodes were processed into single cell suspensions. After counting, 3×10^5 live cells in 100 µl volume were plated in duplicate wells of a 96-well plate. Cells were incubated in the presence of 10 µM BrdU for 8−12 hours. BrdU-labelled cells were adhered to the plate by centrifugation (300 g for 7 min at room temperature) and then dried to the plate at 60 °C for 1 h. After drying, the plates were stored at 4 °C until assessment of BrdU incorporation by ELISA.

BrdU incorporation was quantified using the BrdU Cell Proliferation ELISA kit and protocol.

Results and discussion

The responses to test substances exposure were characterized by BrdU incorporation into the lymph node cells and the stimulation index at each dose was calculated as the ratio of the mean BrdU labelling index for each treatment group to the mean BrdU labelling index of the concurrent vehicle control group. An SI of 2 indicates a positive threshold response in the assay.

Cinnamaldehyde was shown to be sensitising with an EC2 value of 6.1% in the *in vivo* LLNA:BrdU-ELISA test and with an EC2 value of 6.9% in the *ex vivo* LLNA:BrdU test. Irritation was not observed for cinnamaldehyde (determined by ear thickness, erythema score and differentiation index (DI). Detailed information of the responses of each animal per test group is not presented in the article.

3.1.1.2 STUDY 3 (LLNA)

Study reference:

Niklasson I.B., Delaine T., Islam M.N., Karlsson R., Luthman K., Karlberg A-T.: Cinnamyl alcohol oxidizes rapidly upon air exposure. John Wiley & Sons A/S Contact Dermatitis, 68, 129–138, 2013.

Detailed study summary and results:

Test type

LLNA, comparable to the most recent version of OECD guideline 429, however, with only 3 animals used pr. dose instead of 4.

GLP: Not stated

Test substance

Cinnamaldehyde (Aldrich Chemicals, Sweden), purity > 98%

Test animals

Mice (CBA/Ca), female

3 animals per dose (two-week air-exposed cinnamyl alcohol, epoxy cinnamyl alcohol and epoxy cinnamaldehyde also tested)

Age at study initiation: 8-9 weeks

The mice were housed in HEPA-filtered air flow cages, and kept on standard laboratory diet and water ad libitum.

Administration/exposure

Groups of mice (N=3) were treated daily with 25µl the test substance in vehicle or vehicle alone on dorsum of both ears for three consecutive days (day 0-2). The concentrations used for were cinnamaldehyde 0.1, 0.99, 3.3, 9.9 and 19.8% and the vehicle was acetone-olive oil (AOO). On day 5, all mice were injected intravenously via the tail vein with [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting on a Beckman LS 6000TA instrument.

Results and discussion

Results are expressed as mean dpm/lymph node for each experimental group and as stimulation index (SI), that is, test group/control group ratio. Cinnamaldehyde was shown to be sensitising with an EC3 value of 0.75% wt/vol (57 mM) in the LLNA assay. No information on irritation was reported. Detailed information of the responses of each animal per test group is not presented in the article.

3.1.1.3 STUDY 4 (LLNA)

Study reference:

Ulker O.C., Ates I., Atek A., Karakaya A.: Evaluation of non-radioactive endpoints of *ex vivo* local lymph node assay-BrdU to investigate select contact sensitizers. Journal of Immunotoxicology, 10(1): 1–8, 2013.

Detailed study summary and results:

Test type

ex vivo LLNA: BrdU-ELISA, no OECD guideline exists

GLP: Not stated

Test substance

Cinnamaldehyde (Sigma, St. Louis, MO)

Purity: No information on purity available

Test animals

Mice (BALB/c), female

4 animals per dose

Age at study initiation: 8-12 weeks

The animals were kept at 23 °C and relative humidity 55 % with alternating 12h light and dark. The animals had ad libitum access to water and diet.

Administration/exposure

Five groups of mice (n = 4/group) were exposed topically (on dorsum of both ears) for 3 consecutive days to 25µl of different doses of known sensitizers or vehicle (AOO) alone daily. All mice were rested on Day 4 and then euthanized by cervical dislocation on Day 5 to permit collection of their auricular lymph nodes. The excised right and left lymph nodes from each mouse were pooled and homogenized, and the released cells suspended in 15 ml physiological saline. After culture had occurred for 48 hours at 37°C, BrdU was added to the wells for a 24 hour labelling period. The cells in the wells were then recovered by aspiration and the extent of BrdU incorporation measured by ELISA.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 1.91%. No information on irritation reported.

Calculated stimulation index, cinnamaldehyde

Applied concentration	0.5%	1%	5%	10%
SI	1.85	2.60	4.36	9.19

3.1.1.4 STUDY 5 (LLNA)

Study reference:

Kojima H., Takeyoshi M., Sozu T., Awogi T., Arima K., Idehara K., Ikarashi Y., Kanazawa Y., Maki E., Omori T., Yuasaj A., Yoshimurak I.: Inter-laboratory validation of the modified murine local lymph node assay based on 5-bromo-2'-deoxyuridine incorporation. J. Appl. Toxicol.; 31: 63–74, 2011

Detailed study summary and results:

Test type

LLNA:BrdU-ELISA (*in vivo*) in accordance with OECD 442B

The studies were not conducted under full compliance with GLP. However, all the laboratories were equipped to perform, and competent with, GLP.

Test substance

Trans-Cinnamaldehyde (though the study refers to the CAS no. of cinnamaldehyde, 104-55-2)

Purity: No information on purity available

Test animals

Mice (CBA/JN), female

4 animals per dose

Age at study initiation: 8-12 weeks

The animals were kept at 22±3 °C and relative humidity 30-70 % with artificial light for12 hours. The animals had ad libitum access to water and diet.

Administration/exposure

A minimum of four successfully treated animals was used per dose group, with a minimum of three consecutive doses of the chemical, and one group each for the negative vehicle control (AOO) and positive control (50% hexyl cinnamaldehyde). A 25µl dose of test solution was applied to the dorsum of both ears of the mice for three consecutive days using a micro volume pipette. A single intraperitoneal injection of 0.5 ml of BrdU solution (5mg/mouse/injection) was given to the mice 48 h after the final application. Approximately 24 hours after BrdU injection, the auricular lymph nodes were removed. The lymph nodes

were carefully dissected and trimmed of fascia and fat, weighed and stored individually in a 1.5 ml centrifuge tube at -20°C until BrdU-ELISA measurement.

Results and discussion

The EC2 was defined as the estimated concentration that yielded an SI of 2 from the dose–response curve. EC2 of the weighted average was estimated and classified into the appropriate chemical category. trans-cinnamaldehyde was shown to be sensitising with an average EC2 value of 2.2% for the 3 laboratories. No information on irritation reported.

Calculated stimulation index, trans-cinnamaldehyde

Applied concentration	1%	3%	10%
SI laboratory 2	1.10	2.23	3.37
SI laboratory 4	1.57	2.94	3.49
SI laboratory 5	1.14	2.10	4.11

3.1.1.5 STUDY 6-15 (LLNA, 10 studies cited in SCCS 2012)

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: 2003a, 2003b, 2003c, 2003d, 2003e, 2003f, 2003g, 2003h, 2003i, 2003j.

Detailed study summary and results:

Test type

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

Test substance

Cinnamaldehyde, no information on purity.

Test animals

Mice, n=4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

In all 10 studies cinnamaldehyde was tested in concentrations of 0.1, 0.3, 1.0, 3.0 and 10.0%

Vehicles used were either:

- 3:1 ethanol:diethyl phthalate (EtOH:DEP) (2 studies)
- 0.1% α-tocopherol in 3:1 EtOH:DEP (2 studies)

- 2.0% α-tocopherol in 3:1 EtOH:DEP (2 studies)
- 0.3% antioxidant mix (1:1:1 of α-tocopherol, butylated hydroxytoluene (BHT) and eugenol,) in 3:1 EtOH:DEP (2 studies)
- 0.1% Trolox C in 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 cinnamaldehyde in other LLNA studies. The EC3 values reported by RIFM are in the range 0.2%-1.7%.

3.1.1.6 STUDY 16 (LLNA)

Study reference:

Elahi E. N., Wright Z., Hinselwood d., Hotchkiss S. A. M., Basketter D. A., Pease C. K. S.: Protein Binding and Metabolism influence the Relative Skin Sensitization Potential of Cinnamic Compounds. Chem. Res. Toxicol., 17, 301-310, 2004

Detailed study summary and results:

Test type

LLNA, in accordance with OECD 429

GLP: Not stated

Test substance

trans-Cinnamaldehyde

Purity: 96%

Impurities: Cinnamic acid 3.26% and Cinnamic alcohol 0.71%

Test animals

Mice (CBA/Ca)

4 animals per dose

Age at study initiation: 7-12 weeks (Harlan Olac, U.K.)

Administration/exposure

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone (acetone-olive oil (AOO)) on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 1, 2.5, 5, 10 and 25%. On day 5 after the initiation of the exposure, all mice were injected intravenously via the tail vein with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were

sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 1.3%.

Calculated stimulation index, cinnamaldehyde

Applied concentration	1%	2.5%	5%	10%	25%
SI*	2.5	4.9	8.8	10.2	13

*Based on figure 4 in Elahi et al., 2004

3.1.1.7 STUDY 17 (LLNA)

Study reference:

Basketter D. A., Wright Z. M., E. Warbrick V., Dearman R. J., Kimber I., Ryan C. A., Gerberick G. F., White I. R.: Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis, 45, 89–94, 2001

Detailed study summary and results:

Test type

The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it

GLP: Not stated

Test substance

Cinnamaldehyde

Purity: 99%

Impurities: No information available

Test animals

Mice (CBA/Ca), female

4 animals per dose

Age at study initiation: 6-12 weeks (Harlan Olac, U.K.)

Administration/exposure

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone (acetone-olive oil (AOO)) on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 0.5, 1, 2.5, 5, 10 and 25%. On day 5 after the initiation of the exposure, all mice were

injected intravenously via the tail vein with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells in PBS were prepared with cell strainers. The [^{3}H]methylthymidine incorporation into DNA was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising with an EC3 value of 3.1%.

