Photocontact Allergy to Ketoprofen and Simultaneous Contact Allergies

Marmgren, Victoria

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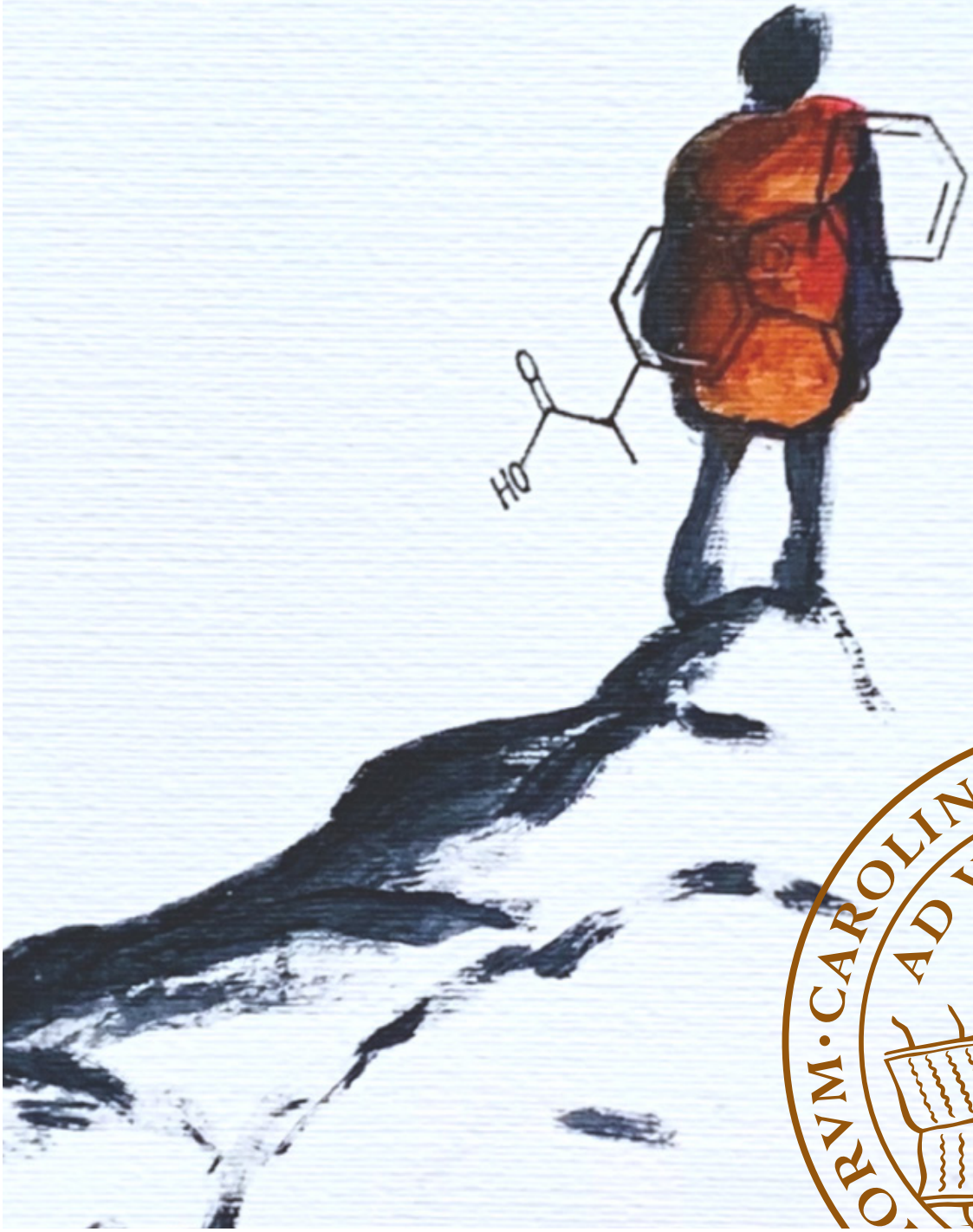

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DOCTORAL DISSERTATION

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Abstract

Ketoprofen is a non-steroidal anti-inflammatory drug with analgesic, antipyretic, and anti-inflammatory properties. It is available in both oral and topical formulations. Photocontact allergy to topically applied ketoprofen has been the most frequent side effect of the formulation, and is fairly well studied. The risk of development of photocontact allergy and of persistent photosensitivity as the result of photosensitization has led to warnings and restricted distribution of ketoprofen-containing gels. Apart from the risk of developing a photoallergic contact dermatitis upon repeated exposure to ketoprofen, the sensitized individuals show higher rates of photocontact and contact allergy to some other sensitizers. Simultaneous photocontact allergic reactions to benzophenones, fentichlor, chlorpromazine, bithionol, tetrachlorosalicylanilide and promethazine were described in patients with photocontact allergy to ketoprofen in the middle of 2000s. Similarly, an overrepresentation of contact allergy to fragrance mix I and *Myroxylon pereirae* in the same group has been known for decades. There is no known common mechanism of simultaneous photocontact and contact allergy, but several research groups have suggested the possibility of cross-reactivity between ketoprofen, which is a substituted benzophenone, and other chemicals with a benzophenone moiety.

This thesis forms a part of the search for an explanation of the phenomenon of simultaneous contact allergies in individuals with photocontact allergy to ketoprofen. A broad perspective is essential in order to understand any phenomenon, which in this case means that we need to obtain better knowledge of which sensitizers individuals with photocontact allergy to ketoprofen may react to more often compared to controls.

Study I examined the possibility of simplifying the procedure of photopatch testing with ketoprofen, and found that reliable results can be obtained by shortening the occlusion time from 24 hours to 1 hour, with no need to change other parameters such as concentration or UVA dose. Studies II, III, and IV were concerned with the epidemiology of simultaneous contact allergy in patients with photocontact allergy to ketoprofen. Study II revealed that patch testing with some of the individual components of fragrance mix I (cinnamal, cinnamyl alcohol, eugenol, and isoeugenol) produced significantly higher numbers of positive patch test reactions in those with photocontact allergy to ketoprofen compared to controls. Similarly, Study IV found that a number of sensitizers tested within the baseline series led to significantly higher rates of contact allergy in those with photocontact allergy to ketoprofen than in dermatitis patients and in the general population. Study III confirmed a clinical suspicion that contact allergy to oxidized linalool and oxidized limonene was indeed overrepresented in the ketoprofen group.

The clinical relevance of these findings is yet to be investigated, but this thesis discusses some of the hypotheses proposed by various researchers in order to explain the phenomenon of simultaneous contact allergies that arise in connection with photosensitization to ketoprofen. Although no definite explanation can be given to date, the main goal of this research is to gain a better understanding of the epidemiology of simultaneous contact allergy, which can act as a building block in future research.

Key words ketoprofen, photoallergy, cross-reaction, simultaneous contact allergy, occlusion time, fragrance, patch testing, photopatch testing

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MADE IN SWEDEN

To my grandparents, Eudokia and Dimitri Wyshemyrski

"Would you tell me, please, which way I ought to go from here?"
"That depends a good deal on where you want to get to," said the Cat.
"I don't much care where—" said Alice.
"Then it doesn't matter which way you go," said the Cat.
"—so long as I get somewhere*," Alice added as an explanation.*
"Oh, you're sure to do that," said the Cat, "if you only walk long enough."

Lewis Carroll
Alice's Adventures in Wonderland

Table of Contents

List of publications

This thesis is based on the following papers, referred to in the text by their Roman numerals. The papers are to be found at the end of the thesis.

I. **Successful photopatch testing with ketoprofen using one-hour occlusion**. Victoria Marmgren, Monica Hindsén, Erik Zimerson, Magnus Bruze. *Acta Derm Venereol* 91(2):131-136

II. **Contact allergy to fragrance mix I and its components in individuals with photocontact allergy to ketoprofen**. Victoria Marmgren, Martin Mowitz, Erik Zimerson, Monica Hindsén, Magnus Bruze. *Contact Dermatitis* 2021; 1- 11. doi:10.1111/cod.13958

III. **Contact allergy to oxidized linalool and oxidized limonene is over-represented in individuals with photocontact allergy to ketoprofen.** Magnus Bruze, Victoria Marmgren, Annarita Antelmi, Monica Hindsén Stenström, Cecilia Svedman, Erik Zimerson and Martin Mowitz. *Acta Derm Venereol* 2021;May 19;101(5); 1-6

IV. **Surprising results of patch testing with the baseline series in patients with photocontact allergy to ketoprofen**. Marmgren V, Mowitz M, Zimerson E, Hindsén M, Bruze M. *In manuscript*

For the published papers, the permission to re-print has been obtained from the publisher.

Thesis at a glance

Paper	Objective	Methods	Main findings
I	To simplify the photopatch testing procedure for photopatch testing with ketoprofen	PPT of 11 patients with known photocontact allergy to ketoprofen and 11 patients with suspected photocontact allergy to ketoprofen with ketoprofen using different concentrations. The occlusion time was both 1h and 24h (standard).	PPT using 1h occlusion gave the results comparable to PPT with 24h occlusion. Neither test concentration of ketoprofen nor UVA dose needed to be adjusted.
II	To investigates the rates of contact allergy to individual components of FM I in patients with photocontact allergy to ketoprofen	PT results in 30 patients with photocontact allergy to ketoprofen patch tested with the baseline series and the individual components of FM I were compared to PT results of 6563 dermatitis patients patch tested with the baseline series and also with 148 of these patients tested with the individual components of FM I during the same period of time, and with general population patch tested with the individual components of FM I. Further comparison was made between ketoprofen-photoallergic and dermatitis patients that were also photopatch tested with the photopatch test series.	The rates of contact allergy were statistically significantly higher in patients with photocontact allergy to ketoprofen for cinnamyl alcohol ($p<0.001$), cinnamal ($p=0.0041$), eugenol ($p<0.001$) and isoeugenol ($p=0.028$), compared to both dermatitis and general population.
III	To compare the rate of contact allergy to fragrance substances oxidized (ox.) linalool and oxidized (ox.) limonene in ketoprofen-photoallergic individuals with the corresponding rates in individuals without photocontact allergy to ketoprofen	Between 2005 and 2015, 4050 dermatitis patients were patch tested with the Swedish baseline patch test series to which ox. linalool was provisionally. Between 2004 and 2015, 3821 dermatitis patients were patch tested with the Swedish baseline patch test series to which ox. limonene was provisionally inserted. None of the patients was patch and/or photopatch tested with ketoprofen. 29 patients were diagnosed with photocontact allergy to ketoprofen during the test period. The rates of contact allergy to ox. linalool and/or ox. limonene in dermatitis patients were compared to the corresponding rates in patients with photocontact allergy to letoprofen.	The rates of contact allergy to both ox. linalool and ox. limonene were significantly higher in patients with photocontact allergy to ketoprofen compared to the dermatitis patients($p<0.001$ for both).
IV	To investigate whether patients with photocontact allergy to ketoprofen show an overrepresentation of simultaneous contact allergy to the sensitizers in the baseline patch test series, other than to FM I and *Myroxylon pereirae*.	The results of PT of 94 patients photoallergic to ketoprofen, patch tested with the baseline series between 1999-2018, were compared with the results of PT of approximately 12800 dermatitis patients, patch tested with the baseline series within the same time frame, and with the results of patch testing with the baseline series of 518 subjects belonging to general population, patch tested in an earlier study.	Significant over-representation was shown for PTBP-F-R, PFR-2, black rubber mix, budesonide (all $p<0.001$), and fragrance mix II ($p=0.02$). The rates of contact allergy to FM I and *Myroxylon pereirae* were significantly higher among patients with photocontact allergy to ketoprofen, as expected based on the results of multiple previous studies.

Abbreviations

ACD	allergic contact dermatitis
PhACD	photoallergic contact dermatitis
PT	patch testing
PPT	photopatch testing
NSAID	Non-steroidal antiinflammatory drug
FM I and II	Fragrance mix I and II
MP	*Myroxylon pereirae*
ICDRG	International Contact Dermatitis Research Group
GC-MS	gas chromatography-mass spectrometry
HPLC	high performance liquid chromatography
ROAT	repeated open application test
LLNA	local lymph node assay
ox.	oxidized
IFRA	International Fragrance Association
IVDK	Information Network of Departments of Dermatology
RIFM	The Research Institute for Fragrance Materials, Inc.
ROS	reactive oxygen species
e g	exempli gratia, for example
i e	id est, that is
h	hour
D	day
v	volume
w	weight
g	gram
mg	milligram
µg	microgram

Sammanfattning på svenska

Fotokontaktallergi mot ketoprofen och samtidiga kontaktallergier

Icke-steroida antiinflammatoriska läkemedel (förkortat NSAIDs, non-steroidal anti-inflammatory drugs) används i stor utsträckning världen runt för behandling av framför allt smärta i muskler och leder samt febertillstånd. Hos vissa individer kan användning av tabletter och kapslar som innehåller NSAIDs leda till att flera biverkningar uppkommer, till exempel magsmärtor och njurproblem. Detta ha lett till att beredningar för utvärtes bruk har introducerats.

Ketoprofen är ett läkemedel som tillhör gruppen NSAIDs. Preparatet är effektivt både i tablettform och som beredning för utvärtes bruk (gel). Vid utvärtes bruk minskar antal biverkningar från inre organ avsevärt. Däremot kan hudbesvär uppstå om det behandlade området exponeras för solljus. Allergi mot ketoprofen i kombination med UV-ljus (fotokontaktallergi) ligger oftast i grunden för dessa besvär, som i princip alltid yttrar sig som ett eksem. I särskilt svåra fall kan eksem i följd av fotokontaktallergi mot ketoprofen vara av så svår grad att sjukhusvård krävs.

Fotokontaktallergi mot ketoprofen har rapporterats sedan preparatets introduktion på marknaden på 1990-talet. Trots relativt begränsad användning har ketoprofen toppat listor på läkemedel som orsakar allergiska reaktioner i huden vid samtidig solexponering (fotosensibiliserande läkemedel) i flera länder där ketoprofenberedningar för utvärtes bruk säljs. 2011 upphörde den receptfria försäljningen av ketoprofeninnehållande geler i Sverige som en följd av beslut av Läkemedelsverket. Preparatet kan fortfarande förskrivas av läkare.

Fotokontaktallergi bör uteslutas om ett eksem uppstår på ett solbelyst område, vare sig någon kemikalieexponering av området är känd eller ej. För att bekräfta eller utesluta fotokontaktallergi bör individen genomgå en så kallad fotolapptestning. En rad kemiska substanser/blandningar som utgör en fotolappserie, och ibland enskilda ämnen, placeras i små behållare av aluminium eller plast och appliceras på ena sidan av individens rygg med hjälp av tejp. En identisk fotolappserie fixeras också på den motsatta sidan av ryggen. Efter 24 timmar tas testsubstanserna bort, och ena sidan av ryggen täcks direkt med ett icke-ljusgenomsläppligt tyg. Denna sida betraktas som kontrollsida. Testsidan belyses med en standartdos av UVA-ljus. Testsidan och kontrollsidan undersöks 3 dagar efter applikation av serien, och ibland också 7 dagar efter. Eventuella reaktioner motsvarande ämnen i testserien bedöms avseende styrka (+, ++ eller +++) samt relevans för den undersökte och fynden dokumenteras.

Vid misstanken om ett kontakteksem, dvs hudbesvär i följd av kontaktallergi mot något kemiskt ämne utan inblandning av ljus, genomförs istället en så kallad lapptestning. Principen för lapptestning liknar den för fotolapptestning, men

innehållet i testserien/testserier är annorlunda och tiden då dessa ämnen får sitta kvar på huden är längre. Dessutom belyses inte testområdet, varför en identisk kontrollserie inte behövs.

Kort tid efter att flera forskargrupper rapporterade om hudbiverkningar av ketoprofenberedningar för utvärtes bruk blev ett nytt fenomen uppmärksammat. Individer som hade blivit allergiska (fotosensibiliserade) för ketoprofen uppvisade också fler positiva reaktioner för en rad andra ämnen i fotolappserien, jämfört med dem utan fotokontaktallergi mot ketoprofen. Ännu mer anmärkningsvärt var det faktum att dessa fotosensibiliserade individer också uppvisade fler samtidiga reaktioner också vid lapptestning. På Yrkes- och miljödermatologiska avdelningen (YMDA) i Malmö genomförs ca 800 lapp- och fotolapptester per år, och dokumentationen över testresultaten är omfattande. Patienter som söker för hudproblem i samband med ljus brukar dessutom undersökas med ett ”vanligt” lapptest tillsammans med ett fotolapptest. Detta ger ofta värdefull information om förekomst av kontaktallergier hos våra patienter. Denna avhandling utgör en genomgång av samtidiga kontaktallergier hos patienter med fotokontaktallergi mot ketoprofen. Förutom att de tidigare kända sambanden bekräftas, presenteras också en rad nya fynd, inte beskrivna i litteraturen tidigare. Proceduren för fotolapptestning granskas, och en förenkling föreslås för testing med ketoprofen.

I *Studie I* undersöks möjlighet till förenkling av testproceduren för fotolapptestning med ketoprofen. Förkortning av tiden mellan applikation och borttagning av testsubstansen/belysning av huden från 24 timmar till 1 timme visade sig ge jämförbara resultat hos de 22 testade patienterna. Denna förkortning innebär att både applikation och borttagning av ketoprofenberedningen/belysning av huden kan genomföras vid ett besök istället för två. I framtiden kan liknande studier genomföras för att om möjligt förenkla testproceduren för de övriga ämnen i fotolappserien.

I Studie II analyseras resultaten av lapptestning med parfymämnen hos patienter med konstaterad fotokontaktallergi mot ketoprofen. Vid jämförelse med en kontrollpopulation visar det sig att patienter med fotokontaktallergi mot ketoprofen reagerar i betydligt större utsträckning för vissa parfymämnen. Uttalat överrepresenterad kontaktallergi gäller parfymämnena kanelalkohol, kanelaldehyd, eugenol och isoeugenol. Ketoprofen är inte kemiskt besläktad med något av dessa ämnen, och teorier kring denna överrepresentation diskuteras i artikeln

I Studie III undersöks en klinisk observation som gjordes på YMDA i Malmö. Vid lapptestning av patienter med fotokontaktallergi mot ketoprofen sågs ofta samtidig kontaktallergi mot två oxiderade parfymämnen, oxiderad limonen och oxiderad linalool. Båda oxiderade ämnen är starkt kontaktallergiframkallande och kan förekomma i parfymerade produkter. Studieresultaten bekräftade den kliniska misstanken. Patienter med fotokontaktallergi mot ketoprofen uppvisar en statistiskt

signifikant ökning av samtidiga kontaktallergier mot de två oxiderade parfymämnena.

Studie IV gjordes med avsikt att kartlägga förekomsten av positiva testreaktioner för ämnen i en så kallad basserie (svensk basserie) hos patienter med fotokontaktallergi mot ketoprofen. Den Svenska basserien innehåller 30 ämnen och blandningar av ämnen som är potentiellt allergiframkallade, och som man kan exponeras för i olika sammanhang i arbets- och vardagslivet. Även i denna studie ses en överrepresentation av kontaktallergiska reaktioner för vissa ämnen hos patienter med fotokontaktallergi mot ketoprofen. Överrepresentation av kontaktallergiska reaktioner för parfymmix I och II, Perubalsam, svart gummimix, budesonid och två fenolformaldehydhartser är av hög statistisk signifikans. Dessa fynd innebär att fotosensibilisering för ketoprofen potentiellt kan leda till en eller flera samtidiga kontaktallergier mot ämnen i vår närmiljö, även om den kliniska relevansen av våra fynd för närvarande är oklar.

Sammanfattningsvis visar denna avhandling att individer som blir fotosensibiliserade mot ketoprofen löper större risk att utveckla ett flertal kontaktallergier till relativt vanligt förekommande ämnen. De flesta av dessa kontaktallergier kan inte förklaras av strukturella likheter mellan dessa ämnen och ketoprofen, även om de flesta av dessa samt ketoprofen tillhör gruppen aromatiska ämnen. Många hypoteser är föreslagna, men någon tydlig förklaring till fenomenet finns inte idag.

Några av slutsatserna är:

Testproceduren vid fotolapptestning med ketoprofen kan förenklas, både för patienten och för sjukvården. En förenkling av testproceduren bör prövas avseende andra ämnen i fotolappserien.

Fotokontaktallergi riskerar att förbises om ljusexponering som en potentiellt viktig faktor inte tas i beaktande. Fotolapptestning bör genomföras vid fynd eller uppgift om eksemliknande hudutslag på ljusexponerade områden, eller om besvären har en tydlig koppling till ljusexponering. Resultaten av Studie II, III och IV talar även för att patienter med fotokontaktallergi mot ketoprofen bör lapptestas med en basserie, och eventuellt också med de oxiderade parfymämnen limonen och linalool.

Vid fynd av kontaktallergi mot något av följande ämnen: parfymmix I eller II, Perubalsam, svart gummimix, budesonid, para tertiärt butylfenolformaldehydhars eller fenolformaldehydharts, bör exponering och eventuella reaktioner för ketoprofeninnehållande läkemedel för utvärtes bruk kartläggas, och fotolapptestning med serie som innehåller ketoprofen övervägas.

Introduction

A brief history of pain

Throughout history, few things have scared us as much as anticipation of pain. A useful signal of danger, pain has always been our companion, ensuring the survival of our species. However, because of the suffering related to pain, we have always tried to understand and explain its nature. Ancient Greek philosophers such as Plato and Aristotle did not believe that the brain played any part in the perception of pain; rather, it was the soul that was seen as a source of both pain and pleasure. René Descartes (1596-1650) suggested that pain was conducted from its origin to the brain via thin threads running through the body, but he also believed that the experience of pain was of dual nature, and that pain should be regarded as either physical or psychological. Many prominent thinkers, including Friedrich Hoffmann, Albrecht von Haller, Pierre Jean Georges Cabanis, and Xavier Bichat, have continued to look into the mechanisms of pain, trying to find a feasible explanation for this phenomenon[1].

One major reason for pain research is, of course, a quest for pain relief. Opium was a popular option in the 18th century. The 19th century saw the emergence of two newer alternatives: ether and chloroform, used mainly as general anaesthetics. Morphine and heroin were introduced in the early 20th century. Although effective, opioids have been found to possess extremely dangerous side effects, which limits their use for management of most types of pain. In 1853, acetylsalicylic acid was synthesized by the chemist Charles Frédéric Gerhardt. Although salicylates had been used for pain management since antiquity, this new non-steroidal anti-inflammatory drug (NSAID) did not find a place on the market until Bayer started an efficient production of the drug, giving it the name Aspirin®. The introduction of acetaminophen (paracetamol) in 1956 and ibuprofen in 1962 further increased the array of available painkillers. However, the quest continues for a drug and delivery route with maximum analgesic properties and minimum side effects.

Attempts to deliver a substance through the skin are countless, and are likely to have been used since the beginning of mankind[2]. The first attempts to actually quantify the transdermal absorption of a drug were made in the first half of the 20th century[3]. Today, nicotine and oestrogen replacement therapies are widely used examples of transdermal drug delivery.

Ketoprofen was patented in 1967 by Rhone-Poulenc Research Laboratories, Paris, and was approved for clinical use in 1973. It belongs to the NSAID group and is used for its analgesic, antipyretic and anti-inflammatory properties. The efficacy of orally administered ketoprofen has been described as superior to ibuprofen and diclofenac[4]. As with most NSAIDs, its side effects include gastro-intestinal and renal disturbances, and so a topical formulation has also been produced[5].

At estimated high efficacy on the site of action, the topical preparation leads to serum levels of the active ingredient being less than 1% of those reported after oral dosing[6].

Ketoprofen

Ketoprofen belongs to a group of (NSAIDs), and is a propionic acid derivative, substituted by a 3-benzoylphenyl group at position 2 [7]. It can also be described as a substituted benzophenone, which implies possession of two benzene rings connected with a ketone group (Figure 1). It indirectly inhibits the synthesis of prostaglandin via inhibition of cylooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway, as well as thromboxane, the latter leading to a decreased platelet aggregation. Ketoprofen is insoluble in water, but soluble in acetone, ethanol, methylene chloride, chloroform, ether and benzene [8,9].

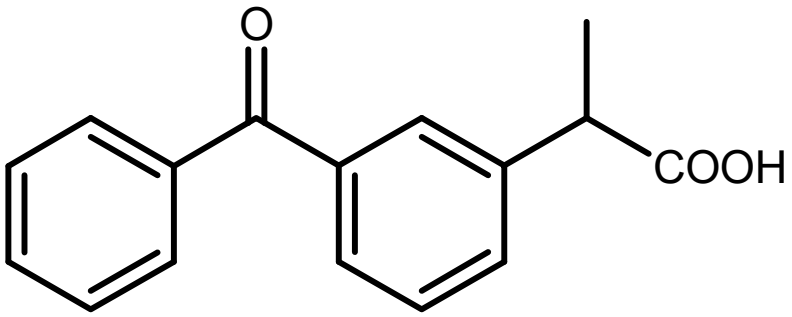


Figure 1. Structural formula of ketoprofen. Cas no: 22071-15-4; MW: 254.

The skin

The skin is our largest organ, accounting for about 15% of the total adult body weight, and has a total area ranging from 1.5 to 2 m^2 in an adult. Its functions include protection against external physical, chemical, and biological factors, as well as

regulation of temperature and water balance. The thickness of the skin differs considerably between parts of the body, depending on the function required.

The skin consists of three layers: the epidermis, the dermis, and subcutaneous fatty tissue (Figure 2). The most abundant cells in the *epidermis* are the keratinocytes. These cells synthesize a protein called keratin, which plays a role in protection of the skin and its appendages. The outer layer of epidermis is called *stratum corneum*. It consists of 15-20 layers of flattened keratinocytes without nuclei, embedded in a matrix of ceramides, cholesterol, and fatty acids. Stratum corneum serves as a barrier that protects the tissue beneath from infection, dehydration and physical stress factors.

The thinnest layer of epidermis, measuring just about 0.1 mm, is found on the eyelids, while the thickest, up to 1.5 mm, covers the soles of the feet. The mucous membranes are the continuation of the skin. After the keratinocytes, the second-largest cell population in the epidermis consists of the subset of immature dendritic cells known as Langerhans cells, a specialized, antigen presenting population of leukocytes. On encountering an antigen, a Langerhans cell migrates to a draining lymph node where it interacts with naïve T cells to induce an immune response to the presented antigen[10]. A number of other cell populations are also present, including the melanocytes and Merkel cells.

The middle layer, the *dermis*, is rich in collagen, a hard, insoluble, fibrous protein that is abundant in the human body. Collagen acts as a supporting structure and gives the skin its strength and elasticity. The thickest dermis is found on the back of the body.

The *skin basement membrane*, or basal lamina, is found between the dermis and epidermis. This is a thin, sheet-like compartment of extracellular matrix that provides support for the tissue and acts as a sentinel between the two layers. It allows a controlled traffic of cells and bioactive molecules in both directions, and serves as a reservoir for the release of different cytokines and growth factors[11].

The *subcutaneous fatty tissue*, found beneath the dermis, contains groups of fat cells known as *lipocytes, which* are divided into groups by septa. The size of lipocytes may vary considerably from person to person.

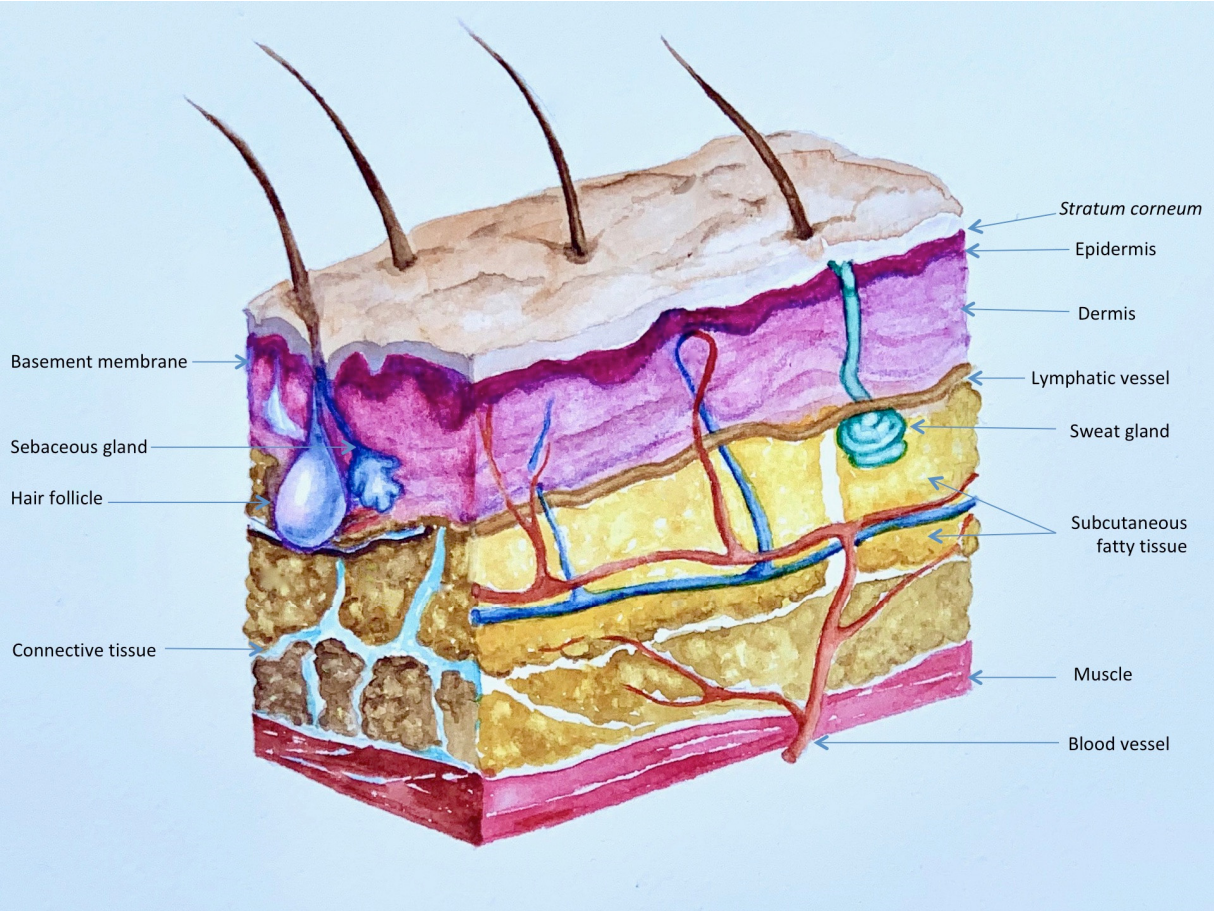


Figure 2. Schematic illustration of the skin structures.
Illustration created by Victoria Marmgren

Light

In terms of physics, light can be described as comprising electromagnetic radiation of different wavelengths in the range of 200 - 2000 nm [12]. An atom possesses a nucleus with protons and neutrons, and a series of electrons orbiting around it. When an electron is excited by external energy, such as electromagnetic radiation, it will strive to return to its lowest energy level by releasing the excess energy in the form of photons. A photon is an elementary form of electromagnetic radiation, a packet of energy, which moves through space until it reaches an object that it can release its energy to. The wavelength of the photon is determined by the amount of energy the excited electron gives off. Our perception of light is limited to only the small spectrum, called “visible light”. The division of electromagnetic radiation depending on the wavelength is presented in Figure 3.

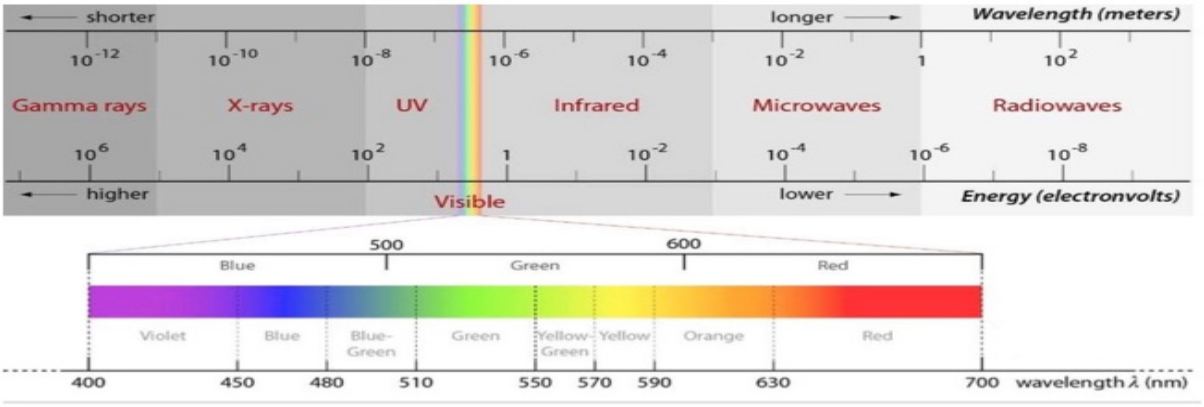


Figure 3. The electromagnetic spectrum.
Illustration by Verhoeven, G., 2017. The reflection of two fields – Electromagnetic radiation and its role in (aerial) imaging. AARGnews 55, 13–18. Copyright by G.Verhoeven.

Ultraviolet (UV) radiation is further divided into UVA, UVB and UVC, depending on its wavelength (Table 1).

Table 1. Wavelength and amounts of UV radiation reaching the surface of the Earth.

	UVC	UVB	UVA
Wavelength (nm)	100-280	280-315	315-400
Amount that reaches the surface of the Earth	Almost non-existent. Does not pass the ozone layer	Mostly absorbed by ozone layer. Accounts for <2% of all UV-radiation	Passes freely though the ozone layer. Accounts for >98% of all UV-radiation

Note: As wavelengths <290 nm are blocked by ozone in the stratosphere [13], wavelengths between 100 nm and 290 nm are sometimes referred to as UVC.

Table adapted from ISO 21348 (process for determining solar irradiances). http://www.spacewx.com/ISO_solar_standard.html.

Light is the main source of energy for all life on the planet. Plants need light for photosynthesis, the process that enables them to survive and produce oxygen. Humans also need light to thrive. Vitamin D is a fat-soluble vitamin, important for the intestinal absorption of calcium, magnesium, and phosphorus [14]. Vitamin D deficiency is known to cause symptoms associated with secondary hyperparathyreoidism, such as rickets in children and osteomalacia with clinical signs of osteoporosis in adults. Furthermore, many studies indicate an association between vitamin D deficiency and some forms of cancer, diabetes, cardiovascular

disease, autoimmune diseases, and depression [14]. Around 90-95% of people are estimated to reach their optimal vitamin D levels from exposure to sunlight [15]. Sun is the main source of electromagnetic radiation, but many artificial sources have also been created.

Exposure of skin to UVB has been shown to increase the concentrations of both serum cholecalciferol, which is a form of vitamin D naturally synthesized by the skin, and serum 25(OH)D3, which is a form of vitamin D produced in the liver by hydroxylation of vitamin D3, albeit to a different extent depending on the area exposed [16]. Studies investigating the effect of both natural and artificial sources of UVB on psoriatic skin have reported improvements in Psoriasis Area and Severity Index (PASI) score as the result of climate therapy and both broadband and narrowband UVB treatment [17-19]. UVB lamps are useful in treating a variety of inflammatory skin conditions, such as eczema and lichen ruber. UVA is also used therapeutically. The combination of UVA and psoralens, which are naturally occurring furocoumarins that increase the skin's sensitivity to light, is known as PUVA, and has historically been used for the treatment of psoriasis. Light sources emitting both UVA and UVB are used for the treatment of different inflammatory conditions in the skin, mainly atopic dermatitis.

Visible light is of immense importance for our wellbeing. Lack of exposure to natural light has been linked to sleep disturbance and depression[20,21], and sources of visible light are used for treatment of mood disturbances. The use of visible light is also an important therapeutic option in the field of dermatology, where it is employed as a part of photodynamic therapy in order to photoactivate protoporphyrin IX and induce a controlled cell death. Photodynamic therapy is used for treatment of pre-tumorous and tumorous skin lesions such as actinic keratosis and basal and squamous cell carcinomas [22].

Alongside these health benefits, exposure to both UV radiation, and in some cases visible light, carries non-negligible risks. A Japanese study found a significant risk of depression in those exposed to visible light at night[23]. Some systemic conditions, such as systemic lupus erythematosus and dermatomyositis, are known to be exacerbated by UV exposure, and common dermatoses, such as atopic and seborrheic dermatitis, rosacea, and some forms of acne may show signs of worsening, or *photoaggravation*, on UV exposure.

In the area of dermatological oncology, exposure to UV radiation has been linked to many forms of skin cancer, including both non-melanoma skin cancer [24-28] and malignant melanoma[24,26,29]. The incidence of malignant melanoma and squamous cell carcinoma is rising, particularly in coastal areas, and UV exposure is considered an important etiological factor [30,31]. UV radiation is thought to cause direct cellular damage to the skin and its immunologic function. DNA damage due to UV radiation may occur via formation of cyclobutane pyrimidine dimers, gene mutations, suppression of the immune responses, oxidative stress, and inflammatory

responses[32]. Additionally, if mutations in p53 tumour suppressor genes are present, UV-induced DNA damage leads to delay in the DNA repair process and apoptosis of cells with substantial DNA damage[33]. The mutated keratinocytes expand, and skin cancer may arise[33,34].

Both UVA and UVB are considered important for the skin carcinogenesis, with UVA playing an important role in the carcinogenic transformation of stem cells [33], and UVB causing DNA damage with subsequent inflammatory responses and tumour formation[34]. UVC radiation may cause acute damage to the skin and the eyes, but the data on long-term exposure to UVC from artificial sources are insufficient. UVC is considered to be carcinogenic to humans [35].

Light in occupational and environmental dermatology

Occupational risks from exposure to UV radiation have been described. [34]. UVC, a possibly cancerogenic part of the UV spectrum [35], is not naturally present close to the surface of the Earth (Table 1) but is emitted by some equipment, such as welding torches and bactericide lamps which are used both in operating rooms and in the industry to kill bacteria and viruses. UVA and UVB exposure are both common due to the presence of these wavelengths in the environment, but occupational risks due to workplace exposure have also been described [36]. A study of the UV exposure of dermatology department staff found that UVB and UVC exposure exceeded the permitted levels [37]. Occupational exposure to UV radiation and risk for squamous cell carcinoma (SCC) have been studied, and dose-response relationship was observed between occupational exposure to solar radiation and incidence of SCC [38].

In welders, ocular exposure to UV radiation (mainly UVB and UVC) has been estimated as four to five times the maximum permissible exposure, and skin exposure as around 3000 times the maximum permissible exposure [36]. The use of adequate protection is therefore mandatory.

Skin reactions may arise if a certain chemical substance comes in contact with the skin, and light may be of a crucial importance for some types of these reactions. If this aspect is not taken into consideration there is a risk that the right diagnosis will be missed. A closer look at different types of skin reactions in relation to such exposure is given in the next chapter.

Types of skin reactions upon occupational and environmental exposure to chemicals

Being a dermatologist is often not too different from being a detective. Different villains (skin diseases) may disguise themselves as anonymous rashes, and meticulous investigations and even Sherlockian deduction are needed to reveal their true identity.

So, what are we dealing with in the area of occupational and environmental dermatology? The website of my department in Malmö gives a clear and simple definition of our aim: "The work is focused on increasing our knowledge of the effect the environment has on the skin, and particularly with regard to contact allergy and allergic contact dermatitis" [39]. The environment mentioned includes both work and leisure. Indeed, most of our efforts are directed towards the diagnosis and prevention of contact dermatitis, with allergic contact dermatitis (ACD) being a very important part of this.

When it comes to terminology, similar terms may be used by dermatologists, allergologists, immunologists and toxicologists, but the meaning of these terms may differ somewhat.

Allergy: While allergologists mainly deal with immunoglobulin E (IgE)-modified, or *immediate type allergic reactions*, the allergic reactions seen by occupational and environmental dermatologists are usually so-called *delayed hypersensitivity reactions* (Table 2).

Table 2. Types of allergic reaction.

Types of allergic reactions	Characteristics	Examples of clinical manifestations
Type I	IgE-mediated, immediate hypersensitivity. Mast cell activation	Allergic rhinitis, asthma, anaphylaxis, contact urticaria
Type II	IgG *or* IgM, antibody-mediated cytotoxic hypersensitivity. Complement-mediated phagocytosis	Drug hypersensitivity, posttransfusion hemolysis, autoimmune anemias, acute graft rejection
Type III	IgG *and* IgM, immune complex-mediated hypersensitivity with subsequent tissue damage	Reactive arthritis, serum sickness, systemic lupus erythematosus
Type IV	T-cell-mediated, antibody-independent, delayed type hypersensitivity. T-cell-induced inflammation or cytotoxicity	Allergic contact dermatitis, multiple sclerosis, chronic graft rejection

Photosensitivity: From a toxicologist's point of view, *photosensitivity* is the ability of a chemical to make skin more sensitive to light. For a dermatologist, photosensitivity means an individual adverse response to light, with or without a chemical involved in the process.

Irritancy versus toxicity: The terms irritancy and toxicity are used in somewhat different way by toxicologists and dermatologists. *Toxicity* is defined as the ability of a chemical substance to damage an organism or a part of an organism, while *irritancy* refers to an inflammatory reaction following an interaction between a chemical substance and an organism.

Phototoxicity and photoirritancy imply that both the UV radiation or visible light and the chemical substance are involved in the onset of the reaction. Strictly speaking, phototoxicity is defined as "a toxic response from a substance applied to the body which is either elicited or aggravated after subsequent exposure to light, or that is induced by skin irradiation after systemic administration of a substance" [40]. In dermatology, the term phototoxicity is used interchangeably with photoirritancy [41], which is a multifactorial, non-immunological inflammatory skin response in the presence of an offending agent/chemical substance and light. For a toxicologist, phototoxicity includes all types of reactions induced by the combination of a chemical and light, and thus includes photoirritant contact dermatitis, photoallergic contact dermatitis (PhACD), and UV-induced DNA damage.

Skin reactions due to phototoxicity/photoirritancy are considered much more common than photoallergic contact skin reactions. The main differences between photoallergic and photoirritant contact skin reactions are described in Table 3.

Table 3. Comparison of photoallergic and photoirritant contact skin reactions.

	Photoirritant contact reaction	Photoallergic contact reaction
Prevalence Risk groups	Common Anyone	Uncommon Susceptible individuals
Type of action	Direct cytotoxic effect	T-cell-mediated immunological reaction
Concentration of contactant	High	Low
Onset	Minutes to hours, first exposure	Hours to days in sensitized, days to weeks in previously non-sensitized individuals
Clinical signs	Well-demarcated redness, vesicles, blisters. Burning sensation	Diffuse redness, papules. vesicles. Pruritus.
Cross-reactivity with other substances	Non-existent	Common
Resolution	Short duration, decrescendo-type reaction	Prolonged duration, crescendo-type reaction

Contact allergy

Contact allergy is a delayed hypersensitivity reaction (type IV reaction), which develops after exposure (usually repeated) to an allergen. More than 4,900 chemicals are considered as potential contact allergens[42,43]. The relevance of many of these allergens has changed over time. For example, elemental mercury and mercury-based substances were important contact sensitizers throughout the 20th century, being found in dental amalgam, disinfectants (earlier formulations of Merthiolate®), eye drops, and topical ointments among others. Since the 1990s, the use of mercury has drastically diminished due to health and safety regulations, influencing the prevalence of clinically significant contact allergy[44]. On the other hand, industrial progress and changes in consumer behaviour can lead to the rise of new allergens. From 2008 to 2015 alone, 172 new allergens were discovered[43].

Many emerging contact allergens are found among fragrances and preservatives [43,45-47].

A substance becomes an *allergen* if it manages to cause an immunological response from the target organism. A chemical needs to become activated in order to become an allergen, and is prior to this is referred to as a *hapten*. Some substances undergo a transformation outside of the skin, for example by reacting with air or light. Haptens are characterized by low molecular weight (defined as <900, or sometimes <500), which allows the molecules to rapidly penetrate the lipophilic stratum corneum and diffuse across cell membranes towards the intercellular space. Furthermore, many haptens need to be able to form stable covalent bands with the protein molecules in the epidermis. In order to elicit an immunological response, the hapten must be combined with a larger molecule, or a *carrier*. The carrier is usually a protein, and together with the hapten forms a hapten-carrier complex (an allergen). The hapten itself is not immunogenic, and protein binding is essential for the immune system to react.

The concept of *prehapten* refers to a non-reactive molecule that can be transformed into hapten by simple chemical transformation with no requirement for a specific enzyme [48,49]. An example of prehapten formation is autooxidation, a spontaneous air-induced oxidation of organic molecules, which proceeds via a free radical chain reaction that leads to the formation of hydroperoxides as primary oxidation products. Fragrance terpenes are examples of substances that are not allergenic until oxidized [50-52].

Another way of hapten formation is via an enzymatic transformation in the skin. The non-protein reactive precursors are referred to as *prohaptens*. Cinnamic alcohol is one example, although the sensitizing metabolites, responsible for positive patch test reactions, are presently not known [53].

The concept of pre- and prohaptens has been questioned, and the notion of electrophiles and proelectrophiles has been suggested as a substitute, largely due to the need for a more precise explanation of sensitization process and the fact that little is known about skin metabolism [54]. At present, however, we continue to use the terms *prehapten, prohapten and hapten* when referring to known or suspected contact and photocontact allergens.

The initial phase of immunological reaction leading to the development of contact allergy is called *sensitization.* The hapten binds to a carrier molecule in the epidermis, and the hapten-carrier complex presents itself to dendritic cells via Toll-like receptors and proinflammatory cytokines. Langerhans cells, also known as antigen-presenting cells, are the only subtype of dendritic cells present in the epidermis. They carry the complex to the regional lymphatic nodes, where it encounters and activates naÏve T-cells. Memory and effector T-cells are formed, and the initial inflammatory response enables them to enter the circulation and migrate back to the initial site of exposure to the hapten-carrier complex.

When re-exposure to the same hapten happens post sensitization, the *elicitation* phase starts. The incubation phase is the time required for the sensitized host organism to prepare for the immunological response, i e elicitation, and usually takes 1-4 days [55]. However, for some substances, such as gold, budesonide, isocyanates and acrylates among others, this phase may be as long as weeks, partly depending on exposure dose [56-59].

Proinflammatory cytokines are released from the effector T-cells, attracting specific and non-specific inflammatory cells, leading to classical signs of dermatitis. Understanding of the elicitation phase is not complete, and the search for the cytokines and chemokines involved in the process is ongoing[60,61].

The possibility of genetic susceptibility to contact sensitization has been confirmed, such as the role of filaggrin mutations in contact sensitization to nickel [62]. Filaggrin mutations are known to be an important factor in atopic eczema and show strong associations with both atopic eczema and contact sensitization to nickel[62,63]. However, a recent multicentre study found no significant correlation between the history of atopic dermatitis and contact sensitization to the components of the baseline series [64].

It is important to distinguish between contact allergy and ACD. ACD is a clinical manifestation of contact allergy that arises when the skin is re-exposed to a chemical to which it has been sensitized. Sensitization does not always lead to elicitation of clinical response; this may be explained by multiple factors, including the state of skin barrier, the number of hapten molecules, and the degree of reactivity to the sensitizer. When the contact allergy is weak, more hapten molecules are needed to elicit an ACD than when a strong allergy is present, but the immunological response is not linearly correlated to the dose of the hapten. Contact allergy that manifests itself at diagnostic testing, but where the subsequent evaluation regarding exposure and assessment of clinical relevance cannot demonstrate any significance for the disease under current or previous investigation, is considered clinically non-relevant[65].

Contact allergy may present itself in many different ways [66]. *Systemic contact dermatitis* occurs when a sensitized individual is re-exposed to the allergen systemically, for example via the oral, parenteral or trans-mucosal route. Systemic contact dermatitis after exposure to mercury was first described in 1895 [67], and the number of chemicals known to cause this type of systemic reaction has since then been increasing steadily. Corticosteroids, cinnamic substances, nickel, neomycin and parabenes are just some of the examples. Systemic contact dermatitis might present with both cutaneous and extracutaneous manifestations. Pain in muscles and joints, abdominal pain and diarrhoea, generalized malaise with subfebrile temperature, and in-stent restenosis are examples of extracutaneous manifestations[66,68].

Photocontact allergy

A remarkable outbreak of skin problems occurred in the United Kingdom and the USA in the 1960s. Rigorous investigations conducted by dermatologists and chemists found that the cause was an increased production and consumption of both industrial and household detergents. The culprit products were discovered to contain halogenated salicylates, such as tetrachlorosalicylanilide, and the dermatitis appeared to be photoinduced with photosensitization presenting as PhACD, sometimes resulting in persistent photosensitivity[69-71]. In the following decades, photosensitizers such as olaquindox (anti-bacterial agent), musk ambrette (fragrance component), and hexachlorophene (disinfectant) became important due to their availability on the market. Today, these photosensitizers have been either removed from the market, or strictly regulated, which has contributed to falling rates of sensitization. Instead, new chemicals are being introduced, some of them possessing photosensitizing properties.

The initial steps of photosensitization and photoirritation share common features (Figure 4). A photosensitizer/photoirritant needs to absorb UV or visible light sufficiently for the phototransformation to occur. Some photosensitizers-/photoirritants used for therapeutic purposes such as photodynamic therapy have their peak absorption at a wavelength between 630 and 700 nm, which makes it possible to achieve the response with visible light [72]. These (e.g. aminolevulinic acid and methyl levulinate) are known to produce reactive oxygen species (ROS), which cause a subsequent DNA-damage; this is considered crucial for phototoxicity, which is a concept that covers both photoallergenicity and photoirritation[73].

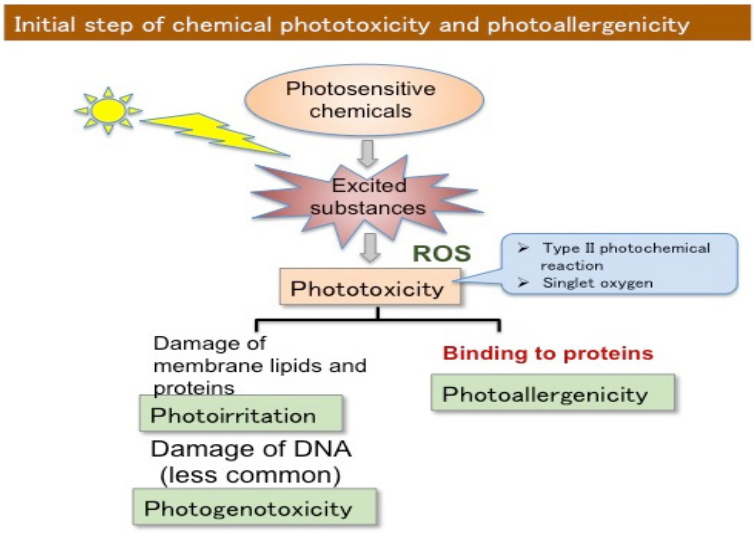


Figure 4. The initial steps of chemical phototoxicity and photoallergenicity

Illustration courtesy of Professor Yoshiki Tokura, MD, PhD, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

The course of sensitization and elicitation concerning a *photocontact allergy* follows the same principles as contact allergy. The main difference is that a chemical needs to undergo a phototransformation by UV or visible light in order to become a hapten. This phototransformation may occur exogenically or upon entering the epidermis. As to the notion of a photoallergen, two main explanation models exist[74]. One model utilizes the concept of a prohapten [75], which is based on the principles described above (see **Contact allergy**). In the setting of photocontact allergy, a prohapten is phototransformed by UV-light into a hapten prior to protein binding. The other explanation model instead proposes the notion of a *photohapten*. A photohapten enters the epidermis, or encounters a protein molecule in some other way, prior to the phototransformation, and the covalent bond with protein is formed upon UV irradiation, via ROS[74]. The exact mechanism of phototransformation is thus still not fully understood. As the standard procedure for photopatch testing (see below) involves the application of a tested substance on the skin before the irradiation, the notion of a photohapten is being used empirically.

Similar to ACD, PhACD implies an immunologic response from the host, which differentiates it from phototoxic and photoirritant contact dermatitis (Figure 5). A chemical needs to possess certain qualities in order to be suitable as a photosensitizer; or, more precisely, as a photoallergen. As in the case of contact allergens, the molecule needs to be of low molecular weight. Aromatic compounds substituted with groups absorbing UV radiation are known to be overrepresented among photosensitizers.

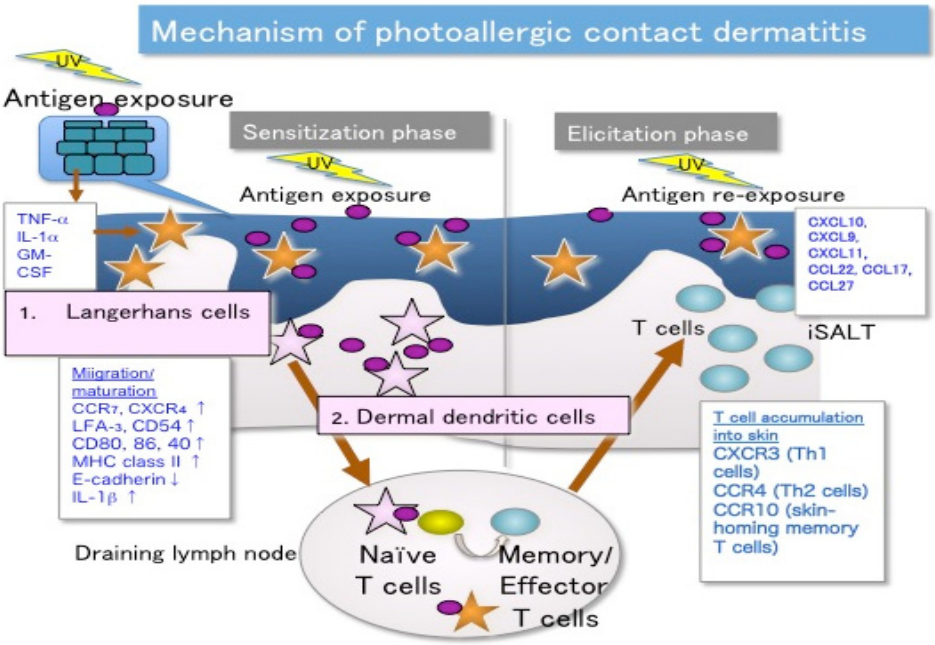


Figure 5. Mechanism of photoallergic contact dermatitis

Illustration courtesy of Professor Yoshiki Tokura, MD, PhD, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Similar to contact allergy, photocontact allergy can manifest itself as the systemic photocontact dermatitis. The most common cause of systemic photodermatitis is photosensitive drugs[76,77]. Antibiotics such as tetracyclines, fluoroquinolones, and sulfonamides are known to cause phototoxic reactions, while the mechanism behind photodermatitis due to hydrochlorothiazide, amiodarone, and chlorpromazine is photoallergic in nature. Doxycycline may cause both phototoxic and photoallergic systemic dermatitis [76,78]. A number of skin conditions has been associated with photocontact allergy, e g polymorphic light eruption, photoaggravated dermatoses (eczema or psoriasis), contact allergic eczema, solar urticaria[79], and even the highly therapeutically challenging actinic reticuloid syndrome[80,81].

Investigative methods

Patch testing

To diagnose contact allergy, a chemical or a mix of chemicals must come into contact with the epidermis and remain in the epidermis for a period of time in order to simulate an actual exposure. The method of controlled allergen application and reaction evaluation known as *patch, or epicutaneous, testing* (PT) was first proposed by a German dermatologist, Josef Jadassohn (1863–1936), in 1895. The procedure of PT is standardized at any given time[82,83], but recommendations for the tested substances, concentrations, and vehicles are constantly being scrutinized, and changes are made when necessary[84-88]. The chemicals used are grouped in series, such as baseline, plastics, dental, or fragrance series, and the option of including additional chemicals or the patient's own products is used when needed. Vehicles used for the sensitizers include petrolatum, ethanol, acetone, and water. A dose of 40 mg/cm² is considered optimal for petrolatum preparations[86], and 30 µl/cm² for liquid preparations[88]. Each chemical or mix of chemicals is placed on an aluminium or plastic chamber and secured to the patient's back with hypoallergenic tape.

Two different application systems are in use. The oldest system uses various types of aluminium or plastic chambers (e.g. Finn chambers, IQ chambers) which have to be loaded with the test preparations before application on the back of the individual to be tested. The Finn chamber method, which has been in use since the 1970s, was designed in 1975 by a member of the International Contact Dermatitis Research Group (ICDRG), professor Veikko Pirilä (1915-1998) at Helsinki University Allergy Hospital. A row of small aluminium chambers is attached to a tape. The chambers are filled with allergens diluted in either petrolatum, ethanol, or water, and then attached to the skin (usually of the upper back) of the tested individual (Figure 6).

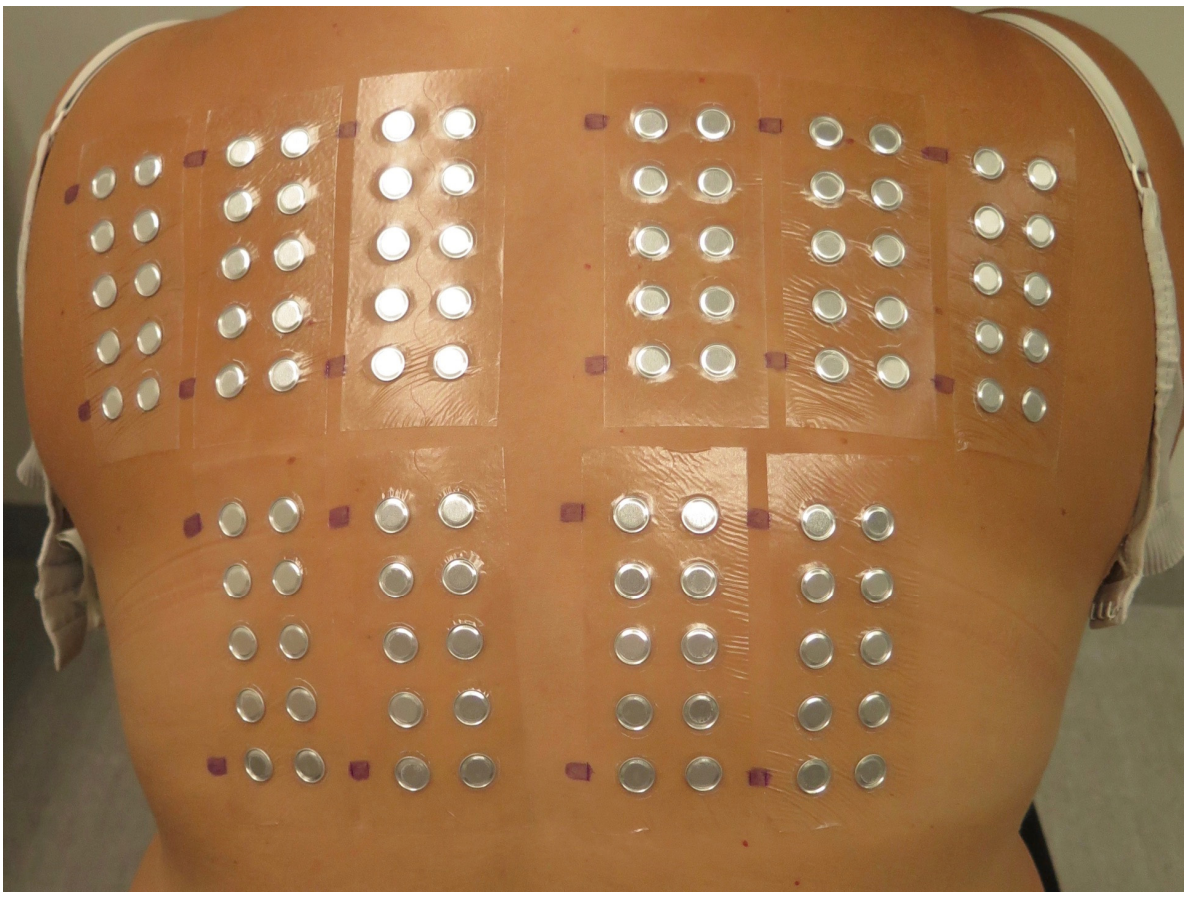


Figure 6: Application of series of contact allergens on the back of a patient, using Finn Chambers.
Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

The other type of application system consists of chambers preloaded with the sensitizers; for example, the thin-layer rapid-use epicutaneous (T.R.U.E) test, which was approved by the United States Food and Drug Administration (FDA) in 1995. The T.R.U.E. test originally contained 23 allergens and a negative control. Five more allergens were added in 2007, and today the test consists of 35 common allergens and a negative control. Major advantages of this ready-to-use test system are the ease of use when there are limited resources, for example in small practices, and the exact hapten dosage. The major limitation is its slowness to adapt to recommendations on changes in the baseline series; for example, the introduction of new sensitizers and changes in the concentrations of sensitizers already present.

The test chambers of the chosen test system are applied to an intact epidermis. The standard occlusion time for PT is 48 hours (h), and the reading of the test is performed after 72 h on day (D) 3 or 4 (96h), and in Sweden also on D7, in order to detect a late-appearing allergic response.

Photopatch testing

If a photocontact allergy is suspected, *photopatch testing* (PPT) should be undertaken. Allergens commonly used for PPT are included in a photopatch test series, such as the Scandinavian (Table 4) or European (Table 5) photopatch test series.

Table 4. The Scandinavian photopatch test series [89].

Substance	Concentration and vehicle
Trichlorocarbanilide	1% pet
Promethazine hydrochloride	1% pet
Para-aminobenzoic acid (PABA)	10% pet
Tribromsalicylanilide	1% pet
Chlorpromazine	0.1% pet
Eusolex 4360 escalol 567	10% pet
Methylcoumarin	1% pet
Bithionol	1% pet
Fentichlor	1% pet
Usnic acid (D-)	0.1% pet
Atranorin	0.1% pet
Wood mix	20% pet
Evernic acid	0.1% pet
Myroxylon pereirae	25% pet
Irgasan BS 200	0.1% pet
Hexachlorophene	1% pet
Chlorhexidine digluconate	0.5% aq
Triclosan	2% pet
Diphenhydramine hydrochloride	1% pet
Fragrance mix I	6% pet

Note: pet = petrolatum, aq = aqua.

Table 5. The European Photopatch Test Series [90].

Substance	Concentration and vehicle
Phenylbenzimidazole sulfonic acid	10% pet
Homosalate	10% pet
Ethylhexyl salicylate	10% pet
Polysilicone-15	10% pet
Benzophenone-3	10% pet
Benzophenone-4	2% pet
Ethylhexyl methoxycinnamate	10% pet
Isoamyl p-methoxycinnamate	10% pet
Butyl methoxydibenzoylmethane	10% pet
Drometrizole trisiloxane	10% pet
2-(4-Diethylamino-2-hydroxybenzoyl)-benzoic acid hexylester	10% pet
Methylene bis-benzotriazolyl tetramethylbutylphenol	10% pet
Diethylhexyl butamido triazone	10% pet
Benzydamine hydrochloride	2% pet
Decyl glucoside	5% pet
Benzophenone-10	10% pet
4-methylbenzylidene camphor	10% pet
Octocrylene	10% pet
Para aminobenzoic acid (PABA)	10% pet
Ketoprofen	1% pet
Ethylhexyl triazone	10% pet
Etofenamate	2% pet
Piroxicam	1% pet
Promethazine hydrochloride	0.1% pet
Bis-ethylhexylphenol methoxyphenol triazine	10% pet

Note: pet = petrolatum.

The European photopatch test series contains 25 allergens, including 18 sunscreen agents and 5 NSAIDs (ketoprofen, etofenamate, piroxicam, diclofenac sodium salt, and ibuprofen). As with PT, a patient's own material can be used in addition to the series. The same application procedure is applicable for PPT as for PT, with chambers containing allergens being applied to the skin on the patient's back. In PPT, however, identical duplicate sets are applied on the left and right side of the back. The occlusion time varies, but according to the ICDRG guidelines and the recommendations of expert groups, 24h is sufficient for PPT [82,89-91]. At this time, 24 h after application (D1), all patches are removed and one side of the back is immediately covered with a UV-opaque material. In order to simulate sun exposure and reflect the action spectrum of most photoallergenic chemicals, the test area is irradiated by UVA[73,92](Figure 7).

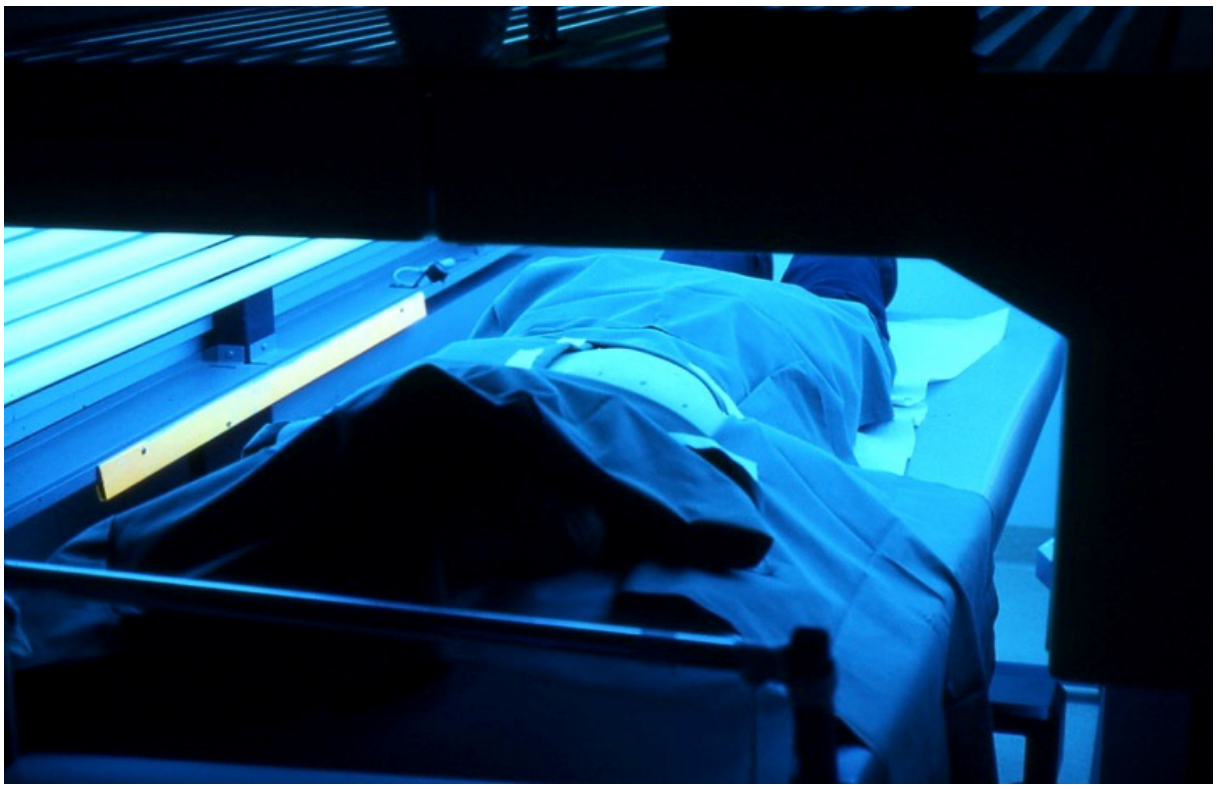


Figure 7. The irradiation of the back of the patient with UVA after the removal of allergen sets. One side of the back is covered with UV-opaque fabric.

Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

The UVA and chemical doses used are standardized to trigger a photoallergic reaction rather than a phototoxic response for phototoxic substances that also may be photosensitizers. The recommended dose for UVA is 5 J/cm^2. Duguid et al. showed that doses of 5 J/cm^2 and down to as little as 1.0 J/cm^2 are sufficient for photoelicitation, but 5 J/cm^2 should remain a standard in order to avoid false negative test results [93]. Another study showed that a UVA dose below 5 J/cm^2 could lead to the loss of significant PPT reactions, but doses between 20–40 J/cm^2 and up to 80 J/cm^2 did not improve the significance of PPT results[94].

In individuals with known or suspected extreme photosensitivity, a UVA test with various doses of UVA including doses lower than 5 J/cm^2 must be performed. This testing can be done at the same time as the PPT. The reading of the UVA test takes place after 24 h. If there is sensitivity to UVA reflected by a lowered threshold for erythema, 50% of the minimal erythema dose of UVA should be used for the PPT irradiation.

Readings should be recorded using the guidelines of ICDRG and the European Society of Contact Dermatitis, with readings on D1 before and immediately after irradiation, and on D3, 48h after irradiation. Further readings on D4 at 72h post irradiation are recommended in order to discover crescendo/decrescendo reactions

[82,89-91,95,96]. For the past decade, an additional mandatory reading of the PPT has been performed in Malmö one week (D7) after the application of the chambers (personal communication with Magnus Bruze, 2021).

Interpretation of the test results

A positive test reaction during PPT may be interpreted in several different ways, depending on its morphology and the relation to the opposite side (whether the reactions occurs on the test or control side or both). A few steps should be taken to establish the nature of the reaction:

1. Is it an irritant reaction? An *irritant* reaction presents itself as a well-demarcated erythema/oedema, occasionally with blistering on both irradiated and non-irradiated sides, without spreading to the surrounding skin. A *photoirritant/phototoxic* reaction (see the chapter on Types of skin reactions upon occupational and environmental exposure to chemicals) will have similar morphology but will only be seen on the irradiated side.

2. Is it an allergic reaction? The morphology of an *allergic* reaction resembles that of eczema, with the erythema, oedema, papules, and vesicles/blisters being present not only at the site of the test chamber, but commonly also on the surrounding skin.

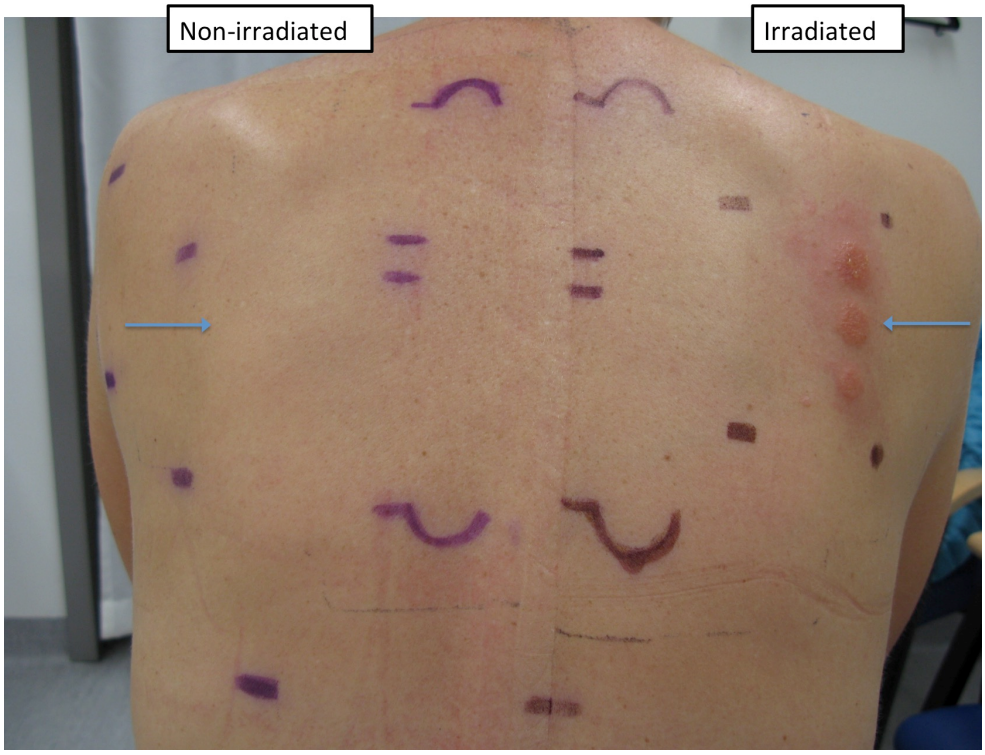
3. What type of allergic reaction is it? Several possible scenarios exist, and the interpretation may be assisted by following the guidelines in Table 6.

Table 6. Interpretation of photopatch test reactions according to the International Contact Dermatitis Group [96]

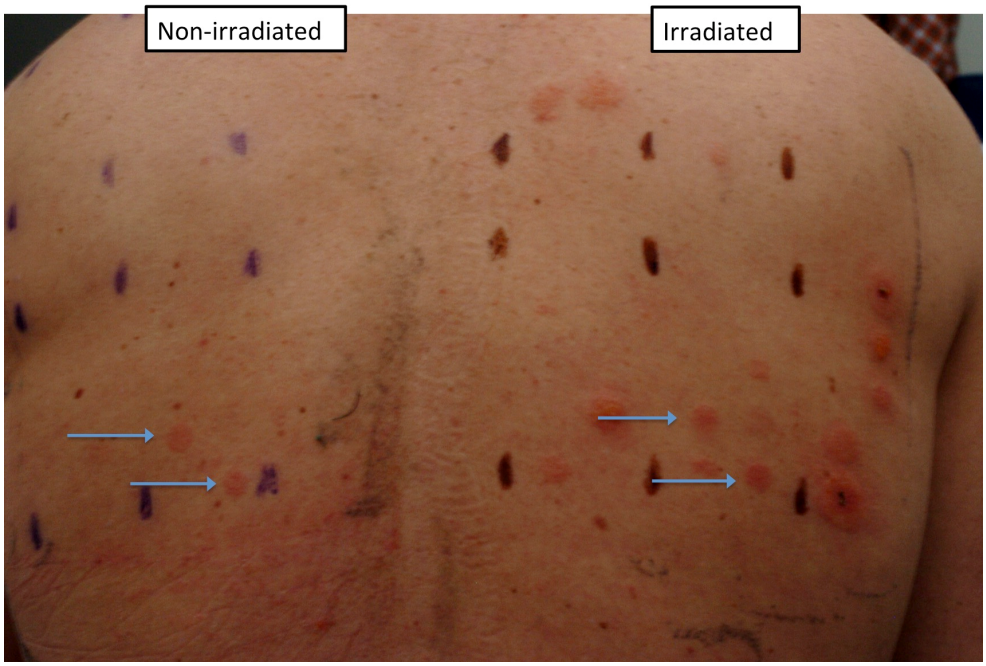
Non-irradiated site	Irradiated site	Interpretation
+	+	Contact allergy
++	++	Contact allergy
+++	+++	Contact allergy
+	+++	Contact allergy and photocontact allergy
+	++	Contact allergy with photoaugmentation
++	+++	Contact allergy with photoaugmentation
+++	++	Contact allergy with photoinhibition
++	+	Contact allergy with photoinhibition
-	+	Photocontact allergy
-	++	Photocontact allergy
-	+++	Photocontact allergy

Some clinical examples of PPT reactions are presented below (Figure 8)

Photocontact reactions



Photocontact and contact reactions



Photocontact, contact reactions and contact reaction with photoinhibition

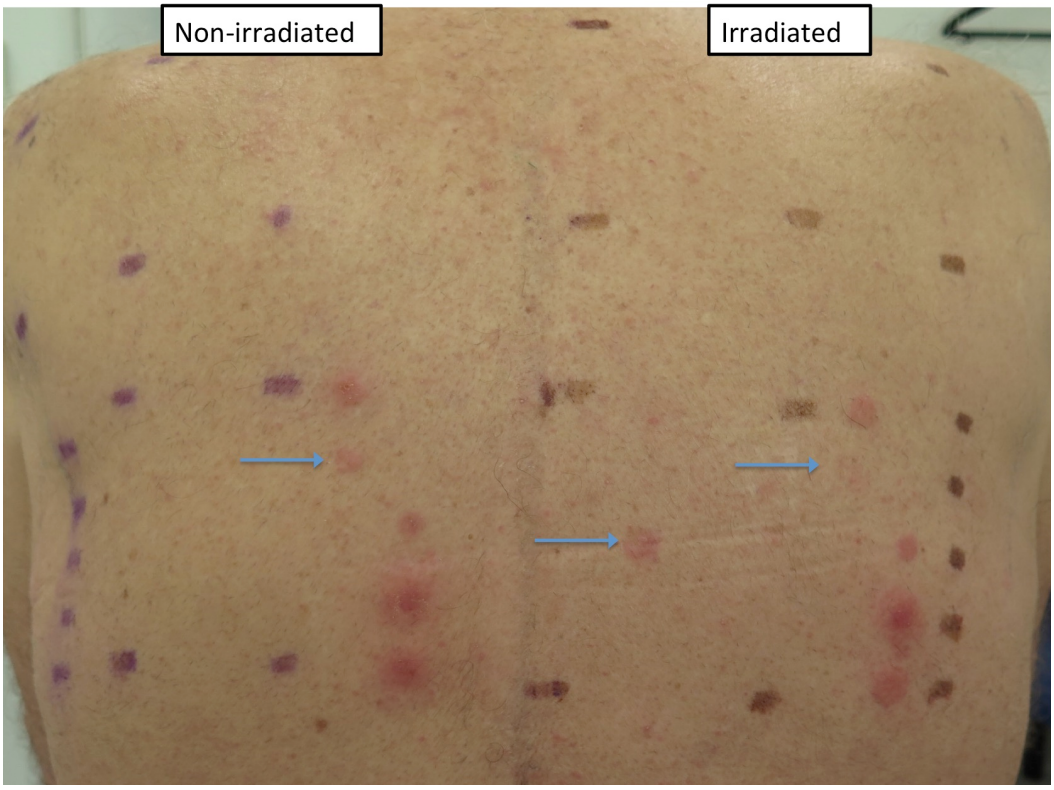


Figure 8. Clinical examples of PPT reactions.
Photos courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

The degree of reactivity is assessed based on the clinical appearance of the reactions, and the reactions are graded as +; ++; or +++; with the latter being the strongest reaction.

A diagnosis of photocontact allergy can be established provided that the reaction patterns on the non-irradiated and irradiated sites are as given for photocontact allergy in Table 6 and the substance in question was an established photoallergic sensitizer tested according to the recommendations. When PPT with a substance/chemical product with unknown photosensitizing capacity results in a reaction pattern suggesting photocontact allergy (Table 6), control testing has to be performed to rule out the possibility that the reaction is a photoirritant reaction mimicking a photoallergic reaction. The imperfectness of the present interpretations of positive PPT reactions was recently discussed in the literature[97].

For both patch and photopatch testing, evaluating the relevance of the results is of utmost importance[97-99]. A relevance scoring system has been proposed by Lachapelle, where both current and past relevance of the test result is assessed as 0=not traced; 1=doubtful; 2=possible; 3 =likely[100].

Scales such as COADEX [101] may also be used to assess the relevance (Table 7).

Table 7. COADEX scale for evaluation of the patch test results

Code	Meaning
C (current)	Exposure to the allergen prior to the current episode of dermatitis, improvement after cessation of exposure
O (old)	Past exposure that resulted in an episode of dermatitis.
A (active sensitization)	Sensitization reaction at present
D (doubtful/unknown)	Unknown relevance and relationship between the positive test result and the dermatitis
E X(exposed/cross-reaction)	Known previous exposure without clinical dermatitis/Cross-reaction with another, clinically relevant allergen, causing positive test reaction

Other investigative methods

The *Repeated Open Application Test* (ROAT) and *Provocative Use Test* (PUT) may be used when the relevance of a positive patch test reaction is unclear; for example, if there is a positive patch test reaction to a chemical but the patient does not experience skin symptoms upon normal use. Reasons behind the absence of reaction upon use might include insufficient exposure time, insufficient percutaneous penetration, or difference in threshold of response at different body sites[102]. The use tests are designed to mimic a real-life exposure[103]. The ROAT is usually conducted by the patient, who applies the tested product on the upper arm, forearm, or scapular area twice daily for 7 days, or until a visible reaction occurs. If no reaction appears, it is advisable to extend the length of the application in order to detect possible late-appearing reactions[104]. The primary goal of the use tests is not to distinguish between irritant and allergic dermatitis, but to confirm the presence of a reaction. The use tests may be conducted with the patient's own products, but no unknown substance should be applied directly to the skin. These use tests are virtually never used for assessment of photoallergenicity.

Intracutaneous tests may be recommended in assessment of doubtful patch test reactions[105]. These tests have mainly been used for metal salts, but nowadays their use is mainly limited to studies. Approximately 0.1 ml of saline solution of the tested substance is injected intracutaneously, and the results are read after 72h. An infiltrated dermal wheal of ≥4 mm is considered a positive test reaction[105,106]. To the knowledge of this author, intracutaneous tests are not used for the establishing of photocontact allergy; one reason for this may be the gradual disappearance of the UV radiation at irradiation of the skin surface.

Assessment of photoallergenicity/allergenicity

The assessment of allergenicity for any given substance is ideally made prior to its introduction to the market, and for most chemicals this is virtually mandatory. Historically, this assessment was made on the basis of reports of adverse reactions. The very first trials included human volunteers, and later laboratory animals became an important part of the assessment. At present, many novel *in vitro* (laboratory tests performed outside a living organism, e.g. in a test tube or culture dish), *in chemico* (abiotic chemical reactivity methods), and *in silico* (tests conducted by means of computer modelling/simulation) diagnostic procedures have made it possible to predict the allergenic potential of a substance without the use of life forms.

The cosmetic products regulation, previously known as the cosmetics directive, has established an animal testing ban on finished cosmetic products since 11 September 2004. The testing ban on ingredients or combination of ingredients and the marketing ban has applied since 11 March 2009[107] for all human health effects except for repeated-dose toxicity, reproductive toxicity, and toxicokinetics, to which the marketing ban has applied since 11 March 2013[107]. This regulation has made the introduction and development of alternative assessment methods even more important.

In vivo models

Human predictive test models such as the Human Repeated Insult Patch Test (HRIPT), the Maximization Test, and the Modified Draize Test are some examples of models used to predict the allergenic potential of a chemical[108-111]. The HRIPT is used to test whether a certain topical dose of a sensitizer induces sensitization after repeated applications over a 3-week period of time[112]. Experimentation on humans is complicated due to both ethical issues and the large number of volunteers needed. The use of human predictive methods for the assessment of phototoxicity and photoallergenicity should be used with caution because of the risk of persistent light reactivity post sensitization.

Guinea pig models for assessment of drug toxicity and allergenicity, but also phototoxicity and photoallergenicity[113-115], have been used since the 1950s. The Draize method was developed by Draize in 1959[116], and was followed by several other techniques. The guinea pig maximization test[117], the cumulative contact enhancement test[118], and Freund's complete adjuvant test[119] have been reported to be the most commonly used predictive guinea pig models in Sweden[120]. Guinea pig models may be used for assessment of both sensitization and elicitation phases[114].

Since the 1980s *predictive test methods in mice* have been developed. These are mainly used for the assessment of contact toxicity and allergenicity, but the use of murine models for the assessment of phototoxicity and photoallergenicity has also been proposed[121]. Predictive test methods in mice include different lymph node assays, such as the popliteal lymph node assay (PLNA) [122], the local lymph node

assay (LLNA) [123], and the sensitive mouse lymph node assay (SLNA) [124], as well as variations on the mouse ear swelling assay[125-127](Figure 9). The results of murine assays are reported to be of greater accuracy than those of guinea pig models[125]. In fact, most of what we know about the pathophysiology of contact dermatitis is derived from murine models[126].

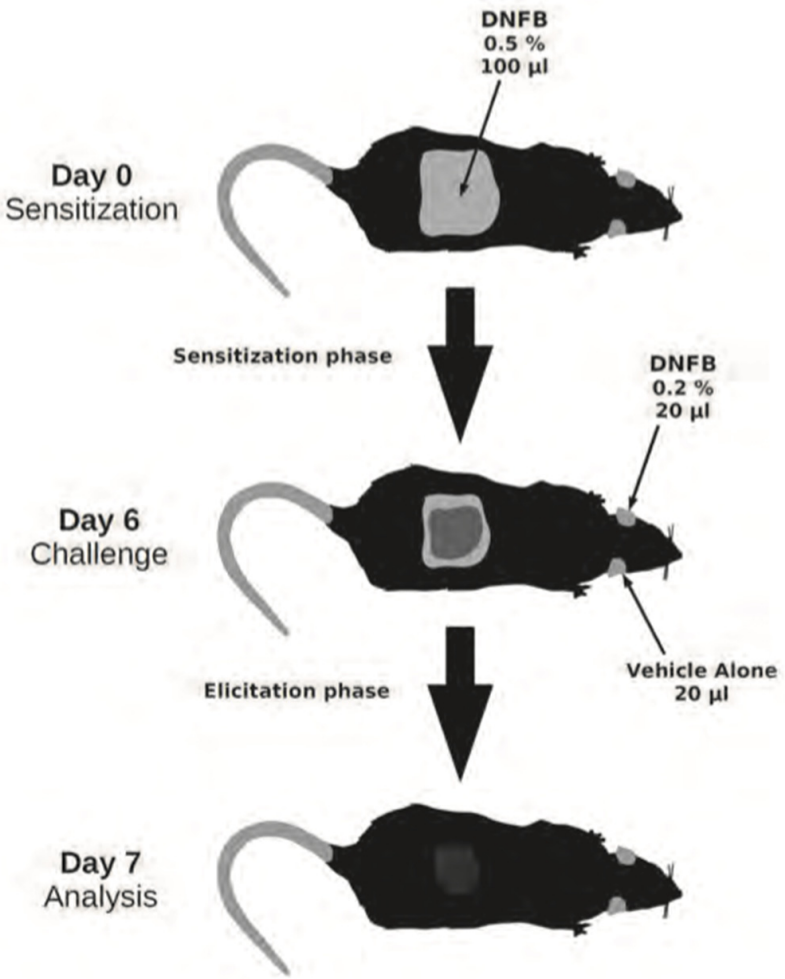


Figure 9. Allergic contact dermatitis experimental protocol. DNFB = 1-fluoro-2,4-dinitrobenzene (Sanger's reagent)

Figure courtesy of Federico Simonetta and Christine Bourgeois (16 December 2011). Animal Models of Contact Dermatitis, Contact Dermatitis, Young Suck Ro, IntechOpen, DOI: 10.5772/29462. Available from: https://www.intechopen.com/chapters/25242

In vitro/in chemico/in silico models

On 14 June 2021, a new guideline on approaches for skin sensitization was published by the Organisation for Economic Co-operation and Development (OECD) [128]. The information provided in the guideline consists of combined data on chemical safety, interpreted using a fixed data interpretation procedure. The approaches include *in chemico, in vitro*, and in some cases *in silico* methods[129] for hazard and potency identification of the chemicals, and are considered to be either

as informative or more informative than LLNA in terms of hazard identification[128]. Similar approaches are under development for the assessment of phototoxicity, including the 3T3 neutral red uptake phototoxicity test (3T3 NRU-PT) [40], the photo hen's egg[130,131] test, and various reconstructed human skin models[132].

When testing a substance for photoallergenicity, the pharmacokinetic profile of the substance should be assessed prior to the study.

After the UV-visual spectral analysis of the substance and the evaluation of superoxide formation via ROS assays, three "key events" are proposed.

Key event 1 is directed to the assessment of the substance's capacity to establish covalent protein binding upon UV exposure.

Key event 2 evaluates the photoactivation of keratinocytes in the presence of the test substance.

Key event 3 is directed towards detection of UV-induced T-lymphocyte activation.

The proposed approach is depicted in Table 8.

Table 8. Proposed safety assessments of phototoxicity and photoallergenicity

Proposed safety assessments of phototoxicity and photoallergenicity of chemicals			
Skin sensitization Adverse Outcome Pathway (AOP) (OECD)		Photo-toxicity	Photo-allergenicity
Photochemical properties UV-VIS spectral analysis ROS assay	UV-VIS absorption of chemicals Generation of $^1O_2$ /superoxide, *in chemico*	+	+
Protein binding capacities Photo-DPRA* Photo-ADRA Photo-SH/NH_2 test	Key event 1 Direct peptide reactivity assay, *in chemico* Amino acid derivative reactivity assay, *in chemico* Changes of cell-surface thiols/amines in THP-1, *in vitro*	(+)	+
Photo-ARE assay Photo-KeratinoSens*	Key event 2 Keap1-Nrf2-ARE pathway induction, KeratinoSens	+	+
APC activation Photo-h-CLAT*	Key event 3 Activation of CD54/CD86 expression, THP-1		+

*OECD approval of ordinary assessments: DPRA, OECD 2015; KeratinoSens, OECD 2018; and h-CLAT; OECD 2018

Table courtesy of Professor Yoshiki Tokura, MD, PhD, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

The schematic of the target sites for the methods described in Table 8 is depicted in Figure 10.

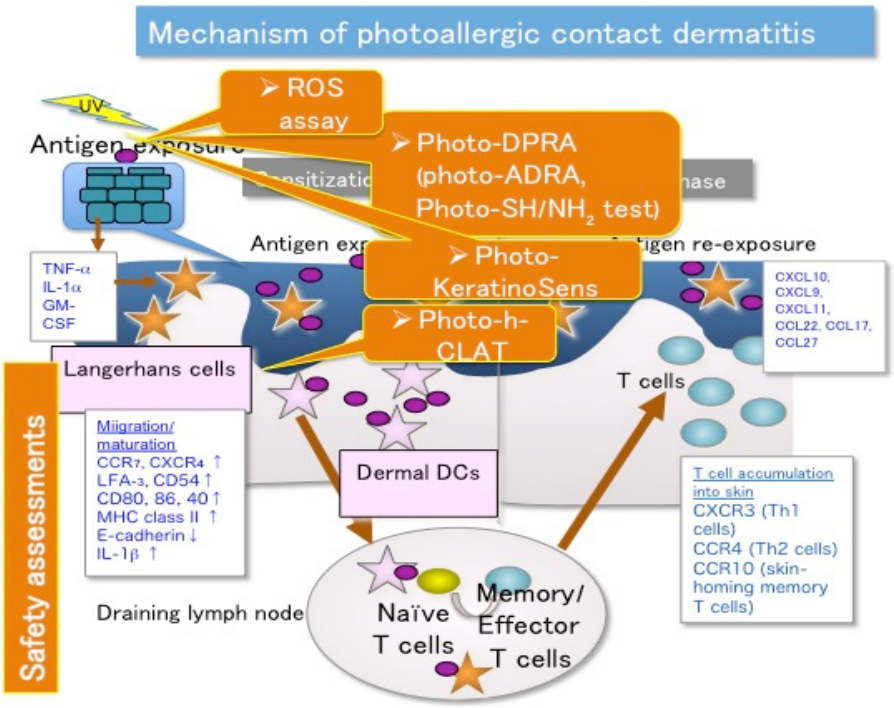


Figure 10. Schematic of ex vivo methods for assessment of phototoxicity and photoallergenicity

Illustration courtesy of Professor Yoshiki Tokura, MD, PhD, Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Ketoprofen as a photoallergen

The molecular weight of ketoprofen is 260, which classifies it as a low weight molecule. Its chemical formula is $C_{16}H_{14}O_3$. Being a substituted benzophenone, ketoprofen possesses two benzene rings (Figure 1). Its peak light absorption in the range 200–320 nm is presented in Figure 11.

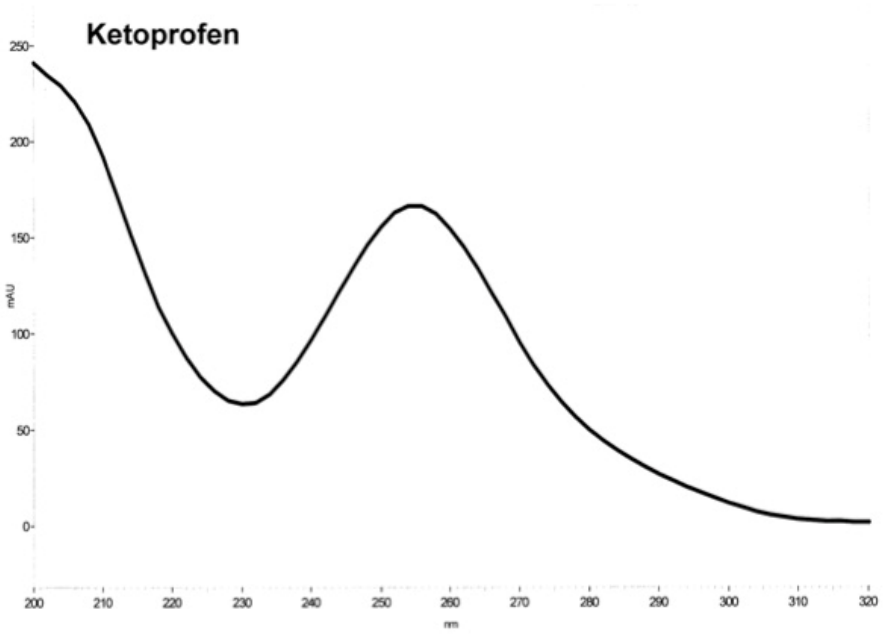


Figure 11. UV absorption by ketoprofen
Illustration by Martin Mowitz

The peak of absorption in the range of 250–260 nm places it in the UVC spectrum. Some absorption continues to occur throughout the UVB and into the UVA spectrum.

After the introduction of topical ketoprofen preparations on the market in the 1990s, reports began to arrive on its ability to cause photodermatitis. The first reports came from research groups in the Mediterranean region and Japan[133-137], and shortly after also from Belgium[138,139]. A multicentre study from Italy described a 10% rate of photocontact allergy to ketoprofen among individuals with a history of PhACD, which placed ketoprofen at the top of the list of the tested photoallergens[140]. Though many reports on the side effects of topical ketoprofen have described a photoallergic pattern, phototoxic, photoaggravated, and even contact allergic[136,141,142] dermatitis have also been reported.

Contrary to the high prevalence of photocontact allergy upon topical exposure to ketoprofen, oral intake does not seem to cause photosensitivity reactions in those without previous cutaneous sensitization.

In Sweden, topical ketoprofen preparations were introduced in 1995. Three gels, all containing 2.5% ketoprofen (Siduro®, Ipex Medical AB, Solna, Sweden; Orudis®, Sanofi-Aventis AB, Bromma, Sweden; Zon®, Antula Healthcare AB, Stockholm, Sweden)) were made available as over-the-counter formulations (Figure 12).

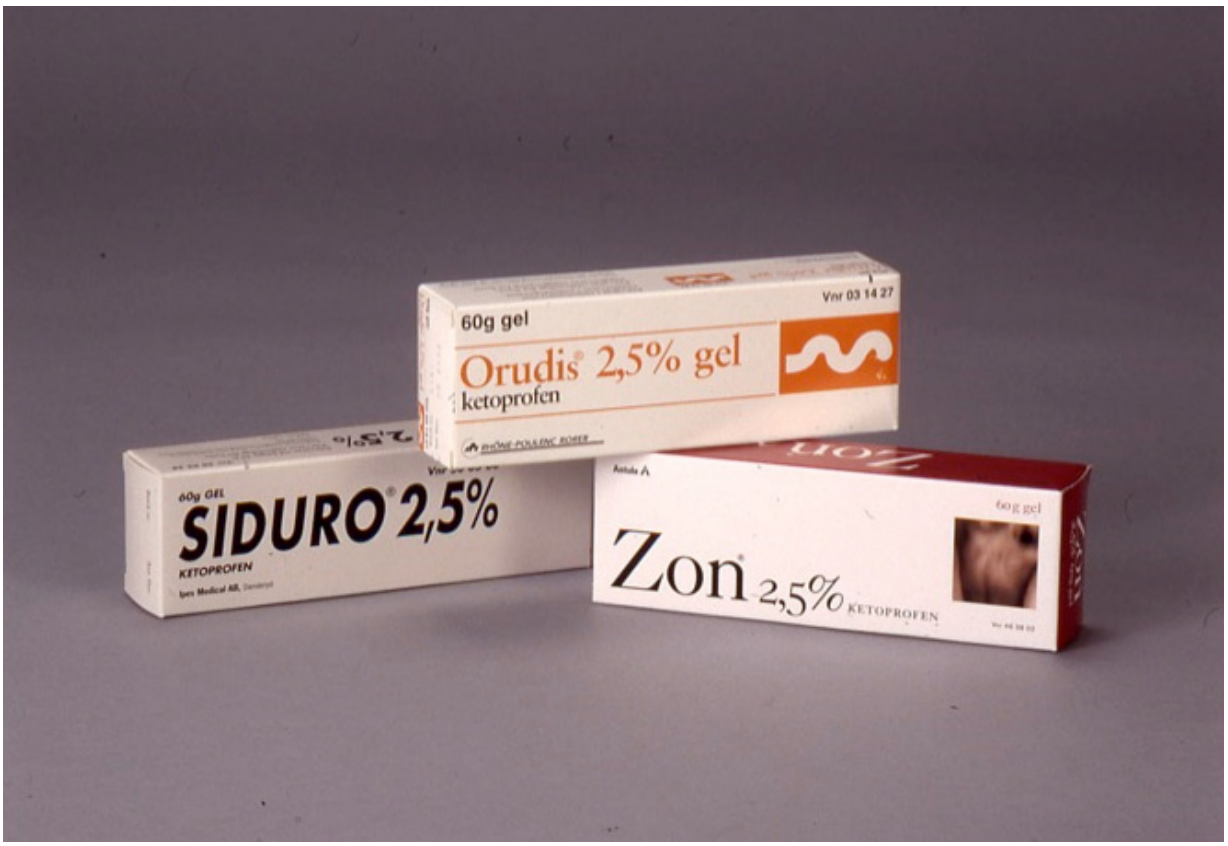


Figure 12. Ketoprofen-containing gels, available in Sweden in the early 2000s.
Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

The over-the-counter distribution of topical ketoprofen was stopped in Sweden by the Swedish Medical Products Agency on February 15th 2011 due to a high rate of reported photocontact allergic reactions. This decision was based on the recommendation from The European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP)(www.ema.europa.eu). Since then, the prevalence of detected photocontact allergy to ketoprofen has diminished (Paper IV). Photocontact allergic dermatitis due to ketoprofen may cause rather severe symptoms, mimicking deep vein thrombosis and erysipelas and requiring hospitalization[143](Figure 13).

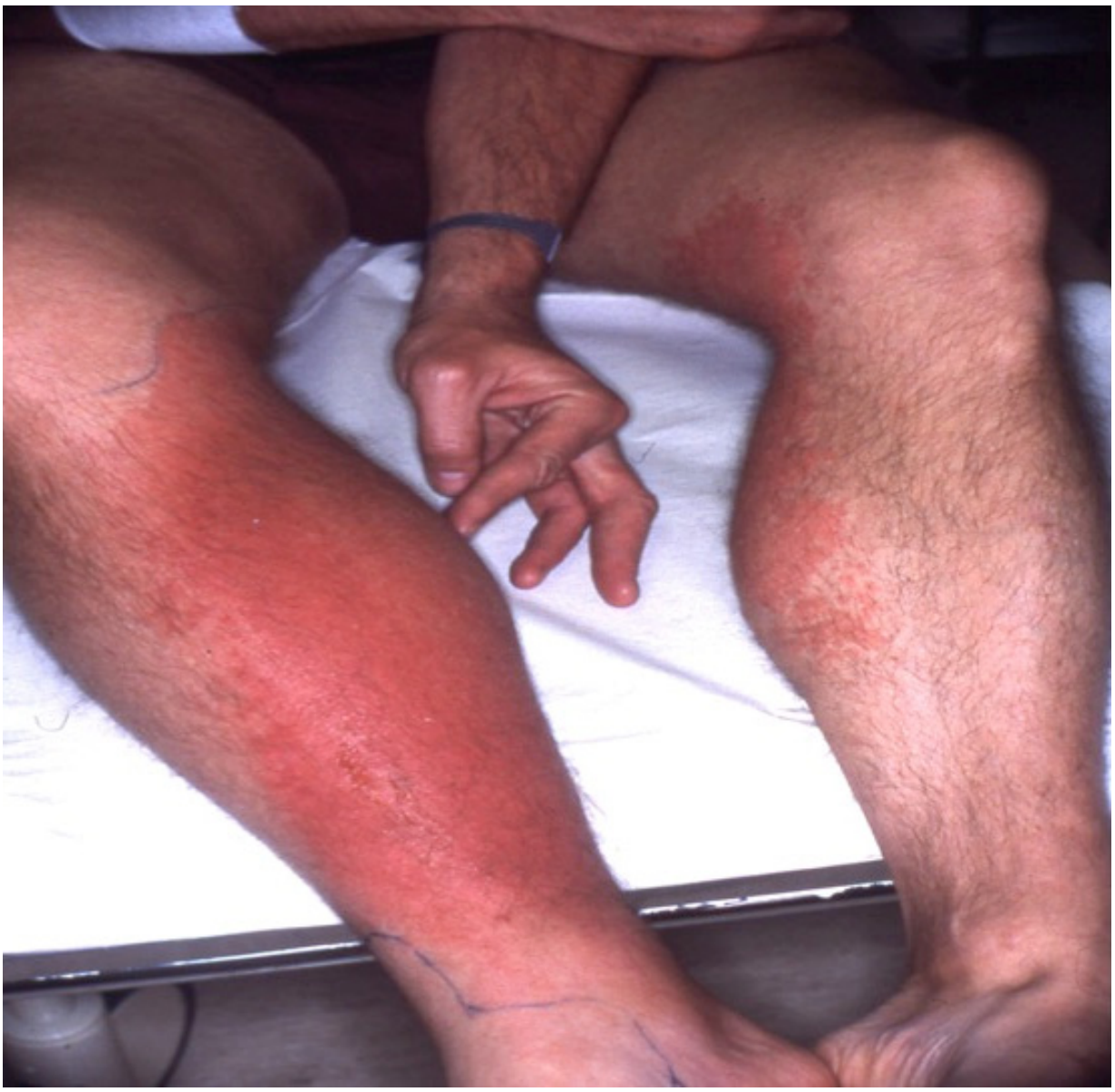


Figure 13. A case of photoallergic contact dermatitis due to ketoprofen, mimicking deep vein thrombosis
Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

The clinically challenging part of photosensitivity to ketoprofen is that the eruption may persist for a long time. A study using skin biopsy samples showed that ketoprofen could be detected in the skin several weeks after the discontinuation of the use of the drug [137]. Not only ketoprofen formulations, but also personal objects with ketoprofen residues can cause severe skin reactions, sometimes months to a year after the use of a ketoprofen formulation (Figure 14).

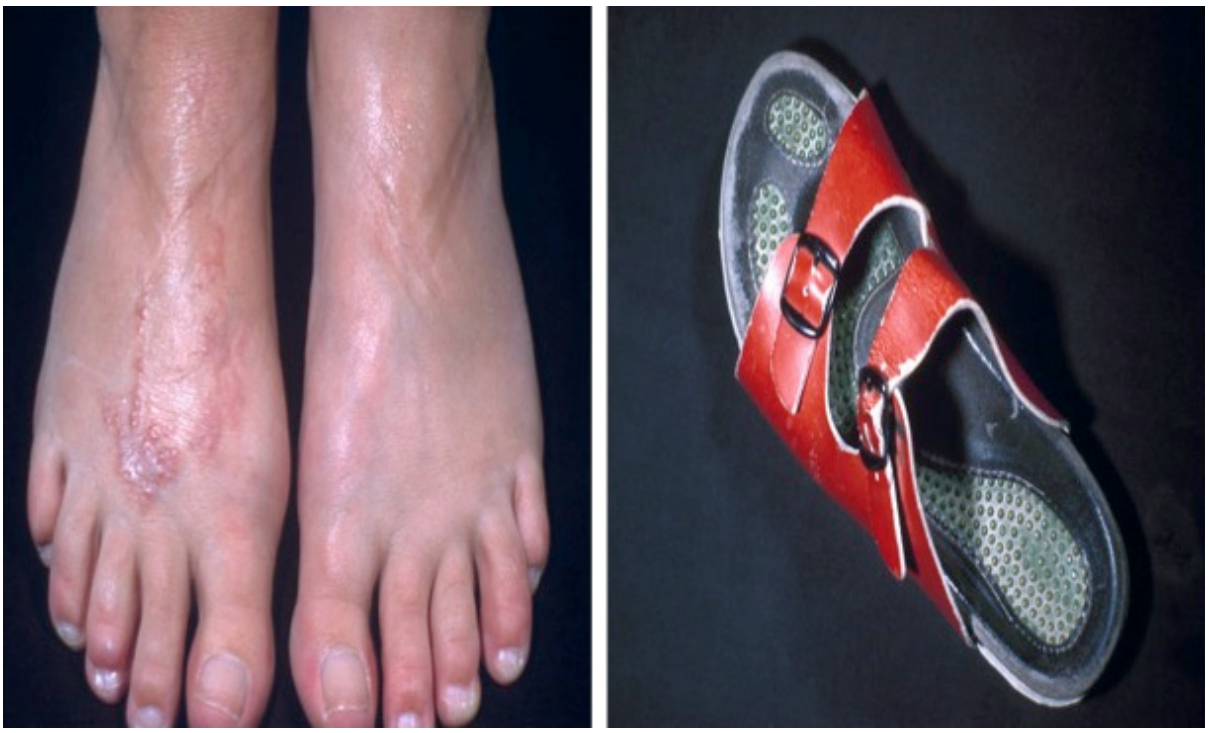


Figure 14. Relapse of photoallergic contact dermatitis from ketoprofen residue in a shoe in one patient (left), and a sandal with ketoprofen residue that was responsible for relapse in another patient (right).

Photos courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

Shoes and bandages may retain traces of ketoprofen, which can be confirmed with thin-layer chromatography and high-pressure liquid chromatography (HPLC) [144], and extracts from these objects have been shown to elicit a positive PPT reaction in the owner (Figure 15). Small amounts of ketoprofen have been detected even in personal objects that have been washed [144], which could make the future use of these objects impossible for individuals with strong photocontact allergy.

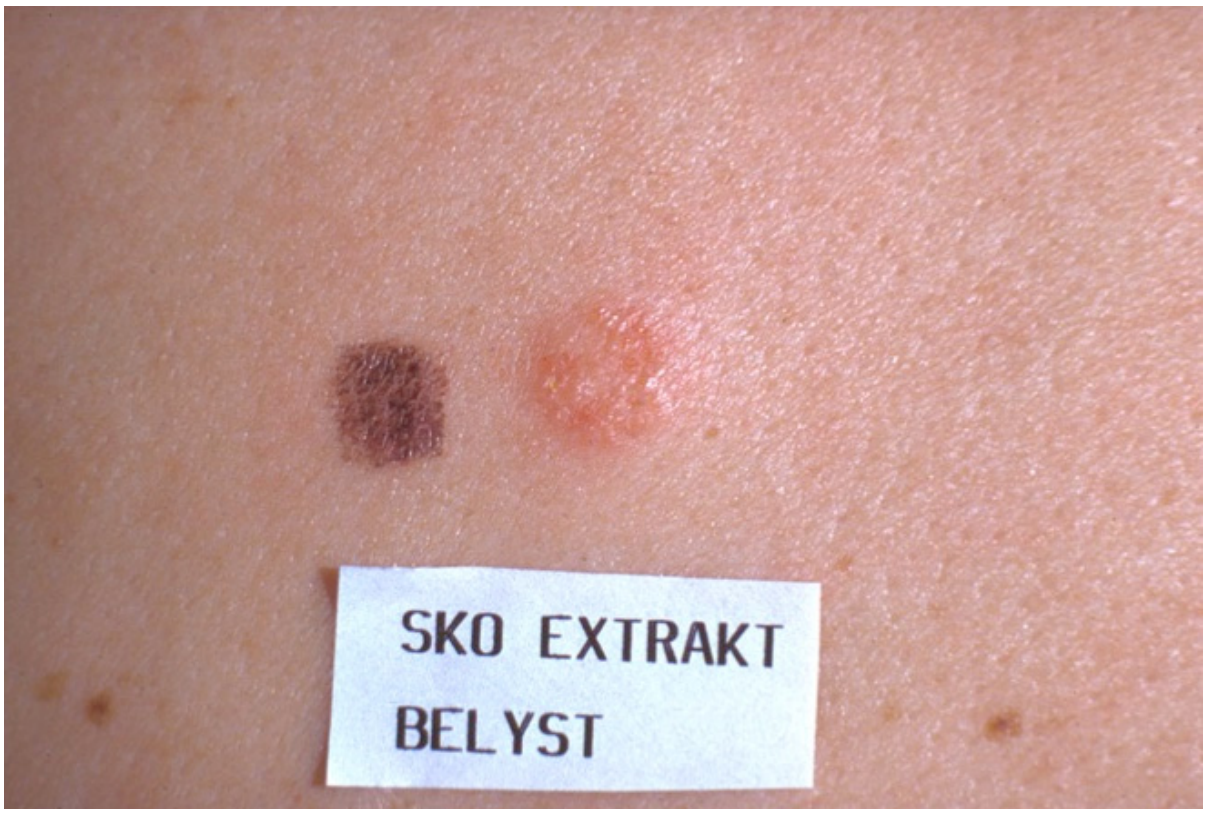


Figure 15. Positive photoallergic reaction in a patient with photocontact allergy to ketoprofen on photopatch testing with an extract from the patient's shoe, containing traces of ketoprofