

Contact allergy to fragrance mix I and its components in individuals with photocontact allergy to ketoprofen

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Conflict of interests: Magnus Bruze is a member of the expert panel for fragrance safety - <http://fragrancesafetypanel.org/>

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Abstract

Background: Contact allergy to fragrance mix I (FM I) is over-represented in patients photoallergic to ketoprofen. The prevalence of contact allergy to two components of FM I, cinnamal and cinnamyl alcohol, in ketoprofen-photoallergic patients is higher than in dermatitis patients.

Objective: To explore the prevalence of contact allergy to FM I and its individual components in patients with photocontact allergy to ketoprofen, and to compare with a dermatitis and the general population.

Methods: Data on patch and photopatch tests performed between 2009-2018 were collected. Ketoprofen-photoallergic patients were compared with dermatitis patients and published data on the general population regarding the prevalence and the distribution of contact allergy to FM I and its components.

Results: A higher prevalence of contact allergy to cinnamyl alcohol compared to cinnamal (23.3% vs 10.0%), and eugenol compared to isoeugenol (23.3% vs 6.7%), was observed in ketoprofen-photoallergic patients, while the relationship was the opposite in dermatitis group (0.7% vs 1.05%; 0.4% vs 0.9%). Overall prevalence of contact allergy to several components of FM I was significantly higher in ketoprofen-photoallergic patients.

Conclusions: Contact allergy to FM I and many of its components is over-represented in patients photoallergic to ketoprofen compared to dermatitis patients and the general population.

Key words: cinnamal, cinnamyl alcohol, eugenol, chemical analysis, contamination, delayed hypersensitivity, fragrance substance, GC-MS, NSAID, ketoprofen, patch testing, photopatch testing

Introduction

The non-steroidal anti-inflammatory drug (NSAID) ketoprofen has become one of the most important photosensitizers that are presently used in both systemic and topical preparations. Reports on photoallergic contact dermatitis from ketoprofen are not rare in the literature¹⁻⁶. In 2001 topical ketoprofen was reported to be the major photosensitizer among topically applied drugs in Sweden⁶. In fact, its over-the-counter distribution was stopped by the Swedish Medical Products Agency in 2011 due to a high rate of reported photocontact allergic reactions⁶. In France, the incidence of photocontact allergy to ketoprofen remained high despite various interventions, including direct information to primary care physicians and warnings on the packages regarding risks associated with sun exposure, according to a pharmacovigilance study⁷. However, as the efficacy of orally administered ketoprofen is described as superior to ibuprofen and diclofenac⁸, ketoprofen-containing drugs, including gels, can still be prescribed by physicians. As photocontact allergy is now a well-known side effect of topical ketoprofen, the under-reporting may be significant. Occupational risk for developing photoallergic contact dermatitis has been described as well⁹.

When a patient is photoallergic to ketoprofen there is a significantly higher risk for a simultaneous contact allergy to fragrance mix I (FM I)^{1,3,4,10-12}. Furthermore, several reports have shown an over-representation of contact allergy to two of the components of the mix, namely cinnamal and cinnamyl alcohol, with higher numbers of contact allergy to cinnamyl alcohol^{3,11,13}. Also those investigated for contact or photocontact allergy other than to ketoprofen (hereafter referred to as “dermatitis patients”), and who are diagnosed with contact allergy to FM I, are known to often react to cinnamal and cinnamyl alcohol, but to a significantly lower degree, and with fairly even distribution of the reactions between cinnamal and cinnamyl alcohol^{1,4,14-17}.

After the initial spike in interest, the research on the photoallergenic properties of ketoprofen has now entered a phase when very few new findings are being published. The phenomenon of simultaneous allergic reactions to non-related chemicals observed in patients with photoallergy to ketoprofen is not, however, close to being fully understood.

Our primary aim was to investigate the distribution of contact allergy to cinnamal and cinnamyl alcohol in patients with contact allergy to FM I with or without photocontact allergy to ketoprofen,

There have been reports on impurities in test preparations, as well as incorrect labelling of test substances¹⁸⁻²⁰. As our secondary aim we wanted to ensure that no mistake in labelling of the test preparations with cinnamal and cinnamyl alcohol had occurred and led to a difference in test results between ketoprofen and dermatitis patients

To our knowledge, there are no studies examining the prevalence of contact allergy to other individual components of FM I in ketoprofen photoallergic patients.

This insight has led us to our third aim - mapping of a distribution of contact allergy to all individual constituents of FM I

Materials and methods

Study design:

All data on ketoprofen-photoallergic/dermatitis patients in this retrospective study were collected from our local database. The database contains information on patch and photopatch testing of patients that have been investigated because of a suspected allergic/photoallergic contact dermatitis.

We have specifically looked at the results of patch testing with FM I and its individual components in 4 main groups (i, ii, iii, and iv) of patients: (ia, ib) photopatch tested patients with photocontact allergy to ketoprofen, (ii) photopatch tested patients without photocontact allergy to ketoprofen, (iii) dermatitis patients patch tested because of a suspected allergic contact dermatitis, and (iv) individuals representing the general population (Fig. 1). The contact allergy rates to FM I and its components were compared between the target group, consisted of patients with photocontact allergy to ketoprofen (ia, ib), and the 3 control groups (ii, iii, iv).

Chemical analyses of patch test preparations with cinnamal and cinnamyl alcohol used for patch testing during the given time period were performed.