Calculated stimulation index, cinnamaldehyde

Applied concentration	0.5	1%	2.5%	5%	10%
SI	1.37	0.9	1.85	7.7	15.75

3.1.1.8 STUDY 18 (LLNA)

Study reference:

Smith C. K., Hotchkiss S. A.: Enzymes and mechanisms of xenobiotic metabolism. Allergic Contact Dermatitis Chemical and Metabolic Mechanisms. Taylor and Francis, London and New York 45-87, 2001. As *cited in:* Scientific Committee on Consumer Safety SCCS OPINION on Fragrance allergens in cosmetic products. June 2012 (SCCS 2012).

Detailed study summary and results:

Test type

LLNA with only two concentrations tested. This is the only deviation reported from with OECD 429 in SCCS 2012

Test substance

Cinnamaldehyde, no information on purity.

Test animals

Mice, n= 4 animals per dose.

No further information available in SCCS 2012.

Administration/exposure

Cinnamaldehyde was tested in concentrations of 1.0 and 2.5% and the vehicle used were 4:1 acetone-olive oil (AOO).

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the study conducted by RIFM the result generally confirm the sensitising properties identified for cinnamaldehyde in other LLNA studies. The EC3 values reported by RIFM are 1.4%.

3.1.1.9 STUDY 19 - 25 (LLNA)

Study reference:

Wright Z. M., Basketter D. A., Blaikie L., Cooper K. J., Warbrick E. V., Dearman R. J., Kimber I.: Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. International Journal of Cosmetic Science, 23, 75-83, 2001

Detailed study summary and results:

Test type

The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it

GLP: Not stated

Test substance

Cinnamaldehyde

Purity: 99%

Impurities: No information available

Test animals

Mice (CBA/Ca), female

4 animals per dose

Age at study initiation: 6-12 weeks (Harlan, U.K.)

Administration/exposure

Five concentrations of cinnamaldehyde were tested in seven different vehicles (50:50 EtOH:water, 90:10 EtOH:water, DMSO, propylene glycol, DMF, MEK and AOO). In order to derive EC3 cinnamaldehyde were re-tested at lower concentrations in DMF and DMSO.

Groups of mice (N=4) were treated daily with 25µl the test substance in vehicle or vehicle alone on dorsum of both ears for three consecutive days. The concentrations used for were cinnamaldehyde 1, 2.5, 5, 10 and 25% and for DMF and DMSO also 0.1, 0.25 and 0.5. On day 5 after the initiation of the exposure, all mice were injected intravenously with 250 µL PBS containing 20 µCi of [^{3}H]methylthymidine and were sacrificed after 5 hours. The draining lymph nodes were excised and pooled for each group, and single-cell suspensions of lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze. The mesh was washed twice with PBS and the cells precipitated in 5% TCA the 4 °C overnight. Each precipitate

were pelleted and re-suspended in 5% TCA and transferred to a scintillation vial with 10 ml scintillation liquid. The [^{3}H]methylthymidine incorporation was measured by β-scintillation counting.

Results and discussion

Cinnamaldehyde was shown to be sensitising in all the tested vehicles. EC3 values depending vehicle are show in the table below.

EC3 values (%v/v) in different vehicles, cinnamaldehyde

Vehicle	AOO	MEK	DMF	PG	DMSO	EtOH:water; 90:10	EtOH:water; 50:50
EC3	1.7	1.1	0.5	1.4	0.9	1.6	1.2

3.1.1.10 STUDY 26-27 (LLNA & GPMT)

Study reference:

Basketter D. A. and Scholes E. W.: Comparison of the Local Lymph Node Assay with the Guinea-pig Maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology, 30, 65-69, 1992.

Also cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic

Detailed study summary and results:

Basketter and Scholes (1992) investigated the potential for cinnamic aldehyde to induce skin sensitisation in a study designed to compare the local lymph node assay with the guinea pig maximisation test for the detection of a range of contact allergens.

Test type:

GMPT: The guinea pig maximization test employed in this study predates the most recent version of OECD guideline 406 but is comparable to it.

LLNA: The local lymph node assay employed in this study predates the most recent version of OECD guideline 429 but is comparable to it.

Test substance

Cinnamaldehyde

Purity: No information available

GMPT vehicle: 70:30 acetone:PEG 400 (A/P)

LLNA vehicle: 4:1Acetone-olive oil (AOO)

Test animals

GMPT: Guinea pig, Albino Dunkin-Hartley. Weight at study initiation approximately 350 g.

LLNA: CBA/Ca mice. Age at study initiation: 8-12 weeks

Administration/exposure:

Preliminary irritation tests were carried out to determine the concentrations of cinnamaldehyde suitable for induction of sensitisation and for sensitisation challenge. Guinea pigs were then treated by a series of six 0.2% cinnamaldehyde intradermal injections in the shoulder region to induce sensitization. After 6-8 days, sensitization was boosted by a 48 hour occluded patch (2.5% cinnamaldehyde) placed over the injection site. Following a rest period of 12-14 days, the animals were challenged on one flank by a 24 hour occluded patch at the maximum non-irritant concentration (0.75% cinnamaldehyde). Challenge sites were scored for erythema (scale 0-3) and oedema 24 and/or 48 hours after removal of the patches. The study does not refer to control animals but the study did identify strong, moderate and mild sensitisers plus a number of non-sensitising chemicals.

Groups of 4 mice received daily topical applications of 5, 10 or 25% cinnamic aldehyde on the dorsal surface of each ear for 3 consecutive days. Control mice were treated with vehicle alone. On day 4 or 5 of the study all mice were injected intravenously in the tail vein with phosphate buffered saline containing tritiated thymidine and killed 5 hours later. The proliferative response of the local lymph node cells was analysed and presented as a ratio of tritiated thymidine incorporation into lymph node cells relative to controls.

Results and discussion

The results of the GMPT study revealed cinnamic aldehyde to be a potent skin sensitiser with 100% of the animals tested judged to have elicited a positive response after 24 or 48 hours.

In the LLNA study a chemical was regarded as a sensitiser if at least 1 concentration of the chemical resulted in at least a 3-fold increase in tritiated thymidine incorporation compared to controls. Cinnamic aldehyde elicited test/control ratios of 12.5, 18.4 and 15.4 for the 5, 10 and 25% concentrations tested respectively and was therefore judged to be a skin sensitiser.

3.1.1.11 STUDY 28 (GPMT)

Study reference:

Basketter D. A.: Skin Sensitization to Cinnamic Alcohol: The role of Skin Metabolism. Acta Derm Venereol, 72, 264-265, 1992.

Also cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43, 799–836, 2005.

The current study includes 2 GPMTs, one of which is referred to in Basketter and Scholes, 1992. Including Study 29, there is a total of 3 GPMTs.

Detailed study summary and results:

Basketter (1992) investigated the potential for trans-cinnamaldehyde, cis- and trans-cinnamic alcohol to induce skin sensitisation in a study designed to investigate the hypothesis that cinnamic alcohol (via oxidation) and cinnamaldehyde gives rise to the same allergen *in vivo*, perhaps via the combination of reactive aldehyde species with skin protein.

Test type:

The guinea pig maximization test employed in this study predates the most recent version of OECD guideline 406 but is comparable to it.

Test substance

trans-Cinnamaldehyde

Purity: No information available.

Test animals

Guinea pig, Albino Dunkin-Hartley

Administration/exposure:

Preliminary irritation tests were carried out in groups of four albino Dunkin-Hartley guinea pig to determine the concentrations of cinnamaldehyde suitable for induction of sensitisation and for sensitisation challenge. Guinea pigs were then treated in the shoulder region by a series of six intradermal injections of 0.2% trans-cinnamaldehyde in combination with Freund's complete adjuvant to induce sensitization. After 6-8 days, sensitization was boosted by a 48 hour occluded patch (2.5% trans-cinnamaldehyde) placed over the injection site. Following a rest period of 12-14 days, the animals were challenged on one flank by a 24 hour occluded patch at the maximum non-irritant concentration (0.75% trans-cinnamaldehyde). A group of four animals treated as above but without cinnamaldehyde served as controls Challenge sites were scored for erythema (scale 0-3) and oedema 24 and/or 48 hours after removal of the patches.

Results and discussion

Sensitisation caused by trans-cinnamaldehyde was observed in 90% (9/10) and in 100% (10/10) of the animals. The mean erythema scores on positive responders (scale 0-3) were 2.0 and 2.2, respectively for the two test groups. The study only found limited evidence for cross reactivity between trans-cinnamaldehyde and trans-cinnamic alcohol.

3.1.1.12 STUDY 29 (GPMT)

Study reference:

Ishihara, M., Itoh, M., Nishimura, M., Kinoshita, M., Kantoh, H., Nogami, T., Yamada, K.: Closed epicutaneous test. Skin Research 28 (Suppl 2), 230–240, 1986
cited in: Bickers D., Calow P., Greim H., Hanifin J.M., Rogers A.E., Saurat J.H., Sipes I.G., Smith R.L., Tagami H.: A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. Food and Chemical Toxicology 43, 799–836, 2005.

Detailed study summary and results:

Test type:

Guinea pig maximation test, no further information available from Bickers et al., 2005

Test substance

Cinnamaldehyde
Purity: No information available

Test animals

Guinea pig. No information on strain, number or sex

Administration/exposure:

Only information from Bickers et al., 2005 is a concentration of 3.0%. It is expected that this concentration refers to the challenge concentration but it is not clear. No information on vehicle.

Results and discussion

Strong sensitisation effect reported (no further details)

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., 30, 264–247, 2015.

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 (FM I and FM II) obtained in the period from 1998-2013 (FMI) and 2005-2013 (FM II). Cinnamaldehyde is a component of FM I (1% cinnamaldehyde). In cases where positive reactions were observed for FM I, testing of the full mix breakdown (and other fragrance allergens) have been done. FM I was patch tested in 141 372 patients in 1998–2013. Of these 13 074 patients (9.25%) had a positive reaction. Time trends were analysed by dividing the time span into eight 2-year periods. The FM I full mix breakdown was tested in 2 798 patients with a positive reaction to FM I. The results obtained with cinnamaldehyde alone are based on patch tests with 1% cinnamaldehyde 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 cinnamaldehyde showed that during the period 1998-2013 10.6% of the 2 798 selected patients were tested positive. The results divided into time spans are listed in the table below (note that the patient counts of the single time periods to not sum up to 1058 as FM I and its single components were tested in different time periods in 66 patients):

IVDK results of retrospective analysis of patch tests with cinnamaldehyde 1% in petrolatum:

Year, patient count	1998-1999 n = 162	2000-2001 n = 139	2002-2003 n = 249	2004-2005 n = 281	2006-2007 n = 285	2008-2009 n = 469	2010-2011 n = 634	2012-2013 n = 513	1998-2013 n = 2 798
Positive reactions	8.6% (4.8-	4.3% (1.6-	10.4% (6.9-	12.1% (8.5-	14.4% (10.5-	9.6% (7.1-	9.6% (7.4-	12.5% (9.7-	**10.6% (9.5-11.8)**

(95% conf. intervals)	14.1)	9.2)	14.9)	16.5)	19.0)	12.6)	12.2)	15.7)	

3.1.2.2 STUDY 2 (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, 307–313, 2013.

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 13 332 patients who had been patch tested in the period from 1990-2011. A total of 13 114 patients were tested with FM I. The number of patients reacting to FM I (which includes 1% cinnamaldehyde) was 1 259. Subsequent patch testing was in done with the individual ingredients of the fragrance mixture.

Description of test method as cited from Nardelli et al., 2013: *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 7% of the selected patients (66/940) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.3 STUDY 3 (Patch test, selected)

Study reference:

Lyons G., Roberts H., Palmer A., Matheson M. Nixon R.: Hairdressers presenting to an occupational dermatology clinic in Melbourne, Australia. Contact Dermatitis, 68, 300–306, 2013

Detailed study summary and results:

Test type

Department of Occupational Dermatology Research and Education Centre, Australia performed a retrospective study of 164 selected hairdressers and hairdressing apprentices diagnosed with occupational contact dermatitis between 1993 and 2010. Patients were patch tested with a number of allergens including 1% cinnamaldehyde in petrolatum.

Description of test method as cited from Lyons et al., 2013: *"The allergens used for patch testing were obtained from Chemotechnique Diagnostics (Vellinge, Sweden) and applied to the back with Finn Chambers on Scanpor tape (Epitest OY, Tuusula, Finland). Patches were removed after 48 hr, and test readings were performed at D2 and D4. Patients were generally tested with an extended European baseline series, cosmetics series, hairdressers' series, and their own samples appropriately diluted. Patients were tested with additional series, for example a rubber series, if clinically relevant. Positive patch test reactions were assessed for relevance by the occupational dermatologist.*

When there was a history of exposure to natural rubber latex, patients were also tested for latex protein allergy, usually with a screening radio-allergosorbent test. Patients were then diagnosed with allergic contact dermatitis, irritant contact dermatitis, contact urticarial (caused by natural rubber latex proteins or ammonium persulfate), endogenous eczema, mucosal atopy, or other conditions. Endogenous eczema included the diagnosis of atopic eczema and other forms of eczema, such as seborrhoeic or discoid eczema. When there were multiple contributory factors, the diagnosis thought to be most contributory to the OCD was referred to as the primary diagnosis. The severity of the skin conditions was assessed on initial presentation with use of the occupational dermatitis disease severity index (ODDI) (20). The ODDI score rates severity of OCD on a scale of 1–5, based on disease course, treatment, clinical signs, and impact on work-related activities."

The results for cinnamaldehyde showed that during the period 1990-2010 1% of 164 selected hairdressers and hairdressing apprentices were tested positive.

3.1.2.4 STUDY 4 (Patch test, selected)

Study reference:

Hession M.T., Scheinman P. L.: The Role of Contact Allergens in Chronic Idiopathic Urticaria. Dermatitis, Vol 23, No 3, 2012

Detailed study summary and results:

Test type

The Dermatology Department at Tufts Medical Center, USA, conducted a prospective study of 23 selected patients with chronic idiopathic urticarial patch tested with a number of allergens including cinnamaldehyde.

Description of test method as cited from Hession and Scheinman, 2012: "*Patch testing was performed using a modified North American Contact Dermatitis Group (NACDG) standard series, as well as cosmetic and fragrance series. Other series were tested if warranted by relevant history or occupational exposure. Textile series were placed when urticaria was in a distribution on trunk and extremities consistent with a possible textile dye or formaldehyde textile resin allergy, a rubber series in patients complaining of hives under bra elastic or waistband elastic, and a hair series for patients with scalp symptoms who had colored their hair, and so on. All allergens were purchased from Chemotechnique Diagnostics (Vellinge, Sweden), except for individual fragrance mix I (FM I) components, which were purchased from Hermal (Reinbek, Germany). Readings were performed at 48 and 72 hours and graded according to the NACDG grading system of 1+ (weak reaction; papules with erythema), 2+ (strong reaction; papules plus edema or vesiculation), or 3+ (extreme reaction; spreading papulovesicles or bullae).*".

The results showed that 13% of the selected patients with chronic idiopathic urticarial (3/23) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.5 STUDY 5 (Patch test, selected)

Study reference:

Turcic P., Lipozencic J., Milavec-Puretic V., Kulisic S. M.: Contact Allergy Caused by Fragrance Mix and *Myroxylon pereirae* (Balsam Of Peru) – A Retrospective Study. Coll. Antropol. 35, 1, 83–87, 2011

Detailed study summary and results:

Test type

Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia concocted a retrospective study of 157 selected patients patch tested with cinnamaldehyde between 2001 and 2005. The 157 patients were chosen out of 509 patients tested positive to FM I.

Description of test method as cited from Turcic et al., 2011: "*Patch-test allergens were applied on the patients' upper back with 2-day occlusion. According to the International Contact Dermatitis Research Group (ICDRG) system, the tests were read 48 and 72 hours after their application 21, 22. The test results were interpreted using the following scale: negative reaction (0); macular erythema (?); erythema/in filtration and possibly papules (1+); erythematous papules and/or vesicles (2+); spreading blisters and/or*

crust with ulceration (3+); and irritant reaction (IR); whereby 1+, 2+ and 3+ were considered positive allergic reactions21. Statistical analysis was performed using the STATISTICA software, Version 7.1. (StatSoft, Inc.).".The results showed that 24.2% of the selected patients (38/157) had positive reactions for cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.6 STUDY 6 (Patch test, selected)

Study reference:

Cuesta L., Silvestre J. F., 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. A total of 86 patients were tested with the Chemotechnique® fragrance series. 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 8.1% of the selected patients (7/86) had positive reactions to cinnamaldehyde 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.

3.1.2.7 STUDY 7 (Patch test, selected)

Study reference:

Uter, W., Geier, J., Frosch, P., Schnuch, A.: Contact allergy to fragrances: current patch test results (2005-2008) from the Information Network of Departments of Dermatology. Contact Dermatitis 63, 254-261, 2010

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 2005-2008, the frequency of contact sensitization to fragrance allergens in patients routinely patch tested for suspected allergic contact dermatitis with the baseline series and special series (including cinnamaldehyde) was investigated in a total of 40709 patients. Cinnamaldehyde was tested as a single constituent in 4527 selected patients as part of a special breakdown series of fragrance mix (FM) I.

Description of patch test as cited from Uter et al., 2010: "*The IVDK (www.ivdk.org), a contact allergy surveillance network in Germany, Switzerland and Austria, has been described elsewhere. Briefly, results for all patients patch tested in the participating departments are electronically recorded, along with important demographic and clinical data. The diagnostic procedure follows international guidelines (9) that have been further refined by the German Contact Dermatitis Research Group (10), of which all IVDK participants are members.*"

Statistical analysis of the data was performed using the statistical software package SAS (version 9.2, SAS Institute, Cary, NC, USA).

The results showed that 2.64% (95% CI: 2.16-3.13%) of the 4527 selected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.8 STUDY 8 (Patch test, selected)

Study reference:

Andersen, K.E., Christensen L. P., Vølund AA., Johansen J. D., Paulsen E: Association between positive patch tests to epoxy resin and fragrance mix I ingredients. Contact Dermatitis, 60, 155–157, 2009

Detailed study summary and results:

Test type

In order to investigate association between positive reactions to epoxy resin and fragrance mix has Andersen et al. conducted a retrospective study of 6115 consecutive eczema patients tested from 1995 to 2007 were

included and test results from all patients tested with fragrance mix ingredients were analysed. 774 of the selected eczema patients were tested with 1% cinnamaldehyde petrolatum.

Description of test method as cited from Andersen et al., 2009: "*Patch tests were applied for 2 days with two readings routinely on D3 and D5–D7. The maximal scoring of test reactions was used in the calculations. Readings were scored according to the International Contact Dermatitis Research Group (ICDRG) ranking scale. A homogenous infiltration was required for a + reading, and ++ to +++ reactions were regarded as positive tests.*"

The results showed that 8.5% (66/774) of the selected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.9 STUDY 9 (Patch test, selected)

Study reference:

Pentinga S. E, Kuik D. J., Bruynzeel D. P., Rustemeyer T.: Do 'cinnamon-sensitive' patients react to cinnamate UV filters? Contact Dermatitis, 60, 210–213, 2009.

Detailed study summary and results:

Test type

Department of Dermatology of the VU University Medical Centre, The Netherlands, conducted a prospective study of 18 selected cinnamon-sensitive patients who were patch tested with 2% cinnamaldehyde in petrolatum.

Description of test method as cited from Pentinga et al., 2009: *"Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Epitest Ltd Oy, Tuusula, Finland) were applied in duplicate on the left and right side of the mid–upper back (avoiding the paravertebral groove) and removed after 2 days. The left side was covered with a light impermeable MoliNea plus D® dressing (Paul Hartmann BV, Nijmegen, the Netherlands). The right side was first exposed to 5 J/cm^2 UVA (Psoralen UVA 800 Unit; Waldmann, FRG) and then covered with MoliNea plus D dressing. Photopatch test readings were scheduled according to the recommendations of the European Taskforce for Photopatch Testing at D0 (2 days after application) before and 15 min after irradiation, D1, and D2, and patch test and photopatch test results were graded according to the scoring system of the International Contact Dermatitis Research Group (12).*

A positive photopatch test was defined as a negative patch test (−) at the non-irradiated side (left) at all readings in combination with a positive patch test (≥+) at the irradiated side (right) for at least one reading.

An 'inverse photopatch test' was defined as a negative patch test (−) at the irradiated side (right) at all readings in combination with a positive patch test (≥+) at the non-irradiated side (left) for at least one reading.".

The results showed that 22% of the selected patients (4/18) had positive reactions for cinnamaldehyde when tested at 2% in petrolatum.

3.1.2.10 STUDY 10 (Patch test, selected)

Study reference:

White J. M. L, White I. R., Kimber I., Basketter D. A., Buckley D. A., McFadden J.P.: Atopic dermatitis and allergic reactions to individual fragrance Chemicals. Journal compilation © 2009 Blackwell Munksgaard Allergy, 64, 312–316, 2009

Detailed study summary and results:

Test type

The study was performed to compare rates of atopic dermatitis between patients with allergic contact dermatitis arising out of individual fragrance chemicals with known oral/cutaneous exposure against exclusively cutaneous exposure. Between 1982 and 2007, 37065 dermatitis patients attending the Department of Cutaneous Allergy at St John's Institute of Dermatology, London, were tested with Fragrance mix (FM) I. Those who were positive were tested for individual fragrance allergy. The patients were either categorised as 'current' atopic dermatitis patients or 'past' atopic dermatitis patients. Cinnamaldehyde was tested at 1% in petrolatum.

Description of patch test as cited from White et al., 2009: "*Allergens were applied to the skin on 8 mm Finn chambers® (Epitest Oy; Tuusula, Finland) under Scanpor® tape (Beiersdorf, Hamburg, Germany). Patch-test readings were performed at days 2/3 and 4/5, according to standard ICDRG criteria (6). A positive (+, ++, +++) patch-test reaction signified allergy. Wherever possible, patients who were allergic (patch-test positive) to FM1 were then patch tested to the individual ingredients of the mix, all at 1% pet.*"

The results of the study showed that 0.98% of the selected patients (364/37065) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.11 STUDY 11 (Patch test, selected)

Study reference:

Vocanson, M., Goujon, C., Chabeau, G., Castelain, M., Valeyrie, M., Floc'h, F., Maliverney, C., Gard, A., Nicolas, J. F.: The skin allergenic properties of chemicals may depend on contaminants - Evidence from studies on coumarin. International Archives of Allergy and Immunology 140, 231-238, 2006.

Detailed study summary and results:

Test type

The aim of the study by Vocanson et al., was to test the importance of purity in the skin allergenic properties of a chemical exemplified by coumarin. A total of 30 patients allergic to their own perfumed product were recruited in 12 months in a multicentre study involving 7 dermatoallergology departments. The inclusion criterion was the presence of a relevant positive patch test to their own perfumed product. Nineteen of the 30

patients were patch tested with the first 8 allergens of the fragrance series (including cinnamaldehyde) in addition to coumarin.

Description of patch test as cited from Vocanson et al., 2006: "*All patients underwent patch testing. Patch testing was done on the skin on the back using Finn Chambers on Scanpor (dc 8 mm).*" ... "*Readings were done after 48/72 h and results were scored using the International Contact Dermatitis Research Group criteria [7] : – = negative; ? = doubtful; + = weak reaction (no vesicle); ++ = strong reaction (edema and vesicles); +++ = extreme reaction (ulceration, bullies); IR = irritant reaction; NT = not tested*."

The results of the study showed that 20% of the 19 patients were tested positive for cinnamaldehyde.

3.1.2.12 STUDY 12 (Patch test, selected)

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 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 were purchased from Chemotechnique Diagnostics, Malmo, Sweden. We selected additional allergens based on past relevant references and information as to usage frequency. 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 1.7% of the selected patients (7/422) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.13 STUDY 13 (Patch test, selected)

Study reference:

Wohrl, S., Hemmer, W., Focke, M., Gotz, M., Jarisch, R., 2001. The significance of fragrance mix, balsam of Peru, colophony, and propolis as screening tools in the detection of fragrance allergy. British Journal of Dermatology 145, 268-273.

Detailed study summary and results:

Test type

The aim of the study by Wohrl *et al.* was to determine the usefulness of adding propolis to the European standard series to test for fragrance allergy. For this purpose between 1997 and 2000 a total of 2660 consecutive patients were patch tested with a standard patch test series. In a prospective study 747 patients suspected of fragrance allergy were tested further with a special fragrance series (including cinnamaldehyde at 1 % in petrolatum and 1% SSO).

Description of patch test as cited from Wöhrl *et al.* 2001: "*The readings were done after 72 h and scored according to the recommendations of the International Contact Dermatitis Research Group (ICDRG).*"

The results of the study showed that 1.7% of the selected patients suspected of fragrance allergy (14/747) were tested positive for cinnamaldehyde.

3.1.2.14 STUDY 14 (Patch test, selected)

Study reference:

Brites, M.M., Goncalo, M., Figueiredo, A., 2000. Contact allergy to fragrance mix - a 10-year study. Contact Dermatitis 43, 181-182.

Detailed study summary and results:

Test type

A total of 2600 consecutive patients were patch tested with fragrance mix (FM) during a 10-year period from 1989 to 1999. A sub-group of 226 selected FM-reactive patients were also tested with the individuel FM constituents including 1% cinnamaldehyde in petrolatum.

The method of patch testing was not described by Brites et al., 2000.

The results of the study showed that 13.3% of the selected FM-reactive patients (30/226) were tested positive for cinnamaldehyde at 1 % in petrolatum.

3.1.2.15 STUDY 15 (Patch test, selected)

Study reference:

Hendriks, S.A., van Ginkel, C.J: Evaluation of the fragrance mix in the European standard series. Contact Dermatitis 41, 161-162, 1999

Detailed study summary and results:

Test type

In a retrospective evaluation of the fragrance mix in the European standard series a total of 757 patients suspected of allergy to cosmetics were patch tested between 1994 and 1998 with the European standard series, including fragrance mix (FM). The results from 50 fragrance-mix-positive/component-positive patients tested with cinnamaldehyde 2% in sorbitan sesquioleate (SSO, 1%) was reported by Hendriks & van Ginkel., 1999.

The method of patch testing was not described by Hendriks & van Ginkel., 1999.

The results of the study showed that 20% of the fragrance-mix-positive/component-positive patients (10/50) were tested positive for 2% cinnamaldehyde in 1% SSO.

3.1.2.16 STUDY 16 (Patch test, selected)

Study reference:

Katsarma, G., Gawkrodger, D.J.: Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. Contact Dermatitis 41, 193-197, 1999.

Detailed study summary and results:

Test type

This study was performed to evaluate the efficacy of fragrance mix (FM) as a screen for fragrance allergy. A total of 91 patients with positive allergic reactions to FM, to 1 of the 8 ingredients of FM, to 1 of 14 other fragrance materials, or to their own perfume were identified out of 744 consecutive unselected patients patch tested in 1994-1995. Cinnamaldehyde was tested in 40 FM-allergic patients identified among the 91 patients with positive allergic reactions to FM, to 1 of the 8 ingredients of FM, to 1 of 14 other fragrance materials, or to their own perfume.

Description of patch test as cited from Katsarma & Gawkrodger 1999: "*The materials were applied in Finn Chambers on Scanpor to the upper back, left on for 2 days (D), and read at D2 and D4, using the International Contact Dermatitis Research Group's grading system. Data were collected from each patient using a form on which were recorded demographic information (i.e., age, sex and occupation), dermatitis site and type, any personal history of atopy, the test results and the final diagnosis.*"

The results of the study showed that 12.5% of the FM-allergic patients (5/40) were tested positive for cinnamaldehyde in petrolatum (concentration not specified).

3.1.2.17 STUDY 17 (Patch test, selected)

Study reference:

Larsen, W., Nakayama, H., Lindberg, M., Fischer, T., Elsner, P., Burrows, D., Jordan, W., Shaw, S., Wilkinson, J., Marks, J., Jr., Sugawara, M., Nethercott, J.: Fragrance contact dermatitis: a worldwide multicentre investigation (Part I). American journal of contact dermatitis: official journal of the American Contact Dermatitis Society 7, 77-83, 1996

Detailed study summary and results:

Test type

The aim of the study by Larsen et al., 1996 was to determine the prevalence of responses to selected fragrance materials in patients with suspect fragrance allergy and to evaluate risk factors and associations with such responses. A total of 167 fragrance sensitive volunteers from seven centres worldwide were patch tested with fragrance mix (FM) and its constituents (including cinnamaldehyde at 1% in petrolatum).

Description of patch test as cited from Larsen et al., 1996: *"The test materials were applied to Finn chambers (Epitest Ltd, Oy, Helsinki, Finland) that were applied to the upper back.[7] The chambers were then further secured to the skin with Scanpor tape (Norgesplaster A/S, Aksjeselskap, Finland). Fifteen to 45 minutes were allowed between the initial patch test removal and the first reading to allow the pressure effect of the patch test appliance to resolve so as not to mask faint responses. The patch test sites were evaluated using the North American Contact Dermatitis Group modification [11] of the International Contact Dermatitis Research Group morphological grading systcm.[12] The patch test sites were evaluated initially at 48 or 72 hours. The test sites were re-examined in the majority of cases, usually between 48 and 120 hours after the first reading. All test site readings were made by the investigators.*"

Statistical analysis of the data was performed using the Statistical Analysis System (release 6.07, SAS Institute, Cary, NC, USA).

The results of the study showed that 14.4% of the 167 selected fragrance sensitive volunteers were tested positive for 1% cinnamaldehyde in petrolatum.

3.1.2.18 STUDY 18-19 (Patch test, selected)

Study reference:

Johansen, J.D., Menne, T.: The fragrance mix and its constituents: a 14-year material. Contact Dermatitis 32, 18-23, 1995.

Detailed study summary and results:

Test type

This study is a review of results from 14 years of patch testing with the fragrance mix (FM) and its constituents and includes 8215 consecutive patients patch tested with FM between 1979 and 1992 at the Department of Dermatology in Gentofte, Denmark. Individual FM constituents were tested in a total of 367 selected patients reacting to the fragrance mix between 1979 and 1992. Of these 105 were tested with 2% cinnamaldehyde in petrolatum, 1979-1983 and 160 were tested with 1% cinnamaldehyde in petrolatum, 1988-1992.

Description of patch test as cited from Johansen and Menné 1995: "*The patches were occluded using Finn Chambers affixed with Scanpor tape." ..." The test substances were applied to the upper back for 2 days. Readings were made on the 2nd, 3rd and 5th- 7th days. In 1987, the scale of readings was adjusted to conform with ICDRG recommendations; before that, a less rigorous scale was used, defining a positive reaction as a definite erythema.*"

The results of the study showed a significant decrease in the frequency of reaction to cinnamaldehyde at the same time as the test concentration was reduced from 2% to 1% pet.

Frequency of positive reactions to cinnamaldehyde

	Patients age 15-34	Patients age 35-60	Patients age >60
2% Cinnamaldehyde in pet. (1979-1983)	32.5%	31.6	30.8
1% Cinnamaldehyde in pet. (1988-1992)	9.1%	12.8	10.4

3.1.2.19 STUDY 20 (Patch test, selected)

Study reference:

de Groot, A.C., van der Kley, A.M., Bruynzeel, D.P., Meinardi, M.M., Smeenk, G., van Joost, T., Pavel, S.: Frequency of false-negative reactions to the fragrance mix. Contact Dermatitis 28, 139-140, 1993.

Detailed study summary and results:

Test type

The purpose of the study by de Groot et al., was to determine the frequency of false-negative reactions to fragrance mix (FM). Between September 1991 and December 1991 a total of 677 patients were patch tested with FM. Out of the 677 tested patients a total 61 patients were positive to FM. Cinnamaldehyde (2%) as a single constituent was tested in the FM positive patients.

The method of patch testing was not described by de Groot et al., 1993.

The results of the study showed that 34% of the selected FM positive patients (21/61) were tested positive for cinnamaldehyde at 2% in petrolatum.

3.1.2.20 STUDY 21 (Patch test, selected)

Study reference:

Enders, F., Przybilla, B., Ring, J.: Patch testing with fragrance mix at 16% and 8%, and its individual constituents. Contact Dermatitis 20, 237-238, 1989

Detailed study summary and results:

Test type

Enders et al., reports a study of 1845 patients patch tested with a fragrance mix in 1987 at the Dermatologische Klinik und Poliklinik, Germany. A total of 162 of the tested patients with a positive reaction to the fragrance mix were retested with the individual constituents including cinnamaldehyde at 1% (vehicle not reported).

The method of patch testing was not described by Enders et al., 1989.

The results of the study showed that 21% of the 162 selected fragrance mix positive patients were tested positive for cinnamaldehyde at 1%.

3.1.2.21 STUDY 22 (Patch test, selected)

Study reference:

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

As *cited in* Opinion concerning Fragrance Allergy in Consumers. A Review of the Problem. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Adopted 8. December 1999 (SCCNFP 1999).

Detailed study summary and results:

Test type

2455 consecutive patients attending patch test clinics in England, Denmark, Spain, France, Germany and Finland were tested to 8% Hermal/Larsen fragrance mix and 9.5% Hausen fragrance mix. When one or the other of the mixes was positive all the individual fragrance compounds contained in the mixes were tested. Patch test technique and readings were as recommended by the ICDRG and, for positive results; an assessment of clinical relevance was also made.

The overall incidence of fragrance sensitivity was 7.8% using Hermal/Larsen mix and 6.7% with the Hausen mix. In 146 patients a direct comparison of the two fragrance mixes could be made: in 32 of these the

reactions were marginal or weak and in 114 the reactions were 1+ or greater. Among the 114 patients with solid reactions to one or other fragrance mix, 78 were tested to individual fragrance materials.

1% Cinnamaldehyde gave positive reactions in 12.8% (10/78).

3.1.2.22 STUDY 23-24 (Patch test, selected)

Study reference:

Santucci, B., Cristaudo, A., Cannistraci, C., Picardo, M.: Contact dermatitis to fragrances. Contact Dermatitis 16, 93-95, 1987

Detailed study summary and results:

Test type

The aim of the study by Santucci et al., 1987 was to evaluate the incidence of contact dermatitis to fragrances in Roma, Italy, and the influence of limited variations in fragrance and perfume mix concentrations on patch test responses. Two large groups of patients with contact dermatitis were patch tested with a range of mixed fragrances including cinnamaldehyde between 1983 and 1984 (n=1200) and 1984 and 1985 (n=1500). A total of 63 and 54 patients were tested positive in the first and second group, respectively. Patients reacting positive to any of the mixed fragrances were tested after 3 months with the individual components of the mix. In the 1983-1984 group the 2% cinnamaldehyde in petrolatum were used and in the 1984-1985 1% cinnamaldehyde in petrolatum were used.

Description of patch test as cited from Santucci et al., 1987: "*Using Finn Chambers on Scanpor'"'. The tests were read at 48, 72 and 96 h, according to the ICDRG scale; the last reading was taken as definitive*."

The results of the study are showed in the table:

Number of tested patients	Number of positives	Percent positive	Test concentration of cinnamaldehyde
63	9	14.3%	2%
54	3	5.6%	1%

3.1.2.23 STUDY 25 (Patch test, selected)

Study reference:

Adams, R.M., Maibach, H.I.: A five-year study of cosmetic reactions. Journal of the American Academy of Dermatology 13, 1062-1069, 1985.

Detailed study summary and results:

Test type

A total of 713 cosmetic related cases were identified among 13216 patch tested contact dermatitis patients from various sections of the United States between 1977 and 1983. To identify the exact cause of their reactions the patients were patch tested with a range of cosmetic ingredients including the cosmetic products

used by the patient. A sub-group of 403 selected patients were patch tested with single ingredients including cinnamaldehyde.

Description of patch test as cited from Adams et al., 1985: "*Patch tests were applied to the upper back for 48 hours according to the methods outlined in the North American Contact Dermatitis Group (4) and the International Contact Dermatitis Group. Readings were made at 48 hours and 72 hours. In most centres, additional readings at 96 hours or 120 hours were also made. The patch test was either the Al test or the Finn Chamber (Hermal Pharmaceutical Labs., Inc., Oak Hill, NY; Allerderm Laboratories, Mill Valley, CA).*"

The results of the study showed that 1.5% of the selected patients (6/403) were tested positive for cinnamaldehyde (vehicle and concentration not specified).

3.1.2.24 STUDY 26 (Patch test, selected)

Study reference:

Malten, K.E., van Ketel, W.G., Nater, J.P., Liem, D.H.: Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 11, 1-10, 1984.

Detailed study summary and results:

Test type

A total of 182 patients with suspected contact sensitisation to cosmetics were patch tested with a series of 22 fragrance and flavour raw materials including cinnamaldehyde at 0.5% in petrolatum.

Description of patch test as cited from Malten et al., 1984: "*The patch test reactions were read at 48 and 72 h; the last reading was recorded as definitive*."

The results of the study showed that 3.7% of the 182 selected patients were tested positive for 0.5% cinnamaldehyde in petrolatum.

3.1.2.25 STUDY 27 (Patch test, selected)

Study reference:

Larsen W. G.: Perfume dermatitis. a study of 20 patients. Archives of Dermatology 113, 623-626, 1977

Detailed study summary and results:

Test type

A total of 20 perfume-sensitive patients were patch tested with several screening sets of fragrance materials including cinnamaldehyde at 1% in petrolatum.

Description of patch test as cited from Larsen 1977: "*The standard patch-testing technique with use of an aluminium-backed strip was employed. Patch tests were applied to the patient's back and were left for 48 hours. Readings were made at the time of removal or 24 hours after removal. Patients were instructed to*

return if an additional delayed reaction occurred. All the fragrance allergens were tested on 50 control patients with negative results. To avoid the "angry back" phenomenon, patients were tested during a period of several months."

The results of the study showed that 30% of the selected patients (6/20) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.26 STUDY 28 (Patch test, unselected/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, 276–281, 2014.

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 (the26 labelled fragrances and trimethylbenzenepropanol, but excluding HICC) were tabulated with spss™ version 12. Data were also collected for patients who reacted to colophonium and epoxy resin. 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 un-oxidized 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 1.38% (27/1951) (95% CI: 0.9-1.9%) of the selective patients had positive reactions for cinnamaldehyde when tested at 1% 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.

3.1.2.27 STUDY 29-31 (Patch test, unselected/consecutive)

Study reference:

Zug K. A., Pham A. K., Belsito D. V., DeKoven J. G., DeLeo V. A., Fowler, J. F. Jr., Fransway A. F., Maibach H. I., Marks J. G. Jr., Mathias C. G. T., Pratt M. D., Denis Sasseville D., Storrs F. J., Taylor J. S., Warshaw E. M., Zirwas M. J.: Patch Testing in Children From 2005 to 2012: Results From the North American Contact Dermatitis Group. DERMATITIS, Vol 25, No 6, 2014

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) has performed a retrospective study of 41 unselected children age 0-5 years, 838 unselected children age 6-18 years and 17 213 unselected adults (> 18 years) patch tested with a total of 87 different allergens, including 1% cinnamaldehyde in petrolatum, between 2005 and 2012.

Description of test method as cited from Zug et al. 2014: *"Deidentified patch test results from patients aged 18 years or younger who were referred on suspicion of having ACD and underwent patch testing between January 1, 2005 and December 31, 2012, by members of the NACDG were retrieved from a central database. This study qualified for an exempt from review from the Committee for the Protection of Human Subjects at Dartmouth-Hitchcock (CPHS no. 24202). During this test period, the NACDG underwent four 2-year cycles of patch testing (2005-2006, 2007-2008, 2009-2010, and 2011-2012) and 4 slightly modified allergen screening series were used for testing. A total of 87 different allergens, of varying chemical composition, delivery vehicles, or concentrations, were tested from 2005 to 2012. The patch testing was performed using a standard series of 65 (2005-2008; Chemotechnique Diagnostics AB, Malmö, Sweden) or 70 (2009-2012; allergEAZE by SmartPractice, Calgary, Alberta, Canada) allergens individually housed in Finn Chambers (SmartPractice, Phoenix, AZ) and applied to the patients' upper back in the standard fashion. At the clinician's discretion and depending on the clinical situation, a patient may have been patch-*

tested with additional supplemental allergens. Details on the number of supplemental allergens tested, if any, are not part of the data set. One or more allergens may have been omitted from testing in an individual patient if the patient had a known history of strongly reacting to that allergen. The patch tests were removed and then evaluated at 48 hours, and second, delayed final reading and interpretation were performed between days 3 and 7 after placement.".

The results for cinnamaldehyde showed that during the period 2005-2012 4.9% of the 41 unselected children age 0-5 years, 1.2% of the 838 unselected children age 6-18 years and 3.0% of the 17 213 unselected adults were tested positive.

3.1.2.28 STUDY 32 (Patch test, unselected/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 cinnamaldehyde. 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 (FM I and FM II), 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. 1503 patients were patch tested with cinnamaldehyde.

Description of patch test as cited from Heisterberg et al., 2011: "*The patch tests were performed according to international guidelines, with Finn Chambers applied on the back with Scanpor tape 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. 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 to oxidized materials, because several studies have shown that it is the oxidized products that cause allergy. 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. 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 1.3% of the consecutive patients (20/1503) were tested positive for cinnamaldehyde at 1% in petrolatum. 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.

3.1.2.29 STUDY 33 (Patch test, unselected/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, 61, 217–223, 2009.

Detailed study summary and results:

Test type

The Department of Dermatology, University of Groningen, the Netherlands performed a prospective study of patients with eczema 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, Milennium 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 1.6% of the unselected eczema patients (5/320) had positive reactions to cinnamaldehyde when tested at 1% in petrolatum.

3.1.2.30 STUDY 34 (Patch test, unselected/consecutive)

Study reference:

Zug K. A., Warshaw E. M., Fowler Jr J. F., Maibach H. I., Belsito D. L., Pratt M. D., Sasseville D., Storrs F. J., Taylor J. S., Mathias C. G. T., DeLeo V.A., Rietschel R. L.: Patch-Test Results of the North American Contact Dermatitis Group 2005–2006. Dermatitis, Vol 20, No 3, pp 149–160, 2009.

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) performed patch test op 4 454 unselected patients with 26 different allergens, including 1% cinnamaldehyde in petrolatum, between January 1. 2005, and December 31. 2006. Results were compared to previous test cycles (including 2003-2004).

Description of test method as cited from Zug et al. 2009: "*Sixty-five allergens (Chemotechnique Diagnostics AB, Malmo¨, Sweden) were tested by the 13 members of the NACDG in 2005 and 2006. Patch testing was performed with a standardized technique using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster Alpharma A/S, Vennesla, Norway). Patches were left in place for 48 hours. First and second patch-test readings were performed at 48 to 72 hours and 72 to 168 hours, respectively, after initial patch-test placement. Allergic patch-test reactions were graded as +, ++, or +++, based on the intensity of positive reactions manifested by erythematous papules, vesicles, or a spreading reaction (sometimes with crusting and ulceration). Doubtful reactions (macular erythema) were generally coded as nonallergic reactions. If the clinical history suggested relevance, or if other positive reactions to the same allergen but in a different vehicle were found, or if a cross-reacting substance was identified, the investigator had the discretion to make the final determination that the macular erythema represented an allergic reaction. Irritant and allergic reactions were differentiated by each investigator, who considered the morphology and timing of the reaction at each reading.*".

The results for cinnamaldehyde showed that during the period 2005-2006 3.1% of the 4 435 unselected patients were tested positive. These 4435 patients are also included in the retrospective 2005-2012 study, Zug et al. (2014), and does therefore not have a separate entrance in the CLH report under Zug et al., 2009. Zug et al., 2009 does, however, also give results for the 2003-2004 test cycle were 2.4% of 5 138 unselected patients had positive patch test reactions for cinnamaldehyde. These results are included in the CLH report.

3.1.2.31 STUDY 35-40 (Patch test, unselected/consecutive)

Study reference:

Nguyen S. H., Dang T. P., MacPherson C., Maibach H., Maibach H. I.: Prevalence of patch test results from 1970 to 2002 in a multi-center population in North America (NACDG). Contact Dermatitis, 58, 101–106, 2008

Detailed study summary and results:

Test type

The North American Contact Dermatitis Group (NACDG) conducted a retrospective study on more than 34000 unselected allergic contact dermatitis (ACD) patients patch tested between 1970 and 2002. The number of patients tested with 1% cinnamaldehyde (in petrolatum according to Zug et al., 2009) was: year 1984-1985: 1199 patients; year 1985-1989: 3964 patients; year 1992-1994: 3528 patients; year 1994-1996: 3112 patients; year 1996-1998: 3443 patients and year 1998-2000: 4735 patients.

Description of patch test as cited from Nguyen et al., 2007: "*The patients were patch tested in a standardized manner as outlined previously (1–8), using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster Aksjeselskap, Venessia, Norway) applied to the back. Allergens were purchased from Hermal Pharmaceutical Laboratories, Inc. (Delmar, NY, USA) or Chemotechnique Diagnostics AB (Malmo, Sweden). The patches remained in place for 2–3 D and read at 3–7 D after placement. Patch test reactions were interpreted to be a 1+, 2+, or 3+ reaction manifested by erythematous papules, vesicles, or a spreading reaction with crust and ulceration (1–8).*".

The number and percentage of unselected ACD patients tested positive with 1% cinnamaldehyde in petrolatum can be seen in the table:

	1984-1985	1985-1989	1992-1994	1994-1996	1996-1998	1998-2000
Positive	1199	3964	3528	3112	3443	4735
%	5.9	3.1	2.7	2.4	2.8	3.6

3.1.2.32 STUDY 41 (Patch test, unselected/consecutive)

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, 57, 1–10, 2007.

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 and the German Contact Dermatitis Research Group (DKG). 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, further refined by the German Contact Dermatitis Group: 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 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)."

The results showed that 1.0% (95% CI: 0.5-1.5%) of the consecutive patients (21/2063) were tested positive for cinnamaldehyde at 1% in petrolatum.

3.1.2.33 STUDY 42 (Patch test, unselected/consecutive)

Study reference:

Belsito D. V., Fowler Jr J. F., Sasseville D., Marks Jr J. G., De Leo V. A., Storrs F. J: Delayed-Type Hypersensitivity to Fragrance Materials in a Select North American Population. Dermatitis, Vol 17, No 1: pp 23–28, 2006

Detailed study summary and results:

Test type

Belsito et al. conducted a prospective study of 1603 selected patients with eczematous dermatitis patch tested with the North American Contact Dermatitis Groups (NACDG), screening tray (including cinnamaldehyde) addition to HMPCC.

Description of patch test as cited from Belsito et al., 2006: "*Patients were patch-tested in a uniform manner as previously described.(13) They returned for patch-test evaluation at 48 hours after the initial application and for a second evaluation 4 to 7 days after the initial application. Results were assigned scores of 1 to 6, based on reaction morphology as previously described.(13) Patients were considered allergic to a fragrance material if they had a +, ++, or +++ reaction.*"

The results of the study showed that 1.7% (27/1603) of the selected patients was tested positive for cinnamaldehyde.

3.1.2.34 STUDY 43 (Patch test, unselected/consecutive)

Study reference:

Schnuch, A., Geier, J., Uter, W., Frosch, P.J.: Another look at allergies to fragrances: Frequencies of sensitisation to the fragrance mix and its constituents. Results from the Information Network on Departments of Dermatology (IVDK). Exogenous Dermatology 1, 231-237, 2002

Detailed study summary and results:

Test type

The IVDK (InformationsVerbund Dermatologischer Kliniken) 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 1996-1999, fragrance mix (FM) (including cinnamaldehyde) was tested in a total of 35599 unselected patients and its single constituents were tested at 1% in petrolatum in a subgroup of 4900 patients.

Description of patch test as cited from Schnuch et al., 2002: "*The multicentre project 'Information Network of Departments of Dermatology' ('Informationsverbund dermatologischer Kliniken', IVDK) is an instrument of epidemiological surveillance of contact allergy and has been described in detail elsewhere [2, 8, 9]. Basically, patch tests are performed in accordance with the recommendations of the ICDRG, the International Contact Dermatitis Research Group [10] and the DKG, the German Contact Dermatitis Research Group [11]. Patch test material is obtained from Hermal/Reinbek, Germany, and applied for 24 or 48 h. Readings are performed until at least 72 h. All patch test results and a standardised history of all patients tested in the participating centres (see footnote) are recorded and transferred to the data centre in Göttingen.*"

The results showed that 1.9% of the 4900 unselected patients were tested positive for cinnamaldehyde (1% in pet.).

3.1.2.35 STUDY 44-45 (Patch test, unselected/consecutive)

Study reference:

Frosch, P.J., Pilz, B., Burrows, D., Camarasa, J.G., Lachapelle, J.M., Lahti, A., Menné, T., Wilkinson, J.D.: Testing with fragrance mix. Is the addition of sorbitan sesquioleate to the constituents useful? Results of a multicentre trial of the European Environmental and Contact Dermatitis Research Group (EECDRG). Contact Dermatitis 32, 266-272,1995a.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 709 patients tested in 7 centres located in Europe was performed. The study involved testing of two types of fragrance mix (FM), its 8 constituents with 1% sorbitan sesquioleate (SSO) and its 8 constituents without SSO and 20% SSO. The concentration of cinnamaldehyde was 1% when tested as individual constituent.

Description of patch test as cited from Frosch et al., 1995a: *"The series was applied for 2 days to the back with Finn Chambers on Scanpor tape. Readings were made at 2 and 3 days (4 days in some centres), according to published guidelines (3). 7 centres participated in the study: Amersham in England (100 patients), Barcelona in Spain (100 patients), Belfast in Northern Ireland (100 patients), Brussels in Belgium (100 patients), Hellerup in Denmark (124 patients), Oulu in Finland (85 patients) and Dortmund in Germany (100 patients). The patients were unselected consecutive patients patch tested because of suspected contact dermatitis.*"

The results showed that 0.98% (7/702) reacted to the emulsifier 20 % SSO itself. Furthermore, 0.85% (6/702) and 0.14% (1/702) of the unselected patients were tested positive for cinnamaldehyde (1%) with and without SSO, respectively.

3.1.2.36 STUDY 46 (Patch test, unselected/consecutive)

Study reference:

Frosch, P.J., Pilz, B., Andersen, K.E., Burrows, D., Camarasa, J.G., Dooms-Goossens, A., Ducombs, G., Fuchs, T., Hannuksela, M., Lachapelle, J.M., Lahti, A., Maibach, H.I., Menné, T., Rycroft, R.J.G., Shaw, S., Wahlberg, J.E., White, I.R., Wilkinson, J.D.: Patch testing with fragrances: results of a multicentre study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. Contact Dermatitis 33, 333-342, 1995b.

Detailed study summary and results:

Test type

A prospective multicentre study involving a total of 1323 patients tested in 11 centres located in Europe was performed. The study involved testing of 48 frequently used constituents of perfumes, as well as patch testing with a standard series fragrance mix (FM) containing cinnamaldehyde. In 9 centres 1072 patients were patch tested with 1% cinnamaldehyde in pet. with 1% sorbitan sesquioleate (SSO)).

Description of patch test as cited from Frosch et al., 1995b: *"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 patient 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 0.93% (10/1072) of the unselected patients from a total of 9 European centres were patch tested positive for cinnamaldehyde at 1% in petrolatum with SSO.

3.1.2.37 STUDY 47 (ROAT)

Study reference:

Bruze, M., Johansen J. D., Andersen K. E., Frosch P., Lepoittevin J-P., Rastogi S., Wakelin S., White I., Menne T.: Deodorants: An experimental provocation study with cinnamic aldehyde. Journal of the American Academy of Dermatology 48, Number 2, 2003

Detailed study summary and results:

Test type

A clinical study were conducted involving 17 cinnamaldehyde-allergic patients and 20 healthy controls who were tested with a dilution series of cinnamaldehyde in a patch test and a use test - a repeated open application test (ROAT). The aim of the study was to investigate the significance of cinnamaldehyde in deodorants for the development of axillary dermatitis. For the patch test 2.0%, 1.0%, 0.5%, 0.25%, 0.125%, 0.063%, 0.031%, 0.016%, 0.008%, 0.004%, 0.002%, 0.001%, 0.0005%, 0.00025%, 0.00012%, and 0.00006% cinnamaldehyde in ethanol were used. In the first part of the ROAT 8 patients were exposed to unscented and scented deodorants at 3 concentrations (0.32%, 0.1%, and 0.032% wt/vol) in the axilla.

On the basis of the results of the first part of the study, deodorants with cinnamaldehyde at 0.1%, 0.032%, and 0.01% wt/vol were chosen for the second part were 9 patients were exposed in the axilla. Except for the content of cinnamaldehyde, the scented and unscented deodorants were identical with water, aluminium chlorohydrate, polypropyleneglycol-15, stearyl ether, steareth-2, steareth-21, dichlorobenzyl alcohol, and phenoxyethanol as the ingredients.

Description of patch test as cited from Bruze et al., 2003: "*The Finn Chamber technique was used. On each patch unit mounted on Scanpor tape, 15 µL of the respective test solution was applied. The patches were removed from the back after 48 hours and readings took place on day 3 and day 7 according to International Contact Dermatitis Research Group guidelines. Each test patient was tested with 15 ethanol solutions of cinnamic aldehyde, ethanol, and the unscented and scented deodorants. For those having reacted previously with a +++ reaction to cinnamic aldehyde the testing started at 1.0% and for all other test patients the testing started at 2.0%. Besides testing with the unscented deodorant and ethanol, the control patients were only tested with cinnamic aldehyde at 1.0%. The threshold of sensitivity (the minimal eliciting concentration [MEC]) was defined as the lowest concentration eliciting at least a + reaction (16) The positive test reactions were not always continuous. When the number of negative reactions, doubtful reactions, or both*

were followed by the same number or more of positive reactions, the lowest positive concentration was registered as the MEC. In all other situations, the concentration above the first negative or doubtful reaction was registered as the MEC (16).”

Description of use test as cited from Bruze et al., 2003: *“The use test was done as an ROAT (12) using the axillae as test sites. A pair of deodorants were always used with 1 unscented and 1 scented, which were applied twice daily to the respecive axilla that were randomly chosen for each participant. The deodorant to be used in the left axilla always had a red label and the deodorant for the right axilla, a blue label. Evaluation of the ROAT was made once a week, or at the request of the patient, with inspection including assessment of the following morphologic features: erythema, infiltration, papules, vesicles, and scaling. The involved area with dermatitis and the overall impression of the use-test reaction were also assessed (17).”*

Results of the patch test were that all 17 patients had at least 1 positive reaction to cinnamaldehyde. The lowest concentration that gave positive reactions was 0.002% and the highest were 2.0% in ethanol.

Results of the ROAT test were that 8/8 patients in the first part of the study and 8/9 patients in the second part of the study gave positive reactions in the axilla when tested with cinnamaldehyde in deodorants. Positive reactions were seen in 1/9 patients at the lowest concentration teste 0.01%.

3.1.2.38 STUDY 48 (ROAT)

Study reference:

Johansen J. D., Andersen K.E., Rastogi S.C., Menne T.: Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. Contact Dermatitis, 34, 165-171, 1996

Detailed study summary and results:

Test type

A clinical study conducted at Gentofte Hospital and Odense University Hospital, Denmark involved 22 cinnamaldehyde-allergic patients and 20 healthy controls who were tested with a dilution series of cinnamaldehyde in a patch test and a repeated open application test (ROAT). The aim of the study was to provide quantitative information on the eliciting capacity of cinnamaldehyde to be considered in assessment of clinical relevance and health hazard. For the patch test 2%, 1%, 0.5%, 0.1%, 0.05%, 0.02% and 0.01% cinnamaldehyde in petrolatum were used along with 0.02%, 0.1% and 0.8% in ethanol. For the 6 week graded ROAT use test 0.8%, 0.1% and 0.02% cinnamaldehyde in ethanol were used. Ethanol and petrolatum were included as vehicle controls.

Description of patch test as cited from Johansen et al., 1996: “*The eczema patients were patch tested with coded concentrations of cinnamaldehyde applied to the upper back in a random order, changing for each patient. The control persons were tested with fragrance mix 8% pet. only. Scanpor® tape and Finn Chambers® were used. The patches were left in place for 2 days. Blind readings were done at D2, D3 and*

D7 in Gentofte and at D3 and D7 in Odense. The reactions were scored according to ICDRG scale (11). The threshold response was definded as the weakest concentration giving a visible skin reaction in a continuous line of patch test reactions starting with 2% pet."

Description of use test as cited from Johansen et al., 1996: *"The use test was done as a repeated open application test (ROAT) (14), using the outer aspect of the upper arm as test site. The test area was 5X5 cm. The cinnamic aldehyde solution was applied on one arm and the vehicle ethanol as control on the other. The solutions were coded and the solution of cinnamic aldehyde was, in a random, blinded manner, used in half the patients on the right and the other half on the left arm. An atomizer pump giving 0.05 ml per stroke was used for applications (15). The volunteers were instructed to use 0.1 ml 2X a day. The number of applications made by all persons were recorded, and the containers were weighted every 2 weeks.*

The use test was done with graded concentrations of cinnamic aldehyde. For the 1st 2 weeks, 0.02% cinnamic aldehyde was applied, for the next 2 weeks 0.1% and for the last 2 weeks 0.8%. The patients were asked to report if visible skin symptoms occurred at the test sites. The applications were continued until at least erythema was present or a week had passed form the first symptoms. Subjects with persistent skin reactions at the site of cinnamic aldehyde application and a negative control site were classified at positive no matter what the degree of reaction."

Results of the patch test were that 18/22 had at least 1 positive reaction to cinnamaldehyde and 4/22 had doubtful reactions. The lowest threshold concentration (minimum effect level) was 0.02%. Results of the ROAT use test were that 8 patients reacted to 0.1% and 5 to 0.8% cinnamaldehyde in ethanol. None reacted to 0.02% cinnamaldehyde in ethanol.

A total of 13/18 of the patients with a clearly positive patch test reaction to cinnamaldehyde (2% in pet.) also developed a positive reaction in the ROAT test. The 4 patients with doubtful response on patch test to cinnamaldehyde (2% in pet.) were all negative in the ROAT test.

3.1.2.39 STUDY 49 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2004. Repeated insult patch test of cinnamaldehyde. RIFM report number 47158, November 22a. (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 3 in the publicly available part of the REACH registration (

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 94 volunteers (25 male and 69 female) using 0.5% cinnamaldehyde in 3:1DEP:EtOH.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 0.5% cinnamaldehyde in 3:1DEP:EtOH was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal.*"

The results showed that no sensitization reactions were observed in the 94 volunteers when tested with 0.5% cinnamaldehyde in 3:1DEP:EtOH.

3.1.2.40 STUDY 50 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2003a. Topical photoallergy screening test of cinnamaldehyde and cinnamic acid in male albino hairless guinea pigs (Crl: IAF(HA)-hrBR (Outbred), including primary irritation, phototoxicity and contact hypersensitivity evaluations. RIFM Report Number 41273, January 15 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 4 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 28 volunteers using 3% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 3% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol added to prevent peroxide formation) was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff*

except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers® was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal. The same procedure was repeated using 3% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol added)."

Sensitization reactions were observed in 14% (4/28) of the volunteers exposed to 3% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol. Two irritation reactions were observed in the 28 volunteers.

The 3% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol added) study was aborted during the induction phase due to the number of irritant reactions (8/30) observed with this vehicle.

3.1.2.41 STUDY 51-52 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 2002. Repeated insult patch test of cinnamaldehyde. RIFM report number 41692, August 27 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 7 and 8 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 22 volunteers using 0.5% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol) and on 19 volunteers using 0.5% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% α-tocopherol).

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.3 ml aliquot of 0.5% cinnamaldehyde in 3:1DEP:EtOH (with 0.5% α-tocopherol added to prevent peroxide formation) was applied to 25 mm Hilltop Chambers® and volatilized for a 15–40 min period. Patches were applied to the subjects skin between the left scapula and spinal mid-line for 24 h under occlusion. Induction applications were made to the same skin site (unless reactions became so strong that an adjacent site had to be employed) on a Monday–Wednesday–Friday schedule for three consecutive weeks. All patches were applied and removed by the laboratory staff except on Saturday when the individual subjects were instructed to remove them approximately 24 h after application. Reactions were read 24 or 48 h after patch removal. Following a two-week rest period, a 24-h challenge patch using 25 mm Hilltop Chambers® was applied to a previously unpatched (virgin) site. Reactions to challenge were read at 24, 48, 72 and 96 h after patch removal. The same procedure was*

repeated using 0.5% cinnamaldehyde dissolved in a 3:1EtOH:DEP vehicle (with 0.5% a-tocopherol added)."

Sensitization reactions were observed in 0% (0/22) of the volunteers exposed to 0.5% cinnamaldehyde in 3:1DEP:EtOH with 0.5% α-tocopherol. No irritation reactions were observed in the 22 volunteers.

Sensitization reactions were observed in 0% (0/19) of the volunteers exposed to 0.5% cinnamaldehyde in 3:1EtOH:DEP with 0.5% α-tocopherol. No irritation reactions were observed in the 19 volunteers.

3.1.2.42 STUDY 53-56 (HRIPT)

Study reference:

Danneman, P.J., Booman, K.A., Dorsky, J., Kohrman, K.A., Rothenstein, A.S., Sedlak, R.I., Steltenkamp, R.J., Thompson, G.R., 1983: Cinnamic aldehyde: a survey of consumer patch-test sensitization. Food and Chemical Toxicology 21, 721–725.

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Detailed study summary and results:

Human Repeat Insult Patch Tests (HRIPT) was conducted with cinnamaldehyde in ethanol on a total of 130 volunteers. 41 volunteers were tested with 0.1% cinnamaldehyde in ethanol, 38 volunteers were tested with 0.5% cinnamaldehyde in ethanol, 41 volunteers were tested with 1% cinnamaldehyde in ethanol and 10 volunteers were tested with 1.25% cinnamaldehyde in ethanol.

No reactions were observed when 0.1% cinnamaldehyde was tested in 41 volunteers or when 0.5% cinnamaldehyde was tested in 38 volunteers. 1.0% cinnamaldehyde produced 5/41 questionable reactions (subjects reacted at the induction site and not at the primary challenge site) and 5/10 reactions were observed with 1.25% cinnamaldehyde.

3.1.2.43 STUDY 57-58 (HRIPT)

Study reference:

Marzulli, F.N., Maibach, H.I., 1980. Contact allergy: Predictive testing of fragrance ingredients in humans by Draize and maximization methods. Journal of Environmental Pathology and Toxicology 3, 235–245.

and

Marzulli, F.N., Maibach, H.I., 1976. Effects of vehicles and elicitation concentration in contact dermatitis testing. I. Experimental contact sensitization in humans. Contact Dermatitis 2, 325–329.

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Detailed study summary and results:

Test type

Study summary as cited in Cocchiara et al., 2005: "*Using a modified Draize procedure, Marzulli and Maibach (1976) reported the effects of using two different vehicles to test cinnamaldehyde. A total of 108 volunteers were tested with cinnamaldehyde, 55 were tested with 1% cinnamaldehyde in 95% ethanol and 53 were tested with 1% cinnamaldehyde in petrolatum.*"

Description of test procedure as cited in Cocchiara et al., 2005: "*Each subject received ten 48-h (72 h on weekends) occluded applications, which were made 3 times a week to the same site. Two weeks after induction, a 72-h occluded challenge application was made to a new site.*"

1/55 sensitization reactions were observed in volunteers tested with 1% cinnamaldehyde in 95% ethanol.
No sensitization reactions were observed in 53 volunteers tested with 1% cinnamaldehyde in petrolatum.

3.1.2.44 STUDY 59 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1973b. Repeated insult patch test. Unpublished report from IFF Incorporated, 23 January. Report number 12509 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 9 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 41 volunteers using 1% cinnamaldehyde in alcohol SDA 39C.

Description of HRIPT as cited in Cocchiara et al., 2005: "*A 0.5 ml aliquot was applied to semiocclusive patches, which were then applied to the upper arm of each subject for 24 h. After a 24–48 h rest period, subjects were again patched at the same site. A total of nine induction applications were made over a three week period. Approximately two weeks after the last induction patch, a 24-h semi-occluded challenge patch was applied to the same site and to a site not previously exposed. Reactions to challenge were read at 24 and 72 h after patch removal.* "

Sensitization reactions were observed in 12% (5/41) of the volunteers exposed to 1% cinnamaldehyde in alcohol SDA 39C. No irritation reactions were observed in the 41 volunteers.

3.1.2.45 STUDY 60 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1965. Repeated insult patch test. Unpublished report from IFF Incorporated, 1October. Report number 12508 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 6 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 38 volunteers using 0.5% cinnamaldehyde in ethanol.

Description of HRIPT as cited in Cocchiara et al., 2005: *"A 0.5 ml aliquot was applied to semiocclusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 h after application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."*

Sensitization reactions were observed in 0% (0/38) of the volunteers exposed to 0.5% cinnamaldehyde in ethanol. No irritation reactions were observed in the 38 volunteers.

3.1.2.46 STUDY 61 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1964a. Repeated insult patch test. Unpublished report from IFF Incorporated, 3 April. Report number 12511 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 10 in the publicly available part of the REACH registration.

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on 10 volunteers using 1.25% cinnamaldehyde in ethanol.

Description of HRIPT as cited in Cocchiara et al., 2005: *"A 0.5 ml aliquot of 1.25% cinnamaldehyde in ethanol was applied to semi-occlusive patches, which were then applied to the upper arm of each subject. These patches were removed 24 h after application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."*

Sensitization reactions were observed in 50% (5/10) of the volunteers exposed to 0.5% cinnamaldehyde in ethanol. No irritation reactions were observed in the 10 volunteers.

3.1.2.47 STUDY 62 (HRIPT)

Study reference:

Unpublished report by the Research Institute for Fragrance Materials (RIFM), 1964b. Repeated insult patch test. Unpublished report from IFF Incorporated, 29 July and 25 November. Report number 12510 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 5 in the publicly available part of the REACH registration.

Detailed study summary and results:

Test type

Human Repeat Insult Patch Tests (HRIPT) was conducted on in total 41 volunteers using 0.125% cinnamaldehyde in ethanol. The study was conducted in two phases. In the first phase 31 male and female volunteers were tested and in the second phase 10 female volunteers were tested

Description of the first phase HRIPT as cited in Cocchiara et al., 2005: *"In the first phase on 31 male and female volunteers, a 0.5 ml aliquot of 0.125% cinnamaldehyde in ethanol was applied to semi-occlusive patches which were then applied to the upper arm of each subject. These patches were removed 24 h after*

application. After a 24–48 h rest period, subjects were again patched at the same site. Reactions were read 24–48 h after patch removal just prior to application of the next patch. A total of nine applications were made over a three-week period on a Monday–Wednesday–Friday schedule. Approximately two weeks after the last induction patch, a semi-occluded challenge patch was applied to a site not previously exposed and removed after 24 h. Reactions to challenge were read at 24 and 72 h after patch removal."
Description of the second phase HRIPT as cited in Cocchiara et al., 2005: *"In the second phase, 0.125% cinnamaldehyde in ethanol produced no sensitization reactions in 10 female volunteers after nine 24-h semi-occluded induction applications followed approximately two weeks later by a 24-h semi-occluded challenge patch."*

Sensitization reactions were observed in 0% (0/41) of the volunteers exposed to 0.125% cinnamaldehyde in ethanol. No irritation reactions were observed in the 41 volunteers.

3.1.2.48 STUDY 63 (HMT)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), 1974a. Report on human maximization studies. RIFM report number 1779, August 22 (RIFM, Woodcliff Lake, NJ, USA).
As cited in:
Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 2 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

A Human Maximization Test (HMT) was conducted with 3% cinnamaldehyde in butylene glycol on 25 healthy, male and female volunteers.
Description of the HMT as cited in Cocchiara et al., 2005: "*Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Reactions were read at patch removal and again 24 h after patch removal.*"

Sensitization reactions were observed in 12% (3/25) of the volunteers exposed to 3% cinnamaldehyde in butylene glycol.

3.1.2.49 STUDY 64 (HMT)

Study reference:

Unpublished reports by the Research Institute for Fragrance Materials (RIFM), 1973c. Report on human maximization studies. RIFM report number 1802, October 10 (RIFM, Woodcliff Lake, NJ, USA).

As cited in:

Cocchiara J., Letizia C.S., Lalko J., A. Lapczynski, Api A.M.: Fragrance material review on cinnamaldehyde. Food and Chemical Toxicology 43, 867–923, 2005.

Study no. 1 in the publicly available part of the REACH registration

Detailed study summary and results:

Test type

A Human Maximization Test (HMT) was conducted with 2% cinnamaldehyde in petrolatum on 25 healthy male volunteers.

Description of the HMT as cited in Cocchiara et al., 2005: *"Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Following a ten-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Reactions were read at patch removal and again 24 h after patch removal."*

Strong to severe sensitization reactions were observed in 44% (11/25) of the volunteers exposed to 2% cinnamaldehyde in petrolatum.

3.1.2.50 STUDY 65 (Case study)

Study reference:

Guarneri F.: Occupational allergy to cinnamal in a baker. Contact Dermatitis 63, 294–294, 2010.

Detailed study summary and results:

Test type

After having changed his workplace and work habits, switching from production of bread to the preparation of sweet bakery goods, itching eczematous hand lesions were reported for a 33-year old baker. His work required to knead many ingredients, including cinnamon.

Patch tests were performed with the Italian Society of Allergological, Occupational and Environmental Dermatology baseline series, the bakers series, latex and dust mites, in Hayes' chambers. Readings at D2 and D4 according to International Contact Dermatitis Research Group guidelines showed sensitization to fragrance mix I and cinnamaldehyde.

With correct use of individual protection devices (latex, nitrile or polyvinylchloride gloves), resolution of the lesions occurred in about 4 weeks, with no relapses over 6 months.

3.1.2.51 STUDY 66 (Case study)

Study reference:

Decapite T.J., Anderson B. E.: Allergic contact dermatitis from cinnamic aldehyde found in an industrial odour-masking agent. Contact Dermatitis 51, 311–322, 2004

Detailed study summary and results:

Test type

A 47-year-old man suffered from dermatitis of his hands, feet, face and body. He routinely handled a powder used to mask the vinyl odour from vinyl covers used for car seat upholstery. The powder contained cinnamaldehyde. Patch testing with the North American Contact Dermatitis Group standard series was performed. The day 2 readings showed positive reactions to cinnamaldehyde and North American Contact Dermatitis Group standard series.

3.1.2.52 STUDY 67 (Case study)

Study reference:

Diba V. C., Statham B. N.: Contact urticaria from cinnamal leading to anaphylaxis. Contact Dermatitis 46, 115–119, 2003

Detailed study summary and results:

Test type

A 42-year old woman nurse had rash on her arms. She continued to experience irritation developing on her arms at work. A natural rubber latex prick test was negative. She was patch tested to the European standard series, medicaments series, latex gloves and glutaraldehyde. At D4, however, a + reaction to fragrance mix was seen. She was therefore patch tested to the constituents of fragrance mix, which were applied for just 20 min. A strong urticarial reaction was seen to cinnamaldehyde and after 40 min. she developed widespread pruritus and erythema, and 5 min later, started to feel faint. A blood pressure reading was unrecordable. She was treated with 10mg chlorphenamine maleate and 1mg adrenaline intramuscularly and made a good recovery. Review of the 20-min test sites at D4 identified a ++ reaction to cinnamaldehyde. All other tests were negative. It was concluded that she had immediate, as well as delayed, hypersensitivity to cinnamaldehyde and that this constituent of the fragrance mix was the most likely cause of the anaphylaxis.