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:
eugenol; 2-methoxy-4-(prop-2-en-1-yl)phenol

EC Number: 202-589-1

CAS Number: 97-53-0

Index Number: Not available

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1 PHYSICAL HAZARDS

Physical hazards not assessed in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Toxicokinetics not assessed in this dossier.

3 HEALTH HAZARDS

Skin sensitisation was the only hazard class assessed for this dossier.

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 Study 1 A+B (Study 1A: LLNA:BrdU-ELISA. Study 1B *ex vivo* BrdU LLNA)

Study reference:

Williams, W. C., Copeland, C., Boykin, E., Quell, S. J., & Lehmann, D. M. (2015). Development and utilization of an ex vivo bromodeoxyuridine local lymph node assay protocol for assessing potential chemical sensitizers. *Journal of Applied Toxicology*, *35*(1), 29-40.

Detailed study summary and results:

Test type

Study no. 1A: LLNA:BrdU-ELISA – according to the ICCVAM protocol (2010), comparable to the OECD TG 422B.

Study no. 1B: *ex vivo* BrdU LLNA – no OECD guideline exist.

Deviations from the protocol in the *in vivo* LLNA:BrdU-ELISA: Different mice strain than preferred in TG and injection of BrdU on day 6 with termination on day 7.

The test animals, administration/exposure is equal to the procedure of the *in vivo* LLNA:BrdU-ELISA – accordingly to the ICCVAM 2010 protocol which is comparable to the OECD TG 442B. The *ex vivo* method deviates from the LLNA:BrdU-ELISA test on day 6. The animals in the *ex vivo* study is sacrificed on D6, whereas animals in the *in vivo* LLNA:BrdU-ELISA are injected with BrdU and sacrificed on day 7.

The *ex vivo* bromodeoxyuridine labelling are performed on D6, immediately after scarifying the lymph nodes, draining the ears were harvested and placed in room temperature with HEPES and ML-glutamine supplemented with 10% foetal bovine serum and 2% penicillin/streptomycin. Lymph nodes were processed into single cell suspensions. After counting, 3×105 live cells (in 100µl volume) were plated in duplicate wells of a 96-well plate. Cells were incubated in the presence of BrdU for 8–12h. BrdU-labelled cells were

adhered to the plate by centrifugation and then dried to the plate at 60°C for 1h. After drying, the plates were stored at 4°C until assessment of BrdU incorporation by ELISA.

GLP compliance: not reported.

Test substance

Eugenol (Sigma Aldrich, St. Louis, MO, USA). No other identification of the substances was reported (e.g. CAS/EC/batch no.).

Prepared in acetone/olive oil (4:1, unit not reported) (AAO; Sigma Aldrich).

Positive control (PC) was trimellitic anhydride (TMA), prepared in AAO.

Purity: No information on purity available.

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

Test animals

Mice (BALB/c), female.

Six animals per dose group.

Age at study initiation: 8-10 weeks (weight not reported).

The animals were housed as described in the OECD TG 442B (2010).

Administration/exposure

Test substance, eugenol, and the PC (TMA) were prepared in (AOO) (4:1) (Sigma Aldrich).
Eugenol was given in three doses: 2, 20 and 40% (w/v).

In both experiments: ex vivo BrdU LLNA and the in vivo LLNA:BrdU-ELISA, the dosing protocol and was followed according to ICCVAM, 2010. Monitoring of dermal irritation potential was conducted for both experiments: Ear was scored for erythema and ear thickness was measured using a digital calliper before dosing on day 1 (D1), D3 and day 6 (D6). Erythema score ≥ 3 were and/or a change in ear thickness >25% was considered as an excessive local dermal irritation.

Mice included in the in vivo LLNA:BrdU-ELISA study were on D6 injected with 0.5 ml of pyrogen-free saline containing 5 mg BrdU (Sigma Aldrich), and scarified on D7 (24 hours after BrdU injection).

Mice included in the ex vivo BrdU LLNA study were sacrificed on D6 followed by ex vivo BrdU labelling.

BrdU ELISA analysis kit and protocol, Roche Diagnostics Corporation, were performed for both ex vivo BrdU LLNA and in vivo LLNA:BrdU-ELISA.

Results and discussion

The endpoint used in the LLNA:BrdU-ELISA is the cellular proliferation induced in draining lymph nodes following the topical exposure to a chemical.

The statistically significance was defined as P 0.05 as evaluated by one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test.

The BrdU labelling index was calculated for each animal, and the SI, 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. The EC2 is the effective concentration of test article to elicit an SI of 2.

LLNA:BrdU-Elisa showed a EC2 of 8.5%.

Ex vivo BrdU LLNA showed an EC2 of 9.5%.

Excessive irritation was not observed for eugenol (determined by ear thickness, erythema score and differentiation index.

The result of the PC TMA is only depictured in graph and no specific EC2 value is reported. It is however reported as positive.

3.1.1.1 Study 2 (*ex vivo* LLNA:BrdU-ELISA)

Study reference:

Ulker, O. C., Ates, I., Atak, A., & Karakaya, A. (2013). 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.

Detailed study summary and results:

Test type

ex vivo LLNA:BrdU-ELISA – no guideline.

GLP compliance: not reported.

Test substance

Eugenol (Sigma). No other identification of the substances was reported (e.g. CAS/EC/batch no.).

Vehicle AOO (4:1, v/v).

Negative control: vehicle.

No PC reported.

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

Test animals

Mice (BALB/c), female.

Age at initiation: 8-12 weeks (weight not reported).

Four animals in each dose group.

Mice were housed in animal facilities maintained at 23°C, relative humidity 55% and 12 hours light/dark cycle. Diet and water *ad libitum*.

Administration/exposure

Test substance, eugenol was prepared in AOO (4:1).

Eugenol was given in four doses: 2.5, 10, 20 and 50% (w/v). Doses were chosen according to previous standard LLNA studies and OECD guideline LLNA test procedure.

Groups of mice (*n*=4) were treated daily with 25 µl of the test substance (eugenol) in vehicle (AOO) or the vehicle alone on dorsum of both ears for three consecutive days. On day (D) 5 the animals were sacrificed, and their auricular lymph nodes were collected. The two lymph nodes from each mouse were pooled, homogenized, and then suspended in physiological saline. After counting, some cells from the suspension were seeded into 96-well culture plates in RPMI 1640 medium supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin. After 48 hours culture, BrdU was added for 24 hours. The extent of BrdU incorporation was hereafter measured by ELISA (Roche, Penzberg, Germany).

No measurements of dermal irritation potential are reported.

Results and discussion

SI was calculated as the ratio of the mean of *ex vivo* BrdU incorporation (labelling index) for each treatment group vs that of the vehicle group.

All data were analysed with one-way ANOVA. If ANOVA indicated a significant difference, the difference(s) between the vehicle control and each treatment group were analysed using a Dunnett *t*-test.

The EC3 (a measure of relative skin sensitizing potency of a substance) was expressed as the estimated concentration of chemical necessary to produce a 3-fold increase on proliferation in draining lymph nodes compared with the spontaneous proliferation that occurred with cells from vehicle-treated control hosts.

Calculated SI

Dose	*Vehicle (AOO)*	*2.5%*	*10%*	*20%*	*50%*
SI	*1.00*	*1.48*	*1.90*	***3.17***	***4.54***

EC3 (%) for eugenol was calculated to be 16.60.

3.1.1.2 Study 3 (LLNA)

Study reference:

Basketter, D., Kolle, S. N., Schrage, A., Honarvar, N., Gamer, A. O., van Ravenzwaay, B., & Landsiedel, R. (2012). Experience with local lymph node assay performance standards using standard radioactivity and nonradioactive cell count measurements. *Journal of Applied Toxicology*, *32*(8), 590-596.

Detailed study summary and results:

Test type

LLNA – according to OECD TG 429 (2002).

GLP compliant.

Test substance

Eugenol (Sigma Aldrich, Taufkirchen, Germany). CAS no. 97-53-0.

Prepared in AOO (4:1, v/v).

Purity: 99.7%.

The test substance is equivalent to the substance identified in the CLH dossier.

Test animals

Mice: CBA/J female (In the study CBA/CaOlaHsd is also mentioned. However, CBA/J female is one of the specie of choice in the OECD guideline and it is assumed, that the guideline is followed as stated in the study).

Six animals per dose group.

Age at study initiation: 6-12 weeks (weight not reported).

The mice were housed in Makrolon type I or II cages, temperature 20-24ºC, relative humidity 30-70% and a light-dark cycle of 12 hours. Water and food *ad libitum*.

Administration/exposure

Pre-screen: A pre-test for each substance in the study was conducted, in which three mice (per substance) were exposed to a standard concentration of 5%, followed by weighing of ears, as indicators of skin irritation.

Main study: In the main study eugenol was given in three doses: 2.5, 10 and 25% (w/v).

Groups of mice (n=6) were treated daily with 25 µl of the test substance (eugenol) in vehicle (AOO) applied epicutaneously to the dorsal part of both ears for three consecutive days (D1-D3).

On D6 ^{3}H-thymidine (^{3}HTdR) in sterile saline were injected intravenously into a tail vein. After five hours the mice were sacrificed, immediately followed by punching a circular piece of tissue out of the apical part of each ear of all animals. The pieces were all weighed to detect potential inflammatory ear swelling (indicating irritation) and to facilitate the interpretation of borderline lymph node responses.

The lymph nodes were pooled in the further analysis. Single-cell suspensions were prepared by passing the lymph nodes through an iron mesh into phosphate-buffered physical saline. An aliquot of each suspensions was further diluted with Casy®ton, and the remaining suspension was washed twice with phosphate buffered saline and precipated with trichloroacetic acid (TCA). The precipitate was then transferred to scintillation fluid and the amount of the ^{3}HTdR was measured in a β-scintillation counter.

Results and discussion

The response to eugenol (and the other substances) were characterized by lymph node cell count/lymph node pair (LNCC), ^{3}HTdR incorporation into the lymph node cells (LNCs) , lymph node weight and ear weight.

The skin sensitizing potential of a test substance was calculated using both the LNCC and ^{3}HTdR incorporation into the LNC. The results were interpretated using the SI: LNCC with factor >1.5 ; ^{3}HTdR incorporation with factor >3, both comparing with the concurrent vehicle control group .

A test substance was considered a sensitizer if at least one concentration tested caused a concentration-dependent statistically significant. The authors also considered the reliability of LNCC as a measurement of the substance skin sensitizing ability. This is however not a method with a guideline or a method mentioned in the Guidance on the Application of the CLP Criteria or the CLP. The study included results from the standardised LLNA method, that will be used in this dossier.