Patients

3 groups of controls were used according to Fig. 1. In the period 2009-2018, 6846 patients (2256 males, 4590 females, mean age 46.2 years, range 4-99 years) were patch and/or photopatch tested because of a suspected allergic contact dermatitis and/or photoallergic contact dermatitis (Fig. 1). Photopatch testing was performed in 283 patients. Of these a minority, 69 of 283 patients, was only photopatch tested, while 214 of the 283 patients were also patch tested. The majority, 214 patients (mean age 48.2 years, range 4-90 years) had been photopatch tested with the European photopatch test series²¹. Patch testing exclusively was performed in 6563 patients (2158 males and 4405 females; mean age 45.1 years, range 4-99 years) (Fig. 1).

Dermatitis patients who were both patch and photopatch tested (n=214) in the period 2009-2018 constituted both the target groups (photocontact allergy to ketoprofen, ia and ib) and a control group with those who photopatch tested negatively to ketoprofen in the European photopatch test series (n=148). 184 patients were photopatch tested with the European photopatch test series and patch tested with FM I. Of these, 148 were patch tested simultaneously with FM I and also with its constituents, and these patients constituted control group (ii). Dermatitis patients who were only patch tested and not photopatch tested in the period 2009-2018 (n=6563) constituted another control group (iii).

518 volunteers (males 252 and females 266; mean age 53.17 years, range 18-74 years) living in the Malmö metropolitan area represented the general population (control group iv). These volunteers were patch tested within a European study on contact allergy in the general population with a baseline series but also with the components of FM I (Fig. 1). The methodology used and results of the patch testing have been published previously^{22,23}. In this study the results of the testing with petrolatum preparations of FM I and its components are used. The test methodology with small Finn chambers, concentrations, vehicles, doses in mg/cm², manufacturers, and batches used for the preparations of FM I and its components were the same as for the patch and photopatch tested dermatitis patients in the present study.

Photopatch testing:

Test preparations

Initially the test preparation with ketoprofen was made at the Department of Occupational and Environmental Dermatology in Malmö. Ketoprofen from Sigma – Aldrich, Stockholm, Sweden was used to make a solution in ethanol 99.5% (Kemetyl AB, Haninge, Sweden) at 1.0% w/v and a petrolatum (vaselinum album, Apoteket AB, Gothenburg, Sweden) preparation at 1.0% w/w. Ketoprofen in ethanol was photopatch tested in 6 patients and the petrolatum preparation was tested in 277 patients as a part of an extended Scandinavian photopatch test series (Chemotechnique Diagnostics, Vellinge, Sweden)²⁴ or from 2013 as a part of the European photopatch test series (Chemotechnique Diagnostics)²¹.

The light source

The light source used was UV440DT IP20 luminare (ESSHÅ Elagentur AB, Värnamo, Sweden) equipped with 4 Philips PL-L 36W UVA tubes (Philips AB, Sundsvall, Sweden).

The metering device used to ensure that the right UVA dose was given was: Delcomp UV-meter (PUVA Combi Light, Leuven, Belgium).

Procedure:

Photopatch testing was conducted according to standard procedure^{21,24,25}. The testing was performed using duplicates of small (diameter 8 mm) Finn Chambers (SmartPractice®, Phoenix, Arizona, USA) secured to the back with Scanpor tape (Norgesplaster A/S, Vennesla, Norway). The identical 20 mg petrolatum preparations²⁶ or identical 15 µl ethanol solutions²⁷ were used on each side. The test preparations were applied to the chambers immediately before the application on the back²⁸. Occlusion time was 24h^{21,24,25}. One side was immediately covered with black cloth after the removal of the test strips to avoid any unintentional UV irradiation, the other side was irradiated with 5 J/cm² of broadband UVA (see above). Reading was performed on D3 and D7 (Magnus Bruze, personal communication 2021) according to the ICDRG classification^{21,29,30}.

Patch testing:

Test preparations:

Patch testing was performed with the Swedish baseline series (Chemotechnique Diagnostics) in which FM I is present and an extended Malmö baseline series in which the 8 FM I components were included.

The preparations of the FM I components cinnamal, cinnamyl alcohol (Bedoukian, Danbury, Connecticut, USA), hydroxycitronellal, eugenol (Firmenich Inc., Plainsboro, New Jersey, USA), amyl cinnamal, geraniol and isoeugenol (International Flavors &

Fragrances, Union Beach, New Jersey, USA), and *Evernia prunastri* extract (oak moss absolute, Robertet, Grasse, France) were prepared in petrolatum (Snow White Quality E, Apoteket Produktion & Laboratorier, Gothenburg, Sweden) at our department in Malmö. All FM I components were prepared at 2.0% w/w except for cinnamal which was prepared at 1.0% w/w. The petrolatum preparation with FM I used during this period was manufactured by Chemotechnique Diagnostics using substances from the same batches that were used in the individual preparations of the FM I components. Furthermore, the extended baseline series also contained a preparation of the emulsifying agent used in FM I, sorbitan sesquioleate 20% w/w in petrolatum.

Procedure:

20 mg petrolatum preparation²⁶ of the test preparations were placed on small (diameter 8 mm) Finn Chambers immediately before the application on the back using Scanpor tape²⁸. The patches were removed after 48h and the reading was performed twice on day 3 or 4 and 7 with scoring according to the ICDRG classification^{29,30}.

Statistical methods:

Pair-wise comparisons using Fisher's exact test, two-sided were performed between the target groups with photocontact allergy to ketoprofen and the control groups regarding the contact allergy rates to FM I and its constituents (Fig. 1). Patch and photopatch tested dermatitis patients with photocontact allergy to ketoprofen (target group ia) were compared with patch tested but not photopatch tested dermatitis patients (control group iii) as well as with patch tested volunteers representing the general population (control group iv)(Fig. 1). Dermatitis patients with photocontact allergy to ketoprofen, patch and photopatch tested with the European photopatch test series (target group ib) were compared with the dermatitis patients without photocontact allergy to ketoprofen, patch and photopatch tested with the European photopatch test series (control group ii)(Fig. 1) To compare the distribution of test reactions to the chemically closely related substances cinnamyl alcohol and cinnamal, as well as eugenol and isoeugenol, inside the ketoprofen photocontact allergy groups (target groups ia and ib; Fig. 1) and the control groups (ii) and (iii), McNemar's binominal exact test (two-tailed) was used. Fisher's exact test, two-sided, was used to compare the distribution of test reactions to cinnamyl alcohol and cinnamal, as well as eugenol and isoeugenol, between the group with ketoprofen photocontact allergy (target group ia; Fig.1) and the control group (iii) (Table 1 appendix, available as online supplement), A *P*-value <0.05 was regarded as statistically significant.

Ethics:

The study was approved by the Regional Ethical Review Board, Lund Sweden, Dnr 2020/02190.

When patients are patch tested and/or photopatch tested, they are informed that data may be used for comparisons on a group level and approval is mandatory if the patients' data are stored in the computer system.

Chemical analysis:

Gas chromatography-mass spectrometry (GC-MS)

Separation of components in the samples was performed with an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, U.S.A) equipped with an HP-MSI capillary column (Agilent Technologies) with a length of 30 m, an internal diameter of 0.250 mm and a film thickness of 0.25 μm . Helium of alphagaz 2 quality (Air Liquide, Malmö, Sweden) was the carrier gas with a flow rate of 1.0 ml/min. The injection was split-less and the inlet was heated to 250°C. The injection volume was 1 μl . The temperature program was isothermal at 70°C for 3 min, then rose with 8°C $\cdot$ min⁻¹ to a final temperature of 300°C and isothermal at this temperature for 10 min. The gas chromatograph was connected to a Jeol GCmate II mass spectrometer (Jeol Datum Ltd., Tokyo, Japan). Electron-ionization (EI) mass spectra were recorded with m/z from 50 to 600 u, with scan duration 0.3 s and interscan delay 0.2 s. The temperature of the ion source was 250°C and the GC-MS interface temperature was 250°C. The electron energy was 70 eV. The National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, U.S.A.) library of mass spectra was used for the identification of cinnamyl alcohol and cinnamal.

Preparation of samples for chemical analysis

Chemical analyses of the cinnamyl alcohol and cinnamal test preparations that were used in this study were performed. Cinnamyl alcohol, art nr C-013, batch 03215A, exp 2005-11, and cinnamal, art nr C-014, batch 03461B, exp 2006-05, both Chemotechnique Diagnostics, had been analysed already before their expiration date, when the question raised in this study was first asked. Also test preparations of cinnamal and cinnamyl alcohol tested in the period 2009-2012, which were prepared at our department, were analyzed. 100 mg of the respective "pure" petrolatum preparation was dissolved in 2.0 ml of heptane (HiPerSolv Chromonorm, VWR, Leuven, Belgium) in a test tube. This solution was extracted with 2.0 ml of methanol (HiPerSolv Chromonorm, VWR), which was recovered and filtered through a 2 μm syringe filter. The filtered solutions were analysed using GC-MS.

Results

The results of the patch testing in dermatitis patients are based on 2 readings on days 3/4 and 7, while there was only one reading on day 3²² for the general population.

Target group ia versus control group iii

In the period 2009-2018, 283 patients were photopatch tested with ketoprofen (Fig. 1). Photocontact allergy was registered in 34 patients (12.0%). Of 214 both patch and photopatch tested patients photocontact allergy to ketoprofen was diagnosed in thirty (target group ia, Fig. 1). Simultaneous contact allergy to FM I was noted in 16/30 (53.3%). Among dermatitis patients, not photopatch tested but patch tested with FM I (control group iii, Fig. 1), contact allergy was noted in 438/6563 patients (6.7%)(16/30 versus 438/6563, $P < .001$)

The comparison between the group with photocontact allergy to ketoprofen (target

group ia) and the dermatitis group, not photopatch tested but patch tested (control group iii), regarding contact allergy rates to the individual components of fragrance mix I is shown in Table 1 (available as online supplement). The rates of contact allergy were statistically significantly higher in the target group (patients with photocontact allergy to ketoprofen) for cinnamyl alcohol ($P<.001$), cinnamal ($P=.0041$), eugenol ($P<.001$) and isoeugenol ($P=0.028$).

Target group ib versus control group ii

The results for those 214 dermatitis patients who were both photopatch tested with the European photopatch test series and patch tested with FM I and its components (target group ib and control group ii, Fig.1) are presented in Table 2 (available as online supplement). A statistically significant over-representation of contact allergy was shown for FM I ($P<.001$), cinnamyl alcohol ($P<.001$) and eugenol ($P=.0077$) in the patients with photocontact allergy to ketoprofen.

Target group ia versus control group iv, Fig. 1 and Table 3

The comparison between the group with photocontact allergy to ketoprofen (target group ia) and the group of volunteers from the general population not photopatch tested but patch tested (control group iv, Fig. 1) regarding contact allergy rates to FM I and its individual components is shown in Table 3 (available as online supplement). The rates of contact allergy were statistically significantly higher in the target group with photocontact allergy to ketoprofen for FM I ($P<.001$), cinnamyl alcohol ($P<.001$), cinnamal ($P=.0027$) and eugenol ($P<.001$).

Pairs of chemically related substances

When comparing the results of patch testing with the 2 pairs of chemically closely related ingredients of FM I (cinnamal/cinnamic alcohol and eugenol/iseugenol) inside the photopatch tested group with photocontact allergy to ketoprofen (target group ia; Fig. 1) and the patch tested dermatitis group (control group iii; Fig. 1), the rate of contact allergy to cinnamyl alcohol was slightly, but not significantly, higher than to cinnamal ($P=.3$) in the ketoprofen group (ia), and significantly lower ($P=0.023$) in the dermatitis group (iii). Similarly, the rate of contact allergy to eugenol was numerically higher but not statistically higher than to isoeugenol ($P=.15$) in the ketoprofen group (ia), while there was a reverse difference in the dermatitis group (iii) with a significantly higher prevalence of contact allergy to isoeugenol ($P=.0012$).

With regard to the small size of the ketoprofen group we have also made a comparison of the distribution of positive reactions to chemically related pairs of substances (cinnamic alcohol vs cinnamal and eugenol vs isoeugenol) between the ketoprofen and the dermatitis groups (target group ia versus control group iii). Fisher's two-tailed exact test showed a statistically significant change of reaction pattern in both chemical groups towards predominance of the reactions to cinnamic alcohol and eugenol in those with a photocontact allergy to ketoprofen (ia) ($P=.038$ for cinnamic substances and $P=.019$ for eugenol/iseugenol) (Table 1 appendix, available as online supplement).

In those patients that were both patch tested and photopatch tested with the European photopatch test series, the rates of contact allergy to cinnamyl alcohol and eugenol were

higher than to cinnamal and isoeugenol, respectively, but without any significance in those with photocontact allergy to ketoprofen (target group ib; Fig. 1). For these pairs, cinnamyl alcohol/cinnamal and eugenol/isoeugenol, the contact allergy rates were virtually the same within respective pair in those without photocontact allergy to ketoprofen (control group ii; Fig. 1).

Chemical analyses

GC-MS analysis of the patch test preparations of cinnamal and cinnamyl alcohol confirmed that the labelled substance on the respective syringe corresponded to its content. We detected cinnamal as a contaminant in all the investigated preparations of cinnamyl alcohol. The amount corresponded to approximately 14% of the total weight of cinnamyl alcohol and there was no contamination of cinnamyl alcohol in cinnamal (detection limit <0.03%).

Discussion

In the last decades it has become obvious that ketoprofen is a very potent topical photosensitizer. Many simultaneous photocontact allergies have been described in those with photocontact allergy to ketoprofen^{1-4,11,13,31,32}.

Interestingly, there are also many reports on simultaneous contact allergies in patients with photocontact allergy to ketoprofen^{1,3,4,11-13,32,33} (Marmgren et al. Surprising results of patch testing with the baseline series in patients with photoallergy to ketoprofen. *In manuscript*). This type of association between contact allergy and photocontact allergy is considered rare. In the past, it has been reported regarding concomitant contact allergy to thiomersal and photocontact allergy to piroxicam^{21,34,35}. In some cases, e.g. with octocrylene, that is a sunscreen agent and cyanoacrylate representative, both contact and photocontact reactions are described as more prevalent in patients with photocontact allergy to ketoprofen^{1,36-40}. The recent Belgian study has analysed raw octocrylene material as well as 28 octocrylene-containing products for presence of unsubstituted benzophenone. Residues of benzophenone were found in virtually all octocrylene-containing products and in the raw material, and the concentration was shown to be increasing with time, possibly due to additional degradation⁴¹. As ketoprofen is a substituted benzophenone, the cross-reactivity due to benzophenone residues is a reasonable explanation to the prevalent simultaneous photocontact allergic reactions to ketoprofen and "octocrylene".

While the photosensitizing properties of ketoprofen itself are fairly well-studied, the mechanism of the simultaneous reactions remains largely unclear.

One mixture of allergens responsible for a significantly higher number of positive patch test reactions in patients with photocontact allergy to ketoprofen is FM I^{1,3,4,11-}

¹³(Marmgren et al. Surprising results of patch testing with the baseline series in patients with photoallergy to ketoprofen. *In manuscript*). The prevalence of contact allergy to FM I is estimated to 2.6-3.5% in the general population^{22,42} and to 6-10% in dermatitis patients^{16,28,43-46}. In this study, contact allergy to FM I was noted in 53.3% (16/30) of the ketoprofen-photoallergic individuals. When patch testing ketoprofen-photoallergic patients with constituents of FM I we have, when comparing with the dermatitis population, noticed an overrepresentation of positive reaction to several substances, namely cinnamal, cinnamyl alcohol, isoeugenol and eugenol. The same pattern was

shown in the comparison between patients with photocontact allergy to ketoprofen and the general population²². Narrowing the search results to only the photopatch tested patients with and without photocontact allergy to ketoprofen resulted in still significantly higher numbers of contact allergy rates to FM I, cinnamyl alcohol and eugenol in the ketoprofen group, while there were no significant differences in contact allergy rates to cinnamal and isoeugenol.

We are aware that the difference in cohort size between ketoprofen-photoallergic and dermatitis patients is influencing the strength of the association. The level of significance has, however, remained very high for at least cinnamyl alcohol and eugenol, despite narrowing the selection criteria to only patients tested with the photopatch test series, which gives support to our findings.

Several studies have described an over-representation of positive reactions to cinnamyl alcohol in ketoprofen-photoallergic patients^{3,11,13}. We could not find any publications regarding possible over-representation of other constituents of FM I and fragrance mix II in ketoprofen-photoallergic patients. In patch tested dermatitis patients, particularly isoeugenol and *Evernia prunastri* are considered important sensitizers, and they usually also show the highest contact allergy rates among patients positive to constituents of FM I^{14,46,47}. In our material the numbers of contact allergy are even higher in the ketoprofen-photoallergic individuals compared to the dermatitis patients.

Both eugenol and isoeugenol are widely used in perfumes and skin care products, eugenol also being a common additive to endosealers in odontology. Both are found in a number of plants and etheric oils, which broadens user profile. The market share for eugenol is 1-4%, for isoeugenol <1%⁴⁷, which may indicate higher possible exposure to eugenol. According to safety assessments from the Research Institute for Fragrance Materials (RIFM), isoeugenol, based on the results of local lymph node assays, is considered to be a moderate skin sensitizer with application concentration required to provoke a 3-fold increase in lymph node cell proliferation activity compared to control (EC3)<2%, and eugenol a weak one with EC3 25.1%⁴⁸⁻⁵⁰. A sensitization exposure quotient (SEQ) has been calculated for some fragrances⁴⁷ in order to compare the relative frequency of sensitization with the relative frequency of use/labelling. According to the report, isoeugenol has a SEQ of 34.58, which places it at the top part of the list of examined fragrances. Eugenol has a SEQ of 1.99, suggesting a low sensitizing potential with given exposure. The sensitisation rates have been described to be going up for isoeugenol, while remaining constant for eugenol¹⁴. So far, we have not found any reports on contact allergy to eugenol being more prevalent than to isoeugenol in the same patient group, which makes patients with photocontact allergy to ketoprofen to stand out.

As to contact allergy to other constituents of FM I, cinnamal and cinnamyl alcohol are the two substances that have been scrutinized by researchers regarding unusually high rates of contact allergy in ketoprofen-photoallergic individuals.

Pigatto et al have in their study in 1996 seen that the component responsible for most positive reactions was cinnamal¹² that has long been considered as a more potent sensitizer than cinnamyl alcohol^{11,51,52}. Both cinnamal and cinnamyl alcohol occur naturally in a variety of fruit and spices, and are widely used in perfumes and skin care products^{53,54}. The market share for cinnamal is 4.3% and for cinnamyl alcohol 3.7%⁴⁷. According to RIFM fragrance safety assessments, cinnamal is considered to be an extreme skin sensitizer with EC3 0.2%, and cinnamyl alcohol a weak one with EC3 21%⁵⁵. An SEQ for cinnamal has been estimated to 13.44, and for cinnamyl alcohol to

7.55⁴⁷, which indicates that, with given exposure, cinnamal would indeed be a stronger sensitizer than cinnamyl alcohol.

During our testing cinnamyl alcohol gave, however, significantly more positive reactions than cinnamal in those with photocontact allergy to ketoprofen. Similar test results came from other research groups as well^{2,4,11,31,56,57}. As errors in labelling of test preparations, as well as impurities, have been reported by scientists in the past¹⁸⁻²⁰, we decided to ensure that our patients were tested with correct substances. We performed chemical analyses of the patch test preparations of cinnamal and cinnamyl alcohol that verified that the labelled substances were present in the test preparations⁵⁸. There seemed to be some rather significant impurities in our preparations as well, but these impurities basically consisted of some amount of cinnamal in cinnamyl alcohol preparation, and thus, should not explain the over-representation of contact allergy to cinnamyl alcohol in our patients with photocontact allergy to ketoprofen.

The hypotheses regarding sensitizing potential of cinnamal and cinnamyl alcohol include suggestion that cinnamal is the "true" allergen, while cinnamyl alcohol has to be transformed to cinnamal, before contact allergic reactions could occur⁵⁹, or that cinnamal may be a protein-reactive hapten, while cinnamyl alcohol is a "prohapten" that requires a metabolic transformation in order to become cinnamal^{60,61}. Girardin et al suggest however that cinnamyl alcohol may be in fact a separate antigen which does not require transformation into cinnamal to become a sensitizer¹³. The potential role of enzymes in biotransformation of cinnamic chemicals has been studied by Smith et al⁵². Cinnamal is a more frequent sensitizer than cinnamyl alcohol in a dermatitis population^{46,47,62}, which aligns with both its estimated sensitizing potential and the theoretical explanation models presented above. Also in the present study there is a slight, although not statistically significant, predominance of contact allergy to cinnamal versus cinnamyl alcohol with 1.05% and 0.7%, respectively in the dermatitis population (control group iii, Fig. 1). The proportions change, however, significantly in favour of cinnamyl alcohol when the patients photoallergic to ketoprofen are considered, according to our results.

Foti et al have by means of computerized conformational analysis suggested that the structure of cinnamyl alcohol is similar to that of ketoprofen, which suggests that in patients with photocontact allergy to ketoprofen, concomitant positive reactions to cinnamyl alcohol are due to cross-sensitization¹¹. Cheung et al. have shown that cutaneous ADH (alcohol dehydrogenase) and ALDH (aldehyde dehydrogenase) located within defined subcellular compartments, play important roles in the activation and detoxification of cinnamyl alcohol and cinnamal in the skin, which may, by means of different metabolic pathways, lead to interindividual differences, cross-reactivities or co-sensitisation to different cinnamic compounds in the clinic⁶⁰. Niklasson et al have in their study seen a bioactivation of cinnamyl alcohol with subsequent formation of epoxy cinnamal⁶³. The sensitizing properties of epoxides of cinnamyl alcohol and cinnamal have later been studied by Hagvall et al⁶⁴, with the conclusion that the investigated epoxides are not important haptens in contact allergy to cinnamon fragrances. The formation of one of the patch tested epoxides⁶⁴, epoxy cinnamyl alcohol, was suggested when the metabolism of carbon-13 substituted cinnamyl alcohol was investigated in a reconstructed human epidermis (RHE) model coupled with high-resolution magic angle spinning nuclear magnetic resonance⁶⁵. On the other hand, metabolism of cinnamyl alcohol did not result in the formation of cinnamal, and the conclusion was made that cinnamyl alcohol is activated to become a sensitizer via a separate route, independent of cinnamal⁶⁵, which favours the assumption made by

Girardin et al¹³. An interesting issue to explore is whether individuals with photocontact allergy to ketoprofen would patch test positively to epoxy cinnamyl alcohol to a higher degree than dermatitis patients without photocontact allergy to ketoprofen.

Another factor to be taken into consideration was the possibility of different concentrations influencing test results. In FM I, the test concentrations of cinnamal and cinnamyl alcohol are 1%, but when tested separately they are tested at 1% and 2%, respectively^{59,66,67}. Ferguson et al have in their study pointed out that there was a significant difference between the results testing with 1% and 2% cinnamal, where 18.7% of patients reacted to cinnamal 2%, and only 3.3% to 1%⁶⁷. Patch testing with cinnamal at 2% has been considered to result in too many irritant reactions. It is theoretically possible that the difference in concentrations in favour of cinnamyl alcohol influences the outcome of testing between the two groups. A trend towards decreasing of the positive test reactions to cinnamal through recent decades has been reported^{14,68}, possible explanation models being among others the lowered test concentration and changed use profile of the chemical. However, the same concentrations of test preparations are used for testing of the dermatitis population in this study, and thus it does not explain the observed difference in distribution of positive reactions in these two groups. In addition, the difference in test concentration can not explain higher rates of allergy to eugenol and isoeugenol, even though a longitudinal prospective study of sensitization rates paired with user profiles would be needed to map the patterns of sensitization over time.

The over-representation of positive reactions to eugenol came as a surprise. Isoeugenol is considered an important sensitizer, and possesses a great sensitizing capacity together with *Evernia prunastri*^{14,69}. The pattern of a less common contact sensitizer taking the lead in ketoprofen-photoallergic population repeats itself.

What about the possibility of simultaneous sensitization rather than cross-reactivity? Sensitization to the oxidized fragrance terpenes is common⁷⁰⁻⁷⁵, and we have previously reported that the prevalence of contact allergy to these compounds is significantly higher in the population with photocontact allergy to ketoprofen¹⁰. One of the three ketoprofen gels responsible for photocontact sensitization in Sweden, Orudis® (Sanofi-Aventis AB, Bromma, Sweden), contains linalool as a part of lavender oil in the preparation. However, when the patients, positive to both ketoprofen and Orudis® on PPT, were patch tested with the lavender oil, no positive reactions were detected³. Whether there is any oxidized linalool in the preparation is unknown, but the oxidation products usually are found in the air-exposed fragrances and raw terpene preparations⁷⁶⁻⁷⁸, so the probability is high. Singlet oxygen, produced during photocontact sensitization to ketoprofen, may be contributing to the oxidation of linalool and limonene by formation of allylic peroxides^{79,80}. However, limonene is not present in Orudis®, and neither linalool nor limonene are present in the other two ketoprofen gels, present on the Swedish market at the time for possible sensitization of our patients. Simultaneous exposure to other skincare products containing terpenes, either already oxidized or oxidized in the presence of ketoprofen, needs to be looked further into.

Another possible explanation for the over-representation of simultaneous allergies in an individual with photocontact allergy to ketoprofen may be that generalized predisposition to type IV allergy occurs in particular individuals, with or without the collusion of sunlight⁸¹. This assumption may be valid, but it does not explain the over-representation of contact allergy to cinnamyl alcohol and eugenol rather than cinnamal and isoeugenol in ketoprofen-photoallergic patients. Interestingly, most of the constituents of FM I responsible for significantly higher contact allergy rates in

ketoprofen-photoallergic patients are aromatic compounds, and thus in possession of a benzene ring (Fig 2). Geraniol and hydroxycitronellal are, on the other hand, aliphatic compounds. A crude comparison could indicate that the aromates are responsible for the high rates of contact allergy, with the exception of amyl cinnamal.

There are two seemingly different reaction patterns in the ketoprofen-photoallergic patients and dermatitis patients. Considering the possibility that some aromatic substances metabolize with the formation of allergenic end products, cross-reacting with ketoprofen, then the higher rates of contact allergy to (some) aromates may have an explanation. The change in rates in favour of cinnamyl alcohol and eugenol (compared to no difference between cinnamal and cinnamyl alcohol, and higher prevalence of allergy to isoeugenol than eugenol in dermatitis population) is a new phenomenon for us.

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Figure 1: Flowchart demonstrating the process of selecting study patients and controls.
ACD =allergic contact dermatitis, PACD=photoallergic contact dermatitis, PPT=photopatch testing, PT=patch testing, KP=ketoprofen, CG=control group, TG=target group, FM I comp= components of FM I, PPT EUR =European Photopatch test series

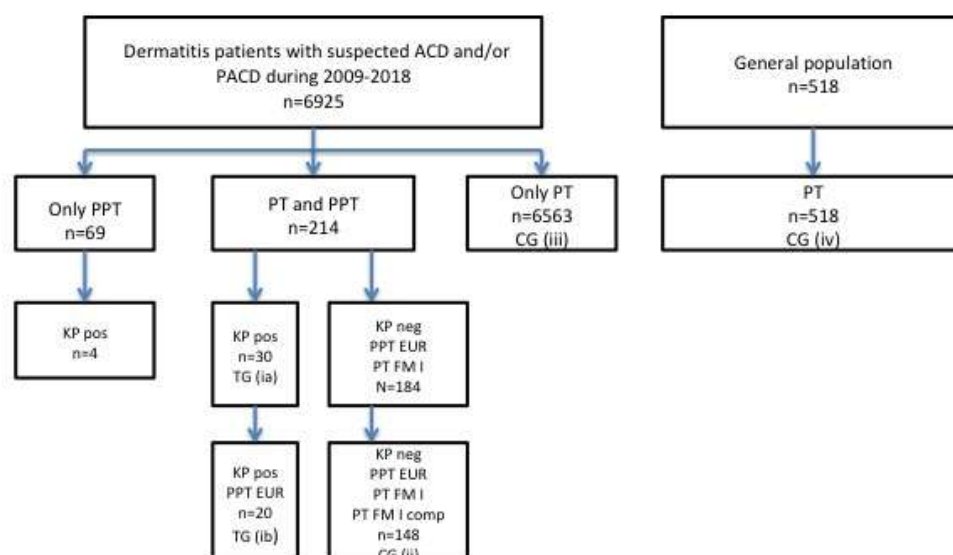


Fig 2. Structural formulas and chemical characteristics of ketoprofen and components of fragrance mix I.

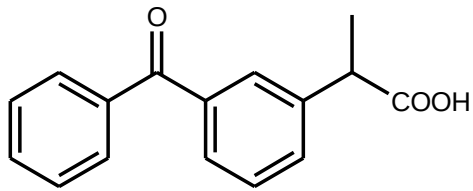
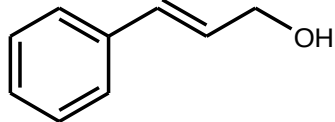
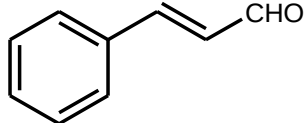
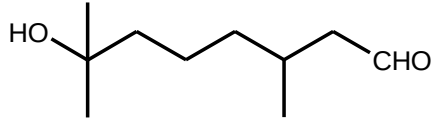
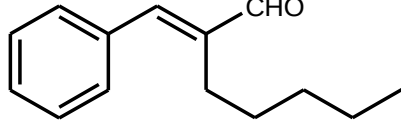
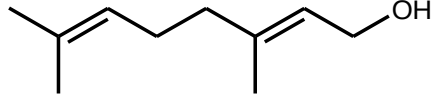
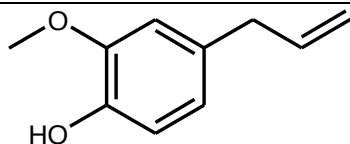
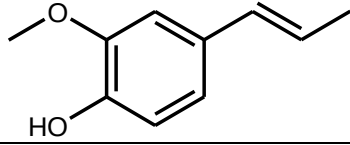
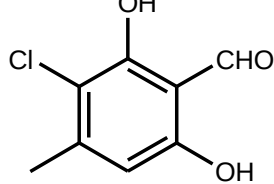
Structural formula	Name, CAS no, MW
	Ketoprofen Cas no: 22071-15-4 MW: 254
	Cinnamyl alcohol; (cinnamic alcohol) Cas no: 104-51-1 MW: 134
	Cinnamal; (cinnamic aldehyde) Cas no: 104-54-1 MW: 132
	Hydroxycitronellal Cas no: 107-75-5 MW: 172
	Amyl cinnamal; (amyl cinnamic aldehyde) Cas no: 122-40-7 MW: 202
	Geraniol Cas no: 106-24-1 MW: 134
	Eugenol Cas no: 97-53-0 MW: 164
	Isoeugenol Cas no: 97-54-1 MW: 164
	Chloroatranol a main allergen in <i>Evernia prunastri</i> Cas no: 57074-21-2 MW: 187

Table 1: Comparison of patch test results to fragrance mix I (FM I) and its components in the period 2009-2018 in 2 groups of dermatitis patients. 6781 patients were patch tested and, of these, 6563 patients were only patch tested (control group iii, Fig. 1) while 214 patients also were photopatch tested. Photocontact allergy to ketoprofen (KP) was diagnosed in 30 of the 214 dermatitis patients (target group ia, Fig. 1). Pos=*positive*.

	KP-photoallergic patients, patch tested with FM I and its components n=30			Dermatitis patients patch tested with FM I and its components n=6563			P-value	Odds ratio (95% CI)
	KP tested	KP pos	% pos	Derm tested	Derm pos	% pos		
FM I	30	16	53.3	6563	438	6.7	<.001	16 (8-33)
Cinnamyl alcohol	30	7	23.3	6563	44	0.7	<.001	45 (18-111)
Cinnamal	30	3	10.0	6563	69	1.05	.0041	11 (3-35)
Hydroxycitronellal	30	1	3.3	6563	32	0.5	.14	7 (0.9-53)
Amyl cinnamic aldehyde	30	0	0.0	6563	8	0.1	1	0 (0, NaN) [†]
Geraniol	30	0	0.0	6563	33	0.5	1	0 (0, NaN)
Eugenol	30	7	23.3	6563	26	0.4	<.001	77 (30-194)
Isoeugenol	30	2	6.7	6563	56	0.9	0.028	8 (2-36)
<i>Evernia prunastri</i> extract	30	2	6.7	6308 [‡]	117	1.9	.108	4 (0.9-16)
Sorbitan sesquioleate	30	0	0.0	6563	24	0.4	1	0 (0, NaN)

[‡]Testing with *Evernia prunastri* extract was not being performed in the beginning of the study period, which explains the difference in number of tested individuals.

[†]NaN – not calculable data

Table 1 appendix: The distribution and comparison of positive patch test reactions to pairs of chemically related substances, cinnamyl alcohol/cinnamal and eugenol/isoegenol, between 30 patients with photocontact allergy to ketoprofen and 6563 dermatitis patients.

Substance	KP-photoallergic population			Dermatitis population			P-value
	Total no of positive reactions	No of unique positive reactions per substance	Positive for both	Total no of positive reactions	No of unique positive reactions per substance	Positive for both	
Cinnamyl alcohol	7	4	4	44	10	34	0.038
Cinnamal	3	0		69	36		
Eugenol	7	6	1	26	11	14	0.019
Isoeugenol	2	1		56	41		

Table 2: Comparison of patch test results to fragrance mix I (FM I) and its components in the period 2009-2018 in 168 patch tested dermatitis patients also photopatch tested with the European photopatch test series divided into those with and without photocontact allergy to ketoprofen (target group ib and control group ii, respectively, Fig. 1).

	Patients with ketoprofen photocontact allergy n=20			Patients without ketoprofen photocontact allergy n=148			P-value	Odds ratio (CI 95%)
	No tested	No pos	% pos	No tested	No pos	% pos		
FM I	20	11	55.0	148	25	16.9	<.001	6 (2-16)
Cinnamyl alcohol	20	7	35.0	148	7	4.7	<.001	11 (3-36)
Cinnamal	20	2	10.0	148	9	6.1	.62	2 (0.4-9)
Hydroxycitronellal	20	0	0.0	148	2	1.4	1	0 (0, NaN)
Amyl cinnamic aldehyde	20	0	0.0	148	0	0.0	1	0 (0, NaN)
Geraniol	20	0	0.0	148	1	0.7	1	0 (0, NaN)
Eugenol	20	4	20.0	148	4	2.7	.0077	9 (2-40)
Isoeugenol	20	1	5.0	148	2	1.4	.32	4 (0.3-45)
<i>Evernia prunastri</i> extract	20	2	10.0	148	10	6.8	.64	2 (0.3-8)
Sorbitan sesquioleate	20	0	0.0	148	1	0.7	1	0 (0, NaN)

Table 3: Comparison of patch test results to fragrance mix I (FM I) and its components in patients photoallergic to ketoprofen (target group ia, Fig. 1) and the general population in Malmö, Sweden (control group iv, Fig. 1) ²². Data presented only for reading 1 on day 3/4 for both groups.

	KP-photoallergic patients patch tested with FM I and its components n=30			General population patch tested with FM I and its components n=518			P-value	Odds ratio (CI 95%)
	No tested	No positive	% positive	No tested	No positive	% positive		
FM I	30	16	53.5	518	13	2.5	<.001	44 (18-110)
Cinnamyl alcohol	30	7	23.3	518	2	0.4	<.001	79 (16-399)
Cinnamal	30	3	10.0	518	3	0.6	.0027	19 (4-99)
Hydroxycitronellal	30	1	3.3	518	1	0.2	.107	18 (1-292)
Amyl cinnamic aldehyde	30	0	0.0	518	1	0.2	1	0 (0, NaN)
Geraniol	30	0	0.0	518	2	0.4	1	0 (0, NaN)
Eugenol	30	6	20.0	518	1	0.2	<.001	129 (15-1117)
Isoeugenol	30	1	3.3	518	1	0.2	.107	18 (1-292)
<i>Evernia prunastri</i> extract	30	2	6.7	518	8	1.6	0.099	5 (0.9-23)
Sorbitan sesquioleate	30	0	0.0	518	1	0.2	1	0 (0, NaN)