Results from the study

Dose	*Vehicle (AOO)*	*2.5%*	*10%*	*25%*
*Cell count SI**		*1.06*	*1.59*	*2.83*
^{3}H-Thymidine SI **		*2.33*	***4.77***	***21.93***

**Cell count SI, the SI derived by dividing test lymph node cell count by that of the concurrent vehicle control. **H-Thymidine SI, the SI derived by dividing the thymidine incorporation of test lymph nodes by the vehicle control.*

No increase in ear weight compared with pretest value was measured for eugenol.

EC3 was calculated to be 4.6%.

3.1.1.3 Study 4 (LLNA)

Study reference:

Fukuyama, T., Kosaka, T., Tajima, Y., Ueda, H., Hayashi, K., Shutoh, Y., & Harada, T. (2010). Prior exposure to organophosphorus and organochlorine pesticides increases the allergic potential of environmental chemical allergens in a local lymph node assay. *Toxicology letters*, *199*(3), 347-356.

Detailed study summary and results:

Test type

LLNA – performed as described by Kimber and Weisenberger (1989) with modifications. The objective of the study was to examine the relationship between immune disorders and the immunosuppression induced by immunosuppressive pesticides, showing that prior exposure to organophosphorus and organochlorine pesticides increases the allergic potential of environmental chemical allergens in a local lymph node assay. The study was conducted by exposing mice to two pesticides four weeks prior to a LLNA with substances known to induce sensitization, of which eugenol was one. The results depictured below, are from mice exposed only to the vehicle of the pesticides (corn oil), functioning as control-groups in the experiments for each pesticide. Thus only results from mice exposed to corn oil prior to the exposure of eugenol have been included in this dossier.

GLP compliance not stated.

Test substance

Eugenol ($C_{10}H_{12}O_2$) (Waka Pure Chemical Industries Ltd. (Oskaka, Japan). (No identification number is reported, e.g. EC/CAS/batch no.).

Eugenol purity > 95%.

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

Corn oil was purchased from Hayashi Chemicals (Tokyo, Japan).

Test animals

CBA/J female mice

Age at initiation (LLNA): 8 weeks. Reported results are of mice exposed to corn oil for five (5) consecutively days, four weeks prior to the LLNA (weight at initiation was not reported).

Six (6) animals where assigned in each group.

Administration/exposure

Dose of eugenol 0, 5, 10, and 25 %.

On day 1-5 corn oil was given orally. On D31, the LLNA was initiated.

Groups of mice (*n*=5) were treated daily with 25 µl of the test substance (eugenol) in vehicle (AOO) applied to the dorsal part of both ears for three consecutive days (D31-33). On D36 ^{3}H-methyl thymidine was injected via the tail vein and 5 hours later the mice were sacrificed followed by removing of both auricular lymph nodes from each animal. The lymph nodes where weight and pooled.

Single-cell suspensions of lymph nodes in PBS (phosphate-buffered saline) were prepared by passage through sterile 70-µm nylon cell strainers. The lymph node cell suspension was washed twice with an excess of PBS, and the cell pellet was incubated in 5% TCA at 4◦C for approximately 18 hours. Each cell pellet was resuspended in TCA and transferred to scintillation fluid. Incorporation of 3H-TdR was measured with a β-scintillation counter as disintegrations per minute for each mouse.

Results and discussion

The data were transformed logarithmically to equalize the variances, and ANOVA was used to evaluate the results. When the ANOVA was significant, the differences between groups were assessed by Dunnett's multiple comparison test. A P value <0.05 was considered to indicate significance.

SIs and EC3 values were calculated from the 3H-TdR incorporation data. The SI was calculated by dividing the mean 3H-TdR incorporation value for each treatment group by that of the control group. The EC3 value is an estimate of the amount of test solution required to induce an SI of 3. In the standard LLNA, the criterion for a positive response is an SI of 3 or greater (Dearman et al., 1999).

Results from the study

Dose	*Vehicle (AOO)*	*5%*	*10%*	*25%*
Final body weight (g; mean ±SD)	*20.3 ± 2.5* *20.9 ± 1.7*	*20.7 ± 1.4* *21.1 ± 1.3*	*20.8 ± 1.2* *21.0 ± 1.1*	*20.5 ± 1.6* *21.2 ± 1.9*
Lymph node weight (mg; mean± SD)	*3.85 ± 1.14* *3.65 ± 0.32*	*5.28 ± 0.77* *5.48 ± 1.85*	*6.15 ± 0.62* *7.60 ± 0.53***	*10.54 ± 1.93*** *11.42 ± 1.76***
H-TdR incorporation DPM (mean± SD)	*304 ± 208* *392 ± 113*	*593 ± 159* *808 ± 536*	*2287 ± 705* *2029 ± 1276*	*8964 ± 2588*** *9476 ± 1707***
SI	*1.0* *1.0*	*2.0* *2.1*	***7.5*** ***5.2***	***29.5*** ***24.2***

*DPM: disintegrations per minute. ** Values for treatment and control groups were compared by Dunnett's multiple comparison test: P<0.01.*

EC3 values were calculated to be 5.28% and 6.45%

3.1.1.4 Study 5 (LLNA:BrdU-FCM)

Study reference:

Jung, K. M., Jang, W. H., Lee, Y. K., Yum, Y. N., Sohn, S., Kim, B. H., Chung, J.H., Park, Y.H & Lim, K. M. (2012). B cell increases and ex vivo IL-2 production as secondary endpoints for the detection of sensitizers in non-radioisotopic local lymph node assay using flow cytometry. *Toxicology letters*, *209*(3), 255-263.

Detailed study summary and results:

Test type

The study is described as a LLNA:BrdU-FCM, comparableto OECD TG 422B (adopted after the publication of the study).

Test substance

Eugenol, CAS no. 97-53-0.

Purity: not reported.

The test substance is equivalent to the substance identified in the CLH dossier.

Test animals

Mice, Balb/b mice, female.

Groups: 4-6 animals.

Age at initiation: 8-9 weeks.

Bodyweight (7-8 weeks): 18-22 g.

Animals were kept at temperature 23±33ºC, relative humidity 50±10%, and a light cycling with 12 hours. Water *ad libitum.*

Administration/exposure

Dose groups: 0, 5, 10 and 25% in AOO (4:1, v/v) .

25 µl of the test substances in vehicle or vehicle alone was applied on the dorsal of both ears daily for 3 consecutive days (Days 1–3). On Day 5 mice were intraperitoneally injected with BrdU and were sacrificed after one day (D6). After sacrifice, auricular lymph nodes were isolated, weighed, and undergone lymphocyte preparation. After bilateral auricular lymph nodes were pooled on individual basis, LNCs were prepared by disaggregation through 70 µm mesh in 1 ml PBS. The LNCs were counted using a haemocytometer after stained with trypan blue.

The LNCs (1.5 × 106/ml) were centrifuged for 5 min in PBS and re-suspended for fixation and permeabilization step. Then LNCs were permeabilized using Cytoperm plus buffer, which contains 10% DMSO. After DNA was denatured by incubating in DNase for 1 h, LNCs were washed, and incubated with

FITC conjugated anti-BrdU antibody at a dilution of 1:50 for 20 min at RT in the dark. Cells were washed once more and then re-suspended in 7-AAD solution to label DNA. Ten thousand 7-AAD expressing cells were gated, and the number of the cells expressing BrdU was analysed using BD FACSCaliburTM system.

Results and discussion

The SI in the LLNA: BrdU-FCM is the ratio of the mean number of LNCs with incorporated BrdU from mice in each of the test substance dose groups to the mean number of LNCs with incorporated BrdU from mice in the vehicle control group.

The statistical significant of the differences between groups was determined by the one-way ANOVA. In the ANOVA, when significant differences were detected, Dunnett's method as a post hoc test was used to compare treatment groups with the appropriate vehicle control group. All the statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 13, SPSS Inc., Chicago, IL, USA) and $P < 0.05$ and $P < 0.01$ was considered significant.

The study showed an EC3 of 2.0

3.1.1.5 Study 6 (LLNA - review)

Study reference:

Basketter, D. A., Gerberick, F., & Kimber, I. (2007). The local lymph node assay and the assessment of relative potency: status of validation. *Contact Dermatitis*, *57*(2), 70-75.

Detailed study summary and results:

Test type

The study is a review with the focus of the utility and validity of the relative potency measurements of skin sensitizing chemicals from LLNA dose-response analyses. The study summarises several studies of which most are unpublished.

Four different EC3 values are reported for eugenol, two of which are unpublished results where no guideline is specified, and two are performed according to the protocol by Kimber and Basketter (1992), cited from Loveless et al. (1996). The EC3 values from Loveless et al. (1996) have been recalculated from the stimulation indexes in the original publication using linear extrapolation, as the original EC3 values have been calculated using fitted quadratic equations. In addition, Loveless et al. (1996) reports three additional EC3 values, however, these have been optained by modified protocols and have for that reason not been included in this dossier, nor have they reported in Basketter et al. (2007). The EC3 values reported by Basketter et al. (2007) are 4.9, 7.5, 12.9, and 15.0 % with a mean EC3 value of 10.1 %.

Test substance

Eugenol in AOO (v,v). No further substance information is reported.

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

Test animals

Not reported.

Administration/exposure

Not reported.

Results and discussion

EC3 values (%) is 15.0, 4.9, 12.9, 7.5 in AOO (4:1), with a mean EC3=10.1 ± 2.3%.

3.1.1.6 Study 7 (LLNA)

Study reference:

Lalko, J., & Api, A. M. (2006). Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. *Food and chemical toxicology*, *44*(5), 739-746.

Detailed study summary and results:

Test type

LLNA accordingly to OECD TG 429 (2002).

GLP compliance not stated.

Test substance

Eugenol, CAS no. 97-53-0, EINECS no. 202-589-1. Phenol, 2-mothoxy-4-(2-propenyl)- (Firmenich, Switzerland).

Purity: 99.9%.

The test substance is equivalent to the substance identified in the CLH dossier.

The vehicle, 1:3 ethanol: diethyl phthalate (1:3 EtOH:-DEP).

Test animals

Mice, CBA/Ca, female.

Age at initiation 8-12 weeks.

Bodyweight: 17-21 g. at initiation.

Groups: 4 mice per group.

Mice were housed in groups of four (4). 12 hours light/dark cycle, temperature 19-25°C and humidity 30-70%. Food and water *ad libitum.*

Administration/exposure

The mice were dosed topically on the dorsum of both ears with 25 µL of test material. Each group received one of five test concentrations in 1:3 EtOH:DEP—2.5%, 5%, 10%, 25% or 50% w/v. A vehicle control group was similarly treated with 1:3 EtOH:DEP. Dosing occurred daily for three consecutive days (D1-D3). The animals "rested" for two days and on D6 the mice were injected intravenously in the tail vein with 3H methyl thymidine. Five hours later, the mice were sacrificed, and the draining auricular lymph nodes were excised and pooled for each experimental group. The incorporated 3H methyl thymidine was measured by β-scintillation counting and expressed as disintegrated per minute (dpm) per lymph node for each experimental group.

Results and discussion

For each concentration of test material, a SI relative to the concurrent vehicle-treated control was calculated.

Results from the study

Dose	*Vehicle (1:3 EtOH/DEP)*	*2.5%*	*5%*	*10%*	*25%*	*50%*
DPM/lymph node	*386*	*464*	*1051*	*2322*	*5534*	*7476*
SI	*N/A*	*1.2*	*2.7*	***6.0***	***14.3***	***19.4***

EC3 was calculated to 5.4% for eugenol.

3.1.1.7 Study 8 A+B (Study 8A: LLNA:BrdU-ELISA. Study 8B: GPMT)

Study reference:

Takeyoshi, M., Noda, S., Yamazaki, S., Kakishima, H., Yamasaki, K., & Kimber, I. (2004). Assessment of the skin sensitization potency of eugenol and its dimers using a non-radioisotopic modification of the local lymph node assay. *Journal of Applied Toxicology: An International Journal*, *24*(1), 77-81.

Detailed study summary and results:

Test type

Study 8A: LLNA:BrdU-ELISA – no guideline mentioned, comparable to OECD TG 442B. GLP compliance not stated.

Study 8B: GPMT – no guideline mentioned. The test was conducted according to a method described previously by Magnusson & Kligman, 1969 (cited from Takeyoshi et al. 2004). GLP compliance not stated.

Test substance

Eugenol (lot no. EG0704) (Kanebo Cosmeticts Company, Odawara, Kanagawa, Japan).

Purity of eugenol was > 95%.

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

LLNA:BrdU-ELISA : Eugenol was dissolved in AOO (4;1).

GPMT: Eugenol was dissolved in olive oil.

Test animals

LLNA:BrdU-ELISA: Mice (CBA/JN), female. Four animals per dose group.

GPMT: Hartley guinea pigs, female. 10 animals per dose group.

The animals were housed in animals rooms with temperature of 22±3ºC, humidity 55±15%, ventilation frequency of 10-15 cycles per hour and a light/dark cycle of 12 hours. Diet and water *ad libitum.*

Age and weight of the animals when the study was initiated is not reported.

Administration/exposure

LLNA:BrdU-ELISA (study 8A):

A 25-µl volume of test chemicals at concentrations of 0% (vehicle control), 1%, 6%, 15% or 30% for eugenol was applied to the dorsum of both ears of the mice daily for three consecutive days (D1-D3). The concentration ranges of each test chemical were decided according to the sensitization potencies classified by the results of GPMT (study 8B). A single intraperitoneal injection of BrdU was given on day (D4). On day 5 (D5), the animals were sacrificed, and the draining auricular lymph nodes were removed, weighed, and stored at −20 °C until ELISA analysis was used to measure the BrdU incorporation.

The incorporation of BrdU into LNCs was determined using a commercial cell proliferation assay kit. The lymph nodes were crushed, passed through a nylon mesh and the LNCs were individually suspended in physiological saline. The cell suspension was added to the wells of a flat-bottom microplate in triplicate. After centrifugation, the supernatants were removed. Fix-Denat solution was added to each well and then the plate was allowed to stand for 30 min at room temperature. After removing the Fix-Denat solution, diluted anti-BrdU antibody solution was added to each well and, after rinsing three times with washing buffer, substrate solution containing tetramethylbenzidine was added and allowed to react for 15 min at room temperature. Absorbance at 370 nm was determined with a microplate reader at a reference wavelength of 492 nm. The absorbance was defined as the BrdU labelling index.

GPMT (study 8B):

Guinea pigs received a series of intradermal injections of eugenol or vehicle control in the shoulder region to induce sensitization. After 6–8 days, sensitization was boosted by a 48-h occluded patch of the same compound placed over the injection sites. Fourteen days later, the animals were challenged on a shaved flank by a 24-h occluded patch containing the same compound. All induction and challenge concentrations were set at 5% (maximum non-irritant concentration) in olive oil for all compounds in view of preliminary dose-finding tests. All compounds elicited an apparent irritation at 10% in preliminary tests for intradermal injection and topical application, so an induction and challenge concentrations of 5% for all compounds was used in order to compare the sensitization potency of the three compounds included in the study.

Results and discussion

LLNA:BrdU-ELISA (study 8A):

Data were analysed using the Bartlett test for homogeneity of variance. If the variances were homogeneous at a level of 5% significance, a one way-ANOVA was conducted. If the one-way ANOVA produced a significant difference, the differences between the control group and each of the experimental groups were analysed using the Dunnett test. If the variances were not homogeneous, the Kruskal–Wallis test was employed. If this test produced a significant difference, the difference between the control group and each of the experimental groups was analysed using the non-parametric Dunnett test. The estimated concentration of a chemical required to induce an SI of 3 relative to vehicle-treated controls (EC3 value) was calculated.

Results including SI.

Concentration	*Lymph node weight (mg)*			*BrdU labelling index ($A_{370-490}$)*		
	Mean	*SEM*	*SI*	*Mean*	*SEM*	*SI*
0	*2.150*	*0.155*	*-*	*0.107*	*0.010*	*-*
1	*3.325*	*0.485*	*1.5*	*0.187*	*0.031*	*1.7*
6	*3.325*	*0.578*	*1.5*	*0.161*	*0.031*	*1.5*
15	*5.175**	*0.085*	*2.4*	*0.251**	*0.028*	*2.3*
30	*5.650***	*0.517*	*2.6*	*0.355***	*0.048*	***3.3***

*$P < 0.05$. ** $P < 0.001$

EC3 was calculated to be 25.1%.

GPMT (study 8B):

Chemicals were classified by the sensitization rate for each chemical (0–8%, weak; 9–28%, mild; 29–64%, moderate; 65–80%, strong; 81–100%, extreme) according to the criteria given by Magnusson and Kligman (1969). In the GPMT for eugenol the sensitization response rates were 20% and eugenol was classified as a mild skin sensitizer.

3.1.1.8 Study 9 (LLNA)

Study reference:

Basketter, D. A., Lea, L. J., Dickens, A., Briggs, D., Pate, I., Dearman, R. J., & Kimber, I. (1999). A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. In *Journal of Applied Toxicology: An International Forum Devoted to Research and Methods Emphasizing Direct Clinical, Industrial and Environmental Applications* (Vol. 19, No. 4, pp. 261-266).

Detailed study summary and results:

Test type

LLNA: According to the protocol described by Kimber & Basketter (1992) (with five concentrations).

Test substance

Eugenol.

Vehicle AOO (4;1, v/v).

The test substance is assumed to be equivalent to the substance identified in the CLH dossier.

Test animals

Mice, CBA/Ca. Gender not reported.

Age: 6-12 weeks. (weight not reported).

Administration/exposure

Eugenol was given in the concentration of 0, 2.5, 5.0, 10.0, 25.0, 50.0 % (% w/v).

The test material (25 ml) or an equal volume of the vehicle alone was applied to the dorsum of both ears of groups four mice. Treatment was performed once daily for three consecutive days. Five days following the initiation of exposure, all mice were injected via the tail vein with 250 ml of PBS containing [3]-methyl thymidine. Mice were sacrificed 5 h later and the draining auricular lymph nodes were excised and pooled for each experimental group. A single-cell suspension of LNC was prepared by gentle mechanical disaggregation through 200-mesh stainless-steel gauze. Pooled LNC were washed twice with an excess of PBS and precipitated with 5% TCA at 4°C for 18 h. Pellets were resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase MP; LKB). Incorporation of [3H]TdR was measured by b-scintillation counting dpm for each experimental group.

Results and discussion

Increase in the [3H]TdR incorporation relative to vehicle-treated controls were derived and recorded as stimulation indices (SIs).

Results from the study

Dose	*Vehicle AOO*	*2.5%*	*5%*	*10%*	*25%*	*50%*
DPM node^{-1}	*380*	*596*	*563*	*899*	*2091*	*6100*
SI	*1*	*1.6*	*1.5*	*2.4*	***5.5***	***16.1***

EC3 (derived using linear interpolation method of statistical analysis) was 11.9%.

3.1.1.9 Study 10 (LLNA:DA)

Study reference:

Zhang, H., Shi, Y., Wang, C., Zhao, K., Zhang, S., Wei, L., Don, L., Gu, W., Xu, Y., Ruan, H., Zhi, H. & Yang, X. (2017). An improvement of LLNA: DA to assess the skin sensitization potential of chemicals. *The Journal of Toxicological Sciences*, *42*(2), 129-136.

Detailed study summary and results:

Test type

Two-Stage LLNA:DA

The study is a combination of rLLNA and LLNA:DA. In stage one, potential sensitizers are found by exposing 2 mice to the highest concentration. The second round consist of a LLNA:DA with chemicals showing SI ≥ 1.8 in rLLNA.

LLNA (Stage II) was conducted according to OECD TG 442 (A). The following will only include results from stage II.

Test substance

Eugenol CAS no. 97-53-0 (Sigma-Aldrich).

Vehicle AOO (4:1, v/v).

The test substance is equivalent to the substance identified in the CLH dossier.

Test animals

Mice, BALB/c.

Four animals in each dose group.

Age 8-12 weeks.

Weight 18-22 g.

The animals were housed with temp between 20-24, humidity 60-80 and 12-hrs. light/dark cycles. Food and water *ad libitum.*

Administration/exposure

Three concentrations: 0, 2.5, 5, 10% in AOO.

Groups of four animals per dose groups were treated with 25 µL of the substance or vehicle on the dorsum of both ears. Treatment was performed on D1-3 and D7. The day 8 (D8), the mice were sacrificed and the auricular lymph nodes on both sides were removed.

Results and discussion

Results from the study

Dose	*Vehicle AOO*	*2.5%*	*5%*	*10%*
ATP content (mean)	*5986±230*	*9260±1133[b]*	*11405±673[b]*	*19943±802[*]*
SI	*1*	*1.55*	***1.91***	***3.33***

[*]statistically significant difference in the ATP content between the control group and the test group (P<0.05).

EC1.8 was calculated to 4.24%.

3.1.1.10 Study 11 (LLNA:BrdU-ELISA)

Study reference:

Chen, W., Xing, C., & Hou, F. (2016). Intra-laboratory study to determine the reproducibility of LLNA: BrdU-ELISA for the prediction of the skin sensitizing potential of chemicals. *Journal of pharmacological and toxicological methods*, *82*, 26-30.

Detailed study summary and results:

Test type

LLNA:BrdU-ELISA – OECD TG 442B and the ICCVAM Recommended Test Method Protocol.

Test substance

Eugenol (Lot No.: TA023201 ; Sinopharm Chemical Reagent Beijing Co.). No other identification of the substances was reported, e.g. CAS/EC no.

Vehicle: AOO (4:1, v/v.)

Test animals

Mice, CBA/J, female.

Four (4) mice in each group.

Age: 8-10 weeks (weight not reported).

The animals were housed as described in the OECD TG 442B, with the deviation of a light cycle of 10 hours light/14 hours darkness.

Administration/exposure

Ten independent LLNA:BrdU-ELISAS with 25% eugenol were conducted repeatedly in nearly a year, to demonstrate the adequate profiency of the assay in the specific laboratory. In addition, mice were exposed to concentrations of eugenol in 10, 25 and 50% to determine the dose-response relationship and the EC1.6 values.

25 µl of the test substance was applied daily to the dorsum of each ear of the mice for three consecutive days (D1-D3). On day 5 (D5) single intraperitoneal injection of 0.5 ml of BrdU solution (10 mg/ml) was given. On day 6 (D6), a pair of auricular lymph nodes from each mouse was excised and stored at −20 °C until BrdU-ELISA analysis was conducted with a BrdU-ELISA kit (Roche Applied Science, Mannheim, Germany).

Results and discussion

Results for each test group are expressed as the mean SI. The SI is derived by dividing the mean BrdU labelling index / mouse within each test group by the mean BrdU labelling index for the AOO group. The mean SI for the AOO group is one. A positive response is defined as the mean SI of the test group ≥ 1.6.

Results including calculated EC1.6.

Concentration	*BrdU labelling index (mean, n = 4)*	*The mean SI*	*EC1.6*
0 (AOO)	*0.05*	*1*	*12.5%*
10%	*0.06*	*1.2*	
25%	*0.18*	***3.6***	
50%	*0.20*	***4***	

3.1.1.11 Study 12, 13, 14 and 15 (LLNA)

Study reference:

Unpublished summary reports by Research Institute for Fragrance Materials (RIFM), <u>*cited in:*</u> Scientific Committee on Consumer Safety (SCCS). Opinion on Fragrance allergens in cosmetic products. Adopted opinion at 15th plenary meeting, June 2012. RIFM references: RIFM, 2001f, 2001g, 2001h and 2001i.

Detailed study summary and results:

Test type

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

Test substance

Eugenol. CAS no. 97-53-0. No information on purity.

The test substance is equivalent to the substance identified in the CLH dossier.

Test animals

n = 4 animals per dose.

No further information in SCCS 2012.

Administration/exposure

In all four studies eugenol was tested in concentration of 1, 3, 10, 30 and 50% (w/v).

Vehicle: 3:1 EtOH:DEP (2001f); 1:3 EtOH:DEP (2001g); EtOH (2001h); DEP (2001i)

No further information available in SCCS 2012.

Results and discussion

Although detailed information is not available for the studies conducted by RIFM the result generally confirms the sensitizing properties identified for eugenol in other LLNA studies. The vehicle is a deviation from the OECD TG 429 of which the scientific rationale is not reported.

Results studies with EC3 values with eugenol including the lowest EC3.

Reference	*Study no.*	*Vehicle*	*EC3*	
			%	*µg/cm²*
Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM, 2001f)	*12*	*3:1 EtoH:DEP*	*5.3*	*1325*
Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM, 2001g)	*13*	*1:3 EtOH:DEP*	*10.5*	*2625*
Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM, 2001h)	*14*	*EtOH*	*10.7*	*2675*
Unpubl. summary report by RIFM 2009 cited in SCCS 2012 (as RIFM, 2001i)	*15*	*DEP*	*15.1*	*3775*

3.1.2 Human data

3.1.2.1 Study 16 (patch test, selected)

Study reference:

Schnuch, A., Uter, W., Lessmann, H. & Geier, J. (2015). Risk of sensitization to fragrances estimated on the basis of patch test data and exposure, according to volume used and a sample of 5451 cosmetic products. *Flavour and fragrance journal*, 30(3), 208-217.

Detailed study summary and results:

Test type

Frequency of sensitization to fragrances was analysed based on data from IVDK (a network of departments of Dermatology in Germany, Austria, and Switzerland) in the period September 2007 to December 2009.

The frequency of the 26 fragrances to be labelled on cosmetic products according to current EU legislation (including eugenol) was documented in 5451 products (based on the labelling of the ingredients), purchased at random between 2007-2009. The sensitization exposure quotient (SEQ) was calculated as the quotient of the relative frequency of sensitization and the relative frequency of use/labelling.

Testing of single components in 806 patients with positive reactions to FM I and without reactions to sorbitan sesquioleate (SSO). Results of reading at D3 (or D4) showed a positive eugenol response in 6.7% with a test concentration of 1% in petroleum (pet.).

The frequencies of sensitization in the study population were extrapolated from the frequency of reactions to the single compounds (eugenol = 6.7%). From this calculation a frequency of sensitization to Eugenol was equal to 0.74% in the study population, which equals 3% of the total share of positives to the single 26 fragrances. Using the market share of 1.51 % a SEQ equal 1.99 are calculated.

SEQ calculated on the basis of labelling frequencies from the CVUA data set for all products (n = 5451), relating the share of allergic reactions and the share of labelling frequencies. In all products Eugenol was labelled on 486 products (a share of 3.14% of the 26 fragrances in all 5451 products), with a SEQ = 0.96. In leave on products (n=3541) Eugenol was found in 309 products (a share of 3.23 of the 26 fragrances in leave on products), with a SEQ = 0.93.

3.1.2.2 Study 17 (patch test, selected)

Study reference:

Turčić, P., Lipozenčić, J., Milavec-Puretić, V., & Marinović Kulišić, S. (2011). Contact Allergy Caused by Fragrance Mix and Myroxylon pereirae (Balsam Of Peru), a Retrospective Study. *Collegium antropologicum*, 35(1), 83-87.

Detailed study summary and results:

Test type

Patch testing according to the International Contact Dermatitis Research Group (ICDRG) system was conducted during 2001-2005 at the Allergy Clinic of the Department of Dermatology and Venereology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia. Allergens were applied on the patients' upper back with 2-days occlusion and were read 48 and 72 hours after application. The test results were interpretated using the following scale: no reaction (0) ; macular erythema (?) ; erythematous papules and/or vesicles (2+) ; spreading blisters and/or crust with ulceration (3+) ; and irritant reaction (IR). 1+, 2+ and 3+ were considered positive allergic reactions.

The specific mix of fragrances consisted of cinnamal (1%), cinnamyl alcohol (5%), alpha-amyl cinnamal (5%), eugenol (5%), isoeugenol (5%), geraniol (5%), hydroxy citronellal (1%) and Evernia prunastri (oak moss) (2%). Vehicle was petrolatum (pet.). Obtained from the Immunology Institute in Zagreb, Croatia.

A total of 27,815 patients with suspected ACD were included in the study. 3,065 patients with contact dermatitis were patch tested using a specific mix of fragrances (8% fragrance mix (FM) in pet.). 509 patients were positive the specific mix of fragrances. Of the 509 patients 157 were chosen to patch test of the single components (in pet.) of the specific mix of fragrances.

Eugenol was found to be one of the most frequent allergens in the mix of fragrances, with 55.4% positive patients of the total 157.

3.1.2.3 Study 18 (patch test, selected)

Study reference:

Pentinga, S. E., Kuik, D. J., Bruynzeel, D. P., & Rustemeyer, T. (2009). Do ‘cinnamon-sensitive’patients react to cinnamate UV filters?. *Contact dermatitis*, 60(4), 210-213.

Detailed study summary and results:

Test type

Department of Dermatology and Department of Epidemiology and Biostatistics at VU University Medical Center (The Netherlands), conducted a prospective study. Patients (n = 18) were considered cinnamon sensitive if they previously had have positive patch test to FM I and/or II in combination with a positive patch test to *M. pereirae* (n = 10) or if they have had positive patch tests with cinnamon-related fragrances in subsequent testing with individual fragrances (n = 8).

Description of the 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 D1*

dressing (PaulHartmann BV, Nijmegen, the Netherlands). The right side was first exposed to5 J/cm2 UVA (Psor-alen UVA 800 Unit; Waldmann, FRG) and then covered with MoliNea plus D dressing. Photopatch test readings were scheduled accordingto the recommendations of the European Task force 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 Inter-national 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 positivepatch 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. Maximum patch test scores of cinnamon, cinnamon-related fragrances, and M. pereirae were obtained from the non-irradiated patch test side (left)."

Eugenol was used in the concentration of 2% in petrolatum and 17% patients (3/18) showed positive patch tests with eugenol.

3.1.2.4 Study 19 (patch test, consecutive/unselected)

Study reference:

Mann, J., McFadden, J. P., White, J. M., White, I. R., & Banerjee, P. (2014). Baseline series fragrance markers fail to predict contact allergy. *Contact Dermatitis*, 70(5), 276-281.

Detailed study summary and results:

Test type

St John's Institutes of Dermatology, St Thomas' Hospital (UK) conducted a retrospective study of 1951 eczema patients routinely tested with labelled fragrance substance and extended European baseline series in 2011-2012.

Description of the test method as cited from Mann et al. 2014:

"*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.*"

Eugenol was used in the concentration of 2% in petrolatum and 0.62% patients (12/1951) showed positive patch tests with eugenol.

3.1.2.5 Study 20 (patch test, selected)

Study reference:

Nardelli, A., Carbonez, A., Drieghe, J., & Goossens, A. (2013). 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(5), 307-313.

Detailed study summary and results:

Test type

Department of Dermatology, University Hospital (Belgium) and University Centre for Statistics (Belgium) conducted a cross-sectional study on patch test results of 13,332 patients from year 1990-2011. A total of 13,114 patients were tested with FM 1 of which 1259 were positive. Of these, 940 selected patients were patch tested with single FM 1 ingredients, including Eugenol.

Description of the 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"... "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 some times later.*"

12.5% (118/940) selected patients showed positive patch test results to Eugenol.

3.1.2.6 Study 21 (patch test, selected)

Study reference:

Nagtegaal, M. J., Pentinga, S. E., Kuik, J., Kezic, S., & Rustemeyer, T. (2012). The role of the skin irritation response in polysensitization to fragrances. *Contact dermatitis,* 67(1), 28-35.

Detailed study summary and results:

Test type

VU University Medical Centre (the Netherlands) and Unveracity of Amsterdam (the Netherlands), conducted a prospective study of selected patients (n = 100) with European baseline patch test series. Included patients had all shown contact allergy towards FM 1 and/or FM II in previous patch tests.

Description of the test method as cited from Nagtegall et al. 2012:

"*Patch tests were performed in accordance with the recommendations of the ICDRG(12). Preparations of test materials in petrolatum were obtained from Trolab® (Almirall-Hermal, Reinbeck, Germany) or Chemotechnique Diagnostics® (Vellinge, Sweden). Van der Bend® patch test chambers (Van der Bend BV, Brielle, The Netherlands) on Fixomull® tape were used. Test chambers were manually filled by a specially trained investigator. The test substances consisted of 27 commercial patch test materials of fragrance chemicals, including FM I (8%) and FM II (14%), and were coded to ensure that the study could be performed in a double-blind fashion. The materials were supplied in polypropylene syringes, and stored in a refrigerator at 5˚C. The patches were applied for 2days on the upper back, and readings were performed on day 2 (48hr), day 3 (72hr), and day 7 (144hr).*"

7% (7/100) selected patients showed positive patch test results to Eugenol (concentration not reported).

3.1.2.7 Study 22 (patch test, consecutive/unselected)

Study reference:

Heisterberg, M. V., Menné, T., & Johansen, J. D. (2011). Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. *Contact Dermatitis*, 65(5), 266-275.

Detailed study summary and results:

Test type

The National Allergy Research Center of Denmark conducted a retrospective study of patch test results of the 26 single fragrances which must be declared on cosmetics products according to the EU Cosmetics Directive. In all 1508 eczema patients patch tested in the period 2008-2010 were included.

Description of the test method as cited from Heisterberg et al. 2011:

"*The patch tests were performed according to international guidelines (9), with Finn Chambers® applied on the back with Scanpor® tape (Vitalfo Scandinavia, AB, Allerød, Denmark) for a period of 2 days. Readings were performed on days 2, 3 or 4, and 7, according to the recommendations of the International Contact Dermatitis Research Group(10).*"

0.3% (4/1502) selected patients showed positive patch test results to Eugenol (1%, vehicle not reported).

3.1.2.8 Study 23A+B (patch test, study 23A: selected, study 23B: consecutive/unselected)

Study reference:

Uter, W., Geier, J., Frosch, P., & Schnuch, A. (2010). Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis*, 63(5), 254-261.

Detailed study summary and results:

Test type

IVDK, University of Göttingen (Germany) conducted between 2005-2008 patch tests with the German baseline series and special series on 40,709 patients routinely patch tested for suspected allergic dermatitis.

Description of the test method as cited from Uter et al. 2010:

"*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"….." Weak (+) to strong (+++) positive patch test reactions on the third day after application of the test or, if this was not read, after the fourth day were aggregated as 'positive' outcomes and contrasted with non-positive (non-allergic) reactions, comprising negative, doubtful and irritant reactions.*"

Study 23A: 6.7% selected patients (*n* = 655) (already confirmed by patch test to be allergic to FM 1) showed positive patch test results to Eugenol (1%, vehicle not reported).

Study 23B: 0.44% (*n* = 1214) and 1.57% (*n* = 4801) consecutive patients showed positive patch test results to Eugenol (1%, vehicle not reported).

3.1.2.9 Study 24 (patch test, selected)

Study reference:

Cuesta, L., Silvestre, J. F., Toledo, F., Lucas, A., Pérez-Crespo, M., & Ballester, I. (2010). Fragrance contact allergy: a 4-year retrospective study. *Contact dermatitis*, 63(2), 77-84.

Detailed study summary and results:

Test type

Department of Dermatology, Hospital General Universitario, Spain, conducted a retrospective study of patients (*n* = 1253) tested with the Spanish baseline series and/or fragrance series: FM I and II, *Myroxylon pereirae*, and hydroxyisohexyl 3-cyclohexene carboxaldehyde, in the period 2004-2008. A fragrance series was tested in a selected groups of 86 patients: 54 patients with positive marker in the baseline series and 32 patients with clinicals symptoms compatible with fragrance contact dermatitis, despite not having any positive fragrance marker in the baseline series.

Description of the 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). FM I consists of eight components: Evernia prunastri (1%), isoeugenol (1%), eugenol (1%), cinnamal (1%), hydroxycitronellal (1%), geraniol(1%), cinnamyl alcohol (1%), and α-amylcinnamal (1%). Both M. pereirae and FM I contain sorbitan sesquioleate as an emulsifying agent.*"… "*The fragrance series (Chemotechnique®) contains 30 substances (Table 2). We added sorbitan sesquioleate to this series to detect false positives to fragrance chemical allergens prepared using this susbtance.*"..."*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*."

13.9% (12/86) selected patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.10 Study 25 (patch test, consecutive/unselected)

Study reference:

Van Oosten, E. J., Schuttelaar, M. L. A., & Coenraads, P. J. (2009). Clinical relevance of positive patch test reactions to the 26 EU-labelled fragrances. *Contact Dermatitis*, 61(4), 217-223.

Detailed study summary and results:

Test type

Department of Dermatology, University Medical Groningen, University of Groningen, the Netherlands conducted a prospective study, with 320 consecutive patients suspected of having contact allergy to fragrances or cosmetics were patch tested with EU-declared fragrance chemicals (26 fragrance substances), FM I and II.

Description of the 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 FMI, was tested in 295 patients, and the FM II (Her-mal/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; BedoukianResearch Inc., USA; Rhodia, France; Symrise, Ger-many 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)."

1.3% (4/320) consecutive patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.11 Study 26 (patch test, selected)

Study reference:

White, J. M. L., White, I. R., Kimber, I., Basketter, D. A., Buckley, D. A., & McFadden, J. P. (2009). Atopic dermatitis and allergic reactions to individual fragrance chemicals. *Allergy*, 64(2), 312-316.

Detailed study summary and results:

Test type

Department of Cutaneous Allergy, St John's Institute of Dermatology, St Thomas' Hospital, UK, conducted a retrospective study of patch test of 37,065 dermatitis patients (both atopic and nonatopic). Patch test was performed with FM I in the years 1982-2007. Patients with positive patch test to FM I, were subsequently patch tested for individual fragrances.

Description of the test method 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.*"

13.3% (30/225) selected patients (with current atopic dermatitis and FM 1 positive patch test) showed positive patch test with eugenol (1% in pet.). Of these 225 patients, 23.5% (53/225) had past observed atopic dertamitis.

3.1.2.12 Study 27 (patch test, selected)

Study reference:

Foti, C., Bonamonte, D., Conserva, A., Stingeni, L., Lisi, P., Lionetti, N., ... & Angelini, G. (2008). Allergic and photoallergic contact dermatitis from ketoprofen: evaluation of cross-reactivities by a combination of

photopatch testing and computerized conformational analysis. *Current pharmaceutical design*, 14(27), 2833-2839.

Detailed study summary and results:

Test type

Department of Internal Medicine, Immunology and Infectious Diseases, Section of Dermatology, University of Bari, Italy and Department of Medical and Surgical Specialities and Public Health, Section of Clinical, Allergological and Venerological Dermatology, University og Perugia, Italy and L. Rigano Laboratories, Italy, conducted a prospective study with patch testing of 15 patient with allergic contact dermatitis (ACD) and photo-ACD to topical ketoprofen (a nonsteroidal anti-inflammatory drug) were tested with the SIDSAPA (societá Italiana di Dernents), including eugenol.

Description of the test method as cited from Foti et al. 2008:

"*The patients were tested at least 3 months after complete disappearance of the dermatitis. Patch and photopatch tests were performed with the SIDAPA (Società Italiana di Dermatologia Allergologica Professionale ed Ambientale) photopatch test standard series (FIRMA Diagent, Florence, Italy) (Table 1) and the above mentioned KP-containing gels as is. Photo-patch tests were carried out by applying the allergens on one side of the back and leaving them under occlusion for 2 days; readings were performed at 48 and 96 h (D2 and D4). Simultaneously, photopatch tests were carried out by applying the allergens on the other side of the back, taking the bandages of at 24 h and exposing them to UVA rays at the dose of 5 J/cm*^{2} *(UV 801 KL, PUVA/TLOI, Photochemotherapy, Herbert Waldman, Werk für Lichttechnik, Germany), whereas the opposite side of the back was covered with a black cloth. Test reactions were read at 1 and 3 days after irradiation (D2 and D4). All 15 patients were also patch tested with FM and its components (eugenol, isoeugenol, oak moss, geraniol, hydroxycitronellal, amyl cinnamaldehyde, cinnamyl alcohol(Calc), cinnamaldehyde (Cald)) (Table 2) and with Myroxylon pereirae (Euromedical, Calolziocorte, Lecco, Italy). The reactions were classified according to Intemational Contact Dermatitis Research Group (ICDRG) guidelines.*"

13.3% (2/15) selected patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.13 Study 28 (patch test, consecutive/unselected)

Study reference:

Schnuch, A., Uter, W., Geier, J., Lessmann, H., & Frosch, P. J. (2007). Sensitization to 26 fragrances to be labelled according to current European regulation: results of the IVDK and review of the literature. *Contact Dermatitis*, 57(1), 1-10.

Detailed study summary and results:

Test type

The IVDK conducted a retrospective study with 21,325 patients being patch tested with 26 fragrances additionally to the standard series. Period of patch test: 2003-2004.

Description of the test method as cited from Schnuch et al. 2007:

"*Patch tests are performed in accordance with the recommendations of the International Contact Dermatitis Research Group (12) and the German Contact Dermatitis Research Group (DKG) (13). Patch test material is obtained from Hermal/Trolab, Reinbek, Germany. Patch test preparations are applied for 24 or 48 hr. Readings are done until at least 72 hr using the following grading based on international standards (14), further refined by the German Contact Dermatitis Group (13): neg,?, +, ++, +++, irritant, follicular.*"

0.5% (11/2065) consecutive/unselected patients showed positive patch test results to Eugenol (1% in pet.).

3.1.2.14 Study 29 (patch test, selected)

Study reference:

Vocanson, M., Goujon, C., Chabeau, G., Castelain, M., Valeyrie, M., Floc'h, F., ... & Nicolas, J. F. (2006). The skin allergenic properties of chemicals may depend on contaminants–evidence from studies on coumarin. *International archives of allergy and immunology*, 140(3), 231-238.

Detailed study summary and results:

Test type

Several research institutes in France conducted a prospective study testing the importance of contaminants in the sensitizing and allergenic properties of coumarin in mice and humans. The study included three different studies on which study no. 3 included patch test with eugenol on 19 patients. Time frame of patch test is not reported.

Description of the test method as cited from Vocanson et al. 2016:

"*Patch testing was done on the skin on the back using Finn Chambers on Scanpor (dc 8 mm). Two hundred and fifty-two patients of study 1 received a patch test of 2% coumarin only, while 100 other patients received patch tests of 1 and 10% and the European standard allergen series (Chemotechnique Diagnostics, Malmö, Sweden). The patients from studies 2 and 3 received patch tests of 2% coumarin and the first 8 allergens of the fragrance series. 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*."

20% (*n*=19) of the selected patients showed positive path test result to Eugenol (2%).

3.1.2.15 Study 30 (patch test, consecutive/unselected)

Study reference:

Vejanurug, P., Tresukosol, P., Sajjachareonpong, P., & Puangpet, P. (2016). Fragrance allergy could be missed without patch testing with 26 individual fragrance allergens. *Contact dermatitis*, 74(4), 230-235.

Detailed study summary and results:

Test type

Institute of Dermatology (Thailand) conducted a prospective study including 312 consecutive dermatitis patients from the period 2013-2014. The patients were patch tested with the baseline series: FM I and II, Myroxylon pereirae and the 26 individual fragrance allergens stated to be labelled on cosmetic products according to the EU Cosmetic Directive.

Description of the test method as cited from Vejanurug et al. 2016:

" *Allergens (provided by Chemotechnique Diagnostics, Vellinge, Sweden) in Finn Chambers® (SmartPractice®, Phoenix, AZ, USA) were applied on the back. The results were read at day 2 and 4 according to ICDRG criteria.*"

1% (3/312) consecutive patients showed positive patch test results to Eugenol (Concentration and vehicle not reported).

3.1.2.16 Study 31 (patch test, consecutive/unselected)

Study reference:

Diepgen, T. L., Ofenloch, R., Bruze, M., Cazzaniga, S., Coenraads, P. J., Elsner, P., Goncalo, M, Svensson, Å., & Naldi, L. (2015). Prevalence of fragrance contact allergy in the general population of five European countries: a cross-sectional study. *British Journal of Dermatology*, 173(6), 1411-1419.

Detailed study summary and results:

Test type

Occupational and Environmental Dermatology, University Hospital Heidelberg (Germany) have with other Departments and Clinics of Dermatology in Europe, conducted a cross-sectional epidemiological study in the following European countries: Sweden, Germany, the Netherlands, Italy and Portugal, 2008-2011. The project is named the EDEN Fragrance Study and includes subjects from the general population. In all 3119 subjects were patch tested to FM I and II, and their single chemical components (Eugenol is included in FM I).

Description of the test method as cited from Diepgen et al. 2015:

"*A random sample was selected from the general population, based on electoral precincts, aged 18–74 years.[3] The study followed a stratified, proportional sampling with replacement design as described previously.[4] In total, 12 377 subjects were interviewed with a standardized questionnaire, and a random sample (n = 3119) was patch tested to investigate sensitization to various allergens*"… "*Patch testing was performed according to the International Contact Dermatitis Research Group guidelines.[6] Weak (+), strong (++) and extreme (+++) reactions with an allergic morphology are considered positive reactions (Appendix S1; see Supporting Information)*."

0.2% (6/3119) consecutive patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.17 Study 32 (patch test, selected - workplace)

Study reference:

Buckley, D. A., Rycroft, R. J. G., White, I. R., & McFadden, J. P. (2002). Fragrance as an occupational allergen. *Occupational medicine*, 52(1), 13-16.

Detailed study summary and results:

Test type

St. John Institute of Dermatology, St Thomas' Hospital, UK, conducted a retrospective study to address the frequency of fragrance allergy in patch test patients of differing occupations. The Hermal FM (8% in pet.) was used with the constituents of oak moss, isoeugenol, eugenol, cinnamal, cinnamic alcohol, alpha-amyl cinnamal, geraniol, hydroxycitronellal.

All patients were referred to the Institute and specific occupational groups were included if more than 15 patients were fragrance allergic. In total 24,046 patients were included, which were tested between 1984-1998.

1813 were tested positive to the FM and 1112 were tested with the single constituents.

159/1112 workers showed positive patch test results to Eugenol (1% in pet.).

25% (14/55) health care workers showed positive patch test results to Eugenol (1% in pet.).

39.3% (11/29) metalworkers showed positive patch test results to Eugenol (1% in pet.).

3.1.2.18 Study 33 (patch test, consecutive/unselected)

Study reference:

Ung, C. Y., White, J. M. L., White, I. R., Banerjee, P., & McFadden, J. P. (2018). Patch testing with the European baseline series fragrance markers: a 2016 update. *British Journal of Dermatology*, 178(3), 776-780.

Detailed study summary and results:

Test type

Guy's and St Thomas' University Hospital, St John's Institute of Dermatology, UK conducted a retrospective study of patch testing with the European baseline series, extended with the individual fragrance substances during the period 2015-2016, with the aim to investigate the validity of patch testing using the European base line series and the ability to test allergy to the 26 individual fragrance substance for which cosmetic ingredient labelling is mandatory in EU. In total data from 2084 patients were included in the study, all had reacted to either a fragrance marker from the European baseline series or to at least one of the allergens in the fragrance series.

Description of the test method as cited from Ung et al. 2018:

"*Patch testing was performed with aluminium Finn Chambers® (Biodiagnostics Ltd, Upton on Severn, U.K.) and allergens were provided by Biodiagnostics®, Trolab® (Hermal Almirall, Reinbeck, Germany) and Chemotechnique® (Vellinge, Sweden). Allergens were prepared in petrolatum; FMI, M. pereirae and E. prunastri contained additional sorbitan sesquioleate 5% as an emulsifier. All allergens were stored, dispensed and used according to the manufacturers' instructions within the recommended stability periods. Reactions were read on day 2 and day 4, according to the European Society of Contact Dermatitis guidelines[4] Reactions documented as questionable or irritant were considered to be negative.*"

0.5% (11/2084) unselected/consecutive patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.19 Study 34 (patch test, consecutive/unselected)

Study reference:

Bennike, N. H., Zachariae, C., & Johansen, J. D. (2017). Non-mix fragrances are top sensitizers in consecutive dermatitis patients–a cross-sectional study of the 26 EU-labelled fragrance allergens. *Contact Dermatitis*, 77(5), 270-279.

Detailed study summary and results:

Test type

National Allergy Research Centre, University of Copenhagen (Denmark) conducted a cross-sectional study on consecutive dermatitis patients. 6004 patients were included and patch tested with the European baseline series and the 26 fragrances mandatory to label in EU, in the period 2010-2015.

Description of the test method as cited from Bennike et al. 2017:

"*In addition to the European baseline series, baseline patch testing at our department was performed with our fragrance series from Trolab®, provided during the study period by Almirall Hermal (Reinbek, Germany), consisting of the 25 fragrance ingredients and SSO 20% pet."... " Between 45.4–53.1% of patients aged <18years (n=262) were not tested with the single constituents of our fragrance series, but only*

with FM I, FM II, and HICC, owing to the limited space on their back. Patch testing was performed with FinnChambers® (8mm;SmartPractice,Phoenix,AZ,USA) applied on the upper back for 48h with Scanpor® tape (Norgesplaster, Vennesla, Norway). Patch test readings were performed on day (D) 2, D3 or D4, and D7, and the maximum reactions are presented here. Grading of positive allegic reactions as weak (+), strong (++), and extreme(+++), and the scoring of doubtful (?+) and irritantreactions (IRs), were performed according to international guidelines, which, retrospectively, are compliant with the current criteria implemented by the ESCD in 2015(7, 31). In the assessment of concomitant reactivity to FM I and FM II and their single constituents, patch test reactions to the respective mix were grouped as either positive, doubtful (?+), or negative (including IRs)."

In all 5772 consecutive patients were tested concomitantly with FM I and its single constituents. Patients sensitized to the emulsifier were excluded from the statistically analysis of the single constituents.
0.36% (21/5772) consecutive patients showed positive patch test results to Eugenol (1% in pet.).

3.1.2.20 Study 35 (patch test, selected)

Study reference:
Schnuch, A., & Griem, P. (2018). Fragrances as allergens. *Allergo Journal International*, 27(6), 173-183.

Detailed study summary and results:
Test type
Information Network of Departments of Dermatology, Georg-August University, Germany and Department of Medical Informatics have conducted a study to analyse the internal ranking of fragrance allergens from the late 1970s. The study also investigates cross reactivity between selected fragrance compounds. Using data from the IVDK a retrospective analysis was conducted of FM I including data from the period 1998-2003.

Description of the test method as cited from Schnuch et al. 2018:
"*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.*"

The study illustrates a significant decrease in sensitizations to eugenol from 1998/1999 to 2002/2003. However, from 2008/2009 to 2012/2013 a slight increase in sensitization frequency is observed with more or less all eight components in FM 1.

Table 1. Percentages of positive reactions to eugenol in patients with a positive reaction to FM 1, but not to SSO. All patch tests were conducted with Eugenol in 1% with pet.

Year	1998/ 1999	2000/ 2001	2002/ 2003	2004/ 2005	2006/ 2007	2008/ 2009	2010/ 2011	2012/ 2013	1998-2013
n	162	139	249	281	285	469	634	513	2789
% positive	11.7	7.2	4.8	9.3	8.4	6.6	6.3	6.8	7.3

3.1.2.21 Study 36 (patch test, selected)

Study reference:

Silvestre, J. F., Mercader, P., González-Pérez, R., Hervella-Garcés, M., Sanz-Sánchez, T., Córdoba, S., ... & Pastor-Nieto, M. A. (2019). Sensitization to fragrances in Spain: A 5-year multicentre study (2011-2015). *Contact dermatitis*, 80(2), 94-100.

Detailed study summary and results:

Test type

The Spanish Research Group on Contact Dermatitis and Skin Allergy (Consisting of 23 Departments of Dermatology, most on hospitals, in Spain) conducted a retrospective study on patch test conducted with the Spanish baseline series, consisting of FM I, FM II, *Myroxylon pereirae*, and hydroxyisohexyl 3-cyclohexene carboxaldehyde. The study included data from the period 2011-2015 and included in total 19,588 patients.

Two different specific fragrance series with different components and different concentrations for the ingredients of FM I was used. The author states this may affect their results, while they may have underestimated the frequency of sensitization to individual components of FM I.

Description of the test method as cited from Silvestre et al. 2019:

"*Patch tests were performed in accordance with ICDRG/ESCD recommendations*[17]*. Patch test preparations were applied for 48 hours, and readings were performed at least on day (D) 4 according to standard international grading. A dermatologist assessed the patch test results, considering doubtful or irritant reactions as negative*."

7.9% (80/1013) selected patients (had previously reacted positively to a fragrance marker) showed positive patch test results to Eugenol (1-2% in pet.).

3.1.2.22 Study 37 (patch test, consecutive/unselected)

Study reference:

Mowitz, M., Svedman, C., Zimerson, E., Isaksson, M., Pontén, A., & Bruze, M. (2017). Simultaneous patch testing with fragrance mix I, fragrance mix II and their ingredients in southern Sweden between 2009 and 2015. *Contact Dermatitis*, 77(5), 280-287.

Detailed study summary and results:

Test type

Department of Occupational and Environmental Dermatology, Lund University, Sweden performed at retrospective study with patch test data from 4430 consecutive dermatitis patient, tested between 2009 -2015. The patients were patch tested with FM I, FM II, and the 14 single components.

Description of the test method as cited from Mowitz et al. 2017:

" *Between 2009 and 2012, patch testing was performed with FM I and FM II ingredients mixed in pet. (Snow White Quality E; Apoteket Produktion & Laboratorier, Göteborg, Sweden) at our department, and with FM I and FM II preparations prepared by Chemotechnique Diagnostics (Vellinge, Sweden) using substances from the same batches that were used in the individual preparations. Between July 2012 and December 2015, a FM II preparation purchased from Chemotechnique Diagnostics was used. Between January 2013 and December 2015, all fragrance test preparations used were from Chemotechnique Diagnostics."... " Patch testing was performed with Finn Chambers®(diameter, 8mm; Smart Practice, Phoenix, AZ, USA). According to recommendations, 20mg of pet. preparations were applied in the test chambers (6). The patch tests were removed by the patients after 48h, and readings were performed by a dermatologist on day (D) 3 or D4 and on D7 according to ICDRG criteria (7). In order to prevent evaporation of the fragrance substances, all fragrance test preparations were loaded in the test chambers immediately before the test chambers were applied to the back of the patient (8,9)*."… "*Doubtful and irritant reactions were considered to be negative.*"

During the time-frame of the study, it was noted that the positive reactions to eugenol was significantly higher in 2009-2012, whereas significantly more positive reactions to FM I were observed in 2013-2015 (caused by more positive reactions in males). The authors suggest this may be explained by differences in the study population and their exposure to fragrances, or by differences in the patch test material. From the study the positive reactions to eugenol had a rise in 2012 whereafter a decrease in the year 2013-2015 was observed (compared to 2012).

2009-2012: 0.9% (19/2235) consecutive dermatitis patients showed positive patch test results to Eugenol (2% in pet.). 2013-2015: 0.3% (7/2248) consecutive dermatitis patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.23 Study 38 (ROAT)

Study reference:

Svedman, C., Engfeldt, M., Api, A. M., Politano, V. T., Belsito, D. V., Isaksson, M., & Bruze, M. (2012). A pilot study aimed at finding a suitable eugenol concentration for a leave-on product for use in a repeated open application test. *Contact dermatitis*, 66(3), 137-139.

Detailed study summary and results:

Test type

Department of Occupational and Environmental Dermatology, Skåne, Sweden, conducted a prospective study of patients from Sweden. The repeated open application tests (ROATs) were conducted with higher concentrations (maximum allowed) and with a longer ROAT time. A total of 5 patients, previously tested positive to FM I and to eugenol (2%) were included in the study.

Description of the test method as cited from Svedman et al. 2012:

"*The study was thus performed as a serial dilution patch test with eugenol performed twice (first when the ROAT was started, and then at day 49), and, in the time span between the patch tests, a ROAT was performed for 4 weeks.*

Patch testing. The patch test system was Finn Chambers® on Scanpor® tape. The serial dilution used contained 17 dilutions of eugenol in DEP/EtOH 2:98 (vol/vol): 2.0%, 1.32%, 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% (wt/vol). As control preparations, the vehicle 2:98 DEP/EtOH and its separate constituents DEP and EtOH were tested. Fifteen microlitres of test solution was micropipetted onto each filter paper disc in the test chambers. The tests remained on the back of the patients for 48hr. The patch tests were read according to the International Contact Dermatitis Research Group criteria (5) on day 3 and day 7 by a dermatologist.

ROAT. The ROAT was performed with four different solutions: three contained eugenol diluted in the same vehicle as used for patch testing at concentrations of 2.7%, 1.0%, and 0.5%, and one solution contained only the vehicle (DEP/EtOH). ROAT was performed on four sites, two on each arm (3× 3 cm each) on the lower volar aspect. The four solutions were randomized to the four sites of the arms according to a Latin square table. The squares on the arms were colour-coded in the same manner as the bottles used for the provocation solutions, to ensure that the correct solution was used for the correct area. For the ROAT, 5-ml propylene droplet bottles (Chemotechnique Diagnostics®, Vellinge, Sweden) were used, each being filled with 3.0 ml of solution. The bottles were colour-coded. Every week, the participants were supplied with fresh bottles. The

concentration was based on the recommended concentration for use in leave-on products according to IFRA standards. The subjects were instructed on how to apply the solution, and to let the solution dry before dressing. Each solution was applied twice daily by the subjects. Two droplets of solution were placed on the marked sites and distributed evenly on the marked skin with the tip of the bottle. The ROAT reading was performed at least once weekly by a dermatologist, according to a manual described previously(4). In the case of a positive reaction, the application (see below) was stopped for that exposure and continued for the others until a reaction occurred or until the ROAT was terminated after 28 days. The randomization code was not broken until the study had finished."

4/5 patients were positive to concentrations down to 1.32% eugenol in the patch test and the ROAT showed 4/5 patients had positive reactions to 2.7% eugenol and 1/5 showed positive reaction to 1% eugenol.

3.1.2.24 Study 39 (patch test, selected)

Study reference:

An, S., Lee, A. Y., Lee, C. H., Kim, D. W., Hahm, J. H., Kim, K. J., ... & Eun, H. C. (2005). Fragrance contact dermatitis in Korea: a joint study. Contact Dermatitis, 53(6), 320-323.

Detailed study summary and results:

Test type

Nine Departments of Dermatology in different university hospitals of Korea and a cosmetic company have conducted a prospective study in which they determined the frequency of responses to selected fragrances in patients with suspected fragrance allergy and to evaluate the risk factors.

422 patients with suspected cosmetic contacts dermatitis were tested with the Korean standard, additional 18 fragrances and a commercial fragrance series, including eugenol. Patch tests were conducted April 2002 to June 2003.

Description of the test method as cited from An et al. 2005:

"*Finn Chambers on Scanpor fape (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).*"

1.9% (8/422) selected patients showed positive patch test results to Eugenol (2% in pet.).

3.1.2.25 Study 40 (patch test, selected)

Study reference:

Schnuch, A., Geier, J., Uter, W., & Frosch, P. J. (2002). Another look at allergies to fragrances: frequencies of sensitization to the fragrance mix and its constituents. Exogenous Dermatology, 1(5), 231-237.

Detailed study summary and results:

Test type

The IVDK contributed with data to study the frequency of sensitization to the FM and its single compounds (SCs), and evaluated the sensitivity of the FM to diagnose sensitization to SCs.
35,599 unselected patients were patch tested with FM 8% in pet. in 1996-1999, and 4,900 patients were patch tested with the 8 SCs (1% in pet.).

Description of the test method as cited from Schnuch et al. 2002:
"*.. 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*."

1.9% (92/4,900) mostly unselected, but partly selected after being tested with the FM, showed positive patch test results to Eugenol (1% in pet). 57.1% of the 4,900 patients were tested positive to FM and the frequency of sensitization to the FM is therefor higher than in unselected population.

Compared to the other studies in this dossier, the frequency of sensitivity toward eugenol is more similar to studies with selected patients than consecutive/unselected, and the studies if for this reason categorized as including"selected patients".

3.1.2.26 Study 41 (patch test, selected)

Study reference:

Giusti, F., Porcaro, V., & Seidenari, S. (2001). Evaluation of eugenol allergy in a patch-test population. Contact Dermatitis: Short Communications, 44(1), 55-56.

Detailed study summary and results:

Test type

Department of Dermatology, University of Modena (IT), has conducted a prospective study of eugenol sensitization. The study included 1754 selected patients, suspected of having ACD which all were patch tested with the Italian standard series and eugenol (1% in pet.). The patch test were conducted in the period September 1998 to January 2000.

Description of the test method as cited from Giusti et al. 2001:

"*The test substances, provided by Hermal-Trolab (Germany) and FIRMA (Italy), were applied to healthy skin of the back with Finn Chambers (Norgesplaster, Norway) on Scanpor tape (Epitest, Finland) for 3 days. Reactions were evaluated 30 min after removal, according to ICDRG guidelines.*"

1.2% (21/1754) selected patients showed positive patch test results to Eugenol (1% in pet.).

3.1.2.27 Study 42 (patch test, selected)

Study reference:

Wöhrl, S., Hemmer, W., Focke, M., Götz, 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(2), 268-273.

Detailed study summary and results:

Test type

Floridsdorf Allergy Centre in Austria conducted a prospective study to demine the usefulness of adding propolis to the European standard series to test for fragrance allergy. In total 2,660 consecutive patients were included in patch tested with standard patch test series. The standard patch test series consisted of 34 allergens, including FM I (8%). Of the 2,660 patients 747 were suspected of fragrance allergy and were tested further with a special fragrance series, including eugenol in 1% in pet. Patch test were conducted in the period from 1997-2000.

Description of the test method as cited from Wöhrl et al. 2001:

"*The fragrance mix and the fragrance series were acquired from Hermal (Reinbek, Germany); all other allergens were bought from Brial Allergen (Greven, Germany). The readings were done after 72 h and scored according to the recommendations of the International Contact Dermatitis Research Group (ICDRG).*"

2.5% (19/747) selected patients showed positive patch test results to Eugenol (1% in pet.)

3.1.2.28 Study 43 (patch test, selected)

Study reference:

Hendriks, S. A., & Van Ginkel, C. J. W. (1999). Evaluation of the fragrance mix in the European standard series. Contact Dermatitis, 41(3), 161-162.

Detailed study summary and results:

Test type

University Hospital in the Netherlands conducted a retrospective study with 50 selected patients, suspected of allergy to cosmetics. The patients were patch tested with the European standard series, including FM and its 8 separate components (of which eugenol is one).
Patch test period 1994-1998.
No information of the methods is reported.

12% (6/50) selected patients showed positive patch test with Eugenol (2% in SSO1%).

3.1.2.29 Study 44 (patch test, selected)

Study reference:
Katsarma, G., & Gawkrodger, D. J. (1999). Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. Contact Dermatitis, 41(4), 193-197.

Detailed study summary and results:
Test type
Department of Dermatology, Royal Hallamshire Hospital (UK) conducted a retrospective study with patch test data from 744 consecutive patient. Of these consecutive patients, patients with a history of facial, perianal or vulval dermatitis, or with a suspected sensitivity towards cosmetic products (in total 40 patients), were selected to further testing. Patch tests were performed with FM, its single constituents or to an extended series of fragrance. Eugenol is included in FM. The patch test were conducted in the period 1994-1995.

Description of the test method as cited from Katsarma & Gawkrodger:
"*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*..."

2/50 selected patients showed positive patch test with Eugenol (1% in pet.)

3.1.2.30 Study 45 (patch test, selected)

Study reference:
Larsen, W., Nakayama, H., Lindberg, M., Fischer, T., Elsner, P., Burrows, D., ... & Sugawara, M. (1996). Fragrance contact dermatitis: a worldwide multicentre investigation (Part I). American Journal of Contact Dermatitis, 7(2), 77-83.
Also cited in SCCNFP, 1999.

Detailed study summary and results:
Test type

A retrospective study with patch test data from seven centres worldwide containing a total of 167 selected patients. Patients with proven sensitization to fragrance materials established by previous patch tests to fragrance allergens or established to be fragrance sensitive based on historical and clinical grounds were selected to be included in the study. The aim of the study was to determine the prevalence of responses to selected fragrance materials in selected patients and to evaluate risk factors and associations with such responses. The patch tests were conducted with FM and the eight single constituents, 6 other fragrance allergens, balsam of Peru and15 less studied fragrance materials.
The test centres were located in Japan, Northern Ireland, United States, England, Switzerland and Sweden.

Description of the test method 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 system.*[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 sites reading were made by the investigators."

7.8% selected patients showed positive patch test with Eugenol (5% in pet.).

3.1.2.31 Study 46 (patch test, consecutive/unselected)

Study reference:

Frosch, P. J., Pilz, B., Andersen, K. E., Burrows, D., Camarasa, J. G., Dooms-Goossens, A., ... & Wilkinson, J. D. (1995). Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact* Dermatitis, 33(5), 333-342.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Also cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999.

Test type

A prospective European multicentre study of 1072 consecutive patients in 9 centres.
13/1072 (1.2%) had a positive reaction to eugenol 1%.

3.1.2.32 Study 47 (patch test, selected)

Study reference:

Larsen, W. G. (1977). Perfume dermatitis: a study of 20 patients. *Archives of dermatology*, *113*(5), 623-626.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Also cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999.

Test type

20 perfume allergic patients were tested with several screening series og fragrances.
Eugenol 2% gave a positive reaction in 4/20 (20%) patients.

3.1.2.33 Study 48 (patch test, selected)

Study reference:

Adams, R. M., Maibach, H. I., Clendenning, W. E., Fisher, A. A., Jordan, W. J., Kanof, N., ... & Marzulli, F. N. (1985). A five-year study of cosmetic reactions. Journal of the American Academy of Dermatology, 13(6), 1062-1069.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Also cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Detailed study summary and results:

All information retrieved from Bredsdorff & Nielsen, 2016.

Test type

Patch test: Retrospective study of 403 selected patients with cutaneous reactions to cosmetic products patch tested with eugenol. Concentration and vehicle not reported.

Unclear how many patients were tested with eugenol

4/403 (1%) patients were positive.

3.1.2.34 Study 49 (patch test, selected)

Study reference:

Broneck, W., Blondeel, A., Dooms-Goossens, A., & Achten, G. (1987). Cosmetic intolerance. *Contact dermatitis*, *16*(4), 189-194.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Also cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999.

Test type

Patch test study with 156 selected patients with contact allergy to cosmetic products patch tested with eugenol. Concentration and vehicle not reported. 11/156 (7.11%) patients were positive.

3.1.2.35 Study 50 (patch test, selected)

Study reference:

Wilkinson, J. D., Andersen, K., Camarasa, J., Ducombs, G., Frosch, P., Lahti, A., ... & White, I. (1989). Preliminary results on the effectiveness of two forms of fragrance mix as screening agents for fragrance sensitivity. In *Current topics in contact dermatitis* (pp. 127-131). Springer, Berlin, Heidelberg.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Also cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999.

Test type

In a European multicentre study involving 6 countries, 78 patients, positive to one or the other of two different FMs, both containing eugenol, were tested with the individual constituents of the mixes. 8/78 (10.3%) were positive to eugenol 2%.

3.1.2.36 Study 51 (patch test, selected)

Study reference:

Santucci, B., Cristaudo, A., Cannistraci, C., & Picardo, M. (1987). Contact dermatitis to fragrances. *Contact Dermatitis*, *16*(2), 93-95.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Also cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Detailed study summary and results:

All information retrieved Bredsdorff & Nielsen, 2016.

Test type

Retrospective study of patch test data from Istituto Dermatologico Santa Maria e San Gallicano, Italy (1983-1985).

A retrospective study of 63 selected patients with dermatitis tested positive to perfume mixture were patch tested between 1983 and 1984 with eugenol 5% in pet. and 54 selected patients with dermatitis tested positive to perfume mixture were patch tested between 1984 and 1985 with eugenol 1% in pet.

Between 1983 and 1984 8/63 (12.7%) and between 1984 and 1985 9/54 (16.7%) patients were tested positive.

3.1.2.37 Study 52 (patch test, selected)

Study reference:

Johansen, J. D., & Menné, T. (1995). The fragrance mix and its constituents: a 14-year material. *Contact Dermatitis*, *32*(1), 18-23.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

and in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999 and Bredsdorff & Nielsen, 2016.

Test type Retrospective study of patch test data from Department of Dermatology, Gentofte Hospital, Denmark (1979-1983 and 1988-1992).

A total of 367 selected patients were patch tested with eugenol 1-2%.

30/367 (8.2%) patients were tested positive.

3.1.2.38 Study 53 (patch test, selected)

Study reference:

Becker, K., Temesvari, E., & Nemeth, I. (1994). Patch testing with fragrance mix and its constituents in a Hungarian population. *Contact Dermatitis*, *30*(3), 185-186.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Also cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Detailed study summary and results:

All information retrieved from Bredsdorff & Nielsen, 2016.

Test type

Prospective study of patch test data from Departments of Dermatology and Venereology, Hungary. Year not stated.

Study of 50 selected patients positive to FM patch tested with eugenol. Concentration and vehicle not reported. 3/50 (6%) patients were tested positive.

3.1.2.39 Study 54 (patch test, selected)

Study reference:

Enders, F., Przybilla, B., & Ring, J. (1989). Patch testing with fragrance mix at 16% and 8%, and its individual constituents. *Contact Dermatitis*, *20*(3), 237-238.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

and in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999 and Bredsdorff & Nielsen, 2016.

Test type

Prospective study of patch test data from Dermatologische Klinik und Poliklinik, Germany (1991). 162 selected patients positive to FM were patch tested with eugenol 1% in pet.

11/162 (6.8%) patients tested positive.

3.1.2.40 Study 55 (patch test, selected)

Study reference:

Artigou, C., Pecquet, C., Pradalier, A., Leynadier, F., & Dry, J. (1989). Dermite de contact aux parfums. *Médecine et hygiène*, *47*(1788), 947-952.

As cited in: Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. (1999). Fragrance allergy in consumers. A review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens. Adopted 8th of December.

Detailed study summary and results:

All information retrieved from SCCNFP, 1999.

Test type

In France, the frequency of contact allergy caused by eugenol in patients positive to the FM, is reported to be 22%.

3.1.2.41 Study 56 (patch test, selected)

Study reference:

De Groot, A. C., Van der Kley, A. M. J., Bruynzeel, D. P., Meinardi, M. M. H. M., Smeknk, G., Van Joost, T., & Pavel, S. (1993). Frequency of false-negative reactions to the fragrance mix. *Contact Dermatitis*, *28*(3), 139-140.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from Bredsdorff & Nielsen, 2016.

Test type

Prospective study with patch test data from patients positive to a FM, patch tested with eugenol 5% in pet. Conducted by University of Amsterdam and University of Leiden, The Netherlands (1987).

12/61 (19.7%) patients had positive patch test with eugenol.

3.1.2.42 Study 57 (patch test, selected)

Study reference:

Brites, M. M., Gonçalo, M., & Figueiredo, A. (2000). Contact allergy to fragrance mix: a 10-year study. *Contact dermatitis*, *43*(3), 181-182.

As cited in: Bredsdorff, L., Nielsen, E. (2016). Evaluation of selected sensitizing fragrance substances. A LOUS follow-up project. Environmental project No. 1840. Environmental Protection Agency. Ministry of Environment and Food of Denmark.

Detailed study summary and results:

All information retrieved from Bredsdorff & Nielsen, 2016.

Test type

Department of Dermatology, University Hospital, Coimbra, Portugal (1989-1999). Study of 226 selected patients sensitive to FM were patch tested with eugenol 1% in pet.

33/226 (14.6%) patients tested positive.

3.1.2.43 Study 58 (case study)

Study reference:

Hill, H., & Jacob, S. E. (2015). Peri-anal ulcerations in a patient with essential pruritus. Dermatitis, 26(6), 292-293.

Detailed study summary and results:

Test type

A 72-year old man suffering from nonhealing peri-anal erosions. The patient had used a daily self-treatment with an analgesic cream containing menthol-eugenol-methyl-salicylate, to treat his chronic peri-anal pruritus. The patient experienced a blistering reaction 10 days after the daily self-treatment was initiated, and sores developed in the area. The patient treated the sores with a healing spray containing trypsin, balsam of Peru (containing eugenol) and castor oil, which lead to progression of the sores to ulceration. Patch testing with a modified North American Contact Dermatitis Standard Screening Tray was performed. After 96-hours the reading showed positive reactions to eugenol, Balsam of Peru and Granul-derm spray.

3.1.2.44 Study 59 (case study)

Study reference:

Nic Dhonncha, E., & Bourke, J. F. (2020). Allergic contact dermatitis to a “natural analgesic” patch. Contact dermatitis, 83(3), 232-233.

Detailed study summary and results:

Test type

A 14-old female was suffering of a recurrent itchy rash (well-demarcated erythematous vesicular eruption) affecting her left hand and right elbow, on three occasions. The rash had appeared after application of a "natural analgesic" patch to her right elbow. Patch testing with the British Society of Cutaneous Allergy baseline, facial, fragrance, rubber, and plant series. Reading on day 2 showed positive reactions to Balsam of Peru, FM I and the "analgesic". Reaction to eugenol was questionable on day 2, but on day 4 positive reactions to hydroperoxides of eugenol, cinnamyl alcohol, cinnamal, linalool and limonene were seen. Positive reactions observed on day 2 were also observed as positive on day 4.

3.1.2.45 Study 60 (case study)

Study reference:

Hadzavdic, S. L., Jovic, A., Hadzavdic, A., & Grgec, D. L. (2018). Vulvar oedema. Contact Dermatitis, 78, 223-239.

Detailed study summary and results:

Test type

A 22-old female was suffering from pruritus, oedema and erythema of the labia minora 24 hours after using a pleasure gel. Two months after, a patch test with a baseline series, and a semi-open test with the pleasure gel was conducted. On day 3 positive reactions were read for FM I and the pleasure gel. The patch test followed by a patch test with the single components and reading on day 3 showed positive to cinnamyl alcohol, cinnamal, eugenol, isoeugenol, geraniol and hydroxycitronellal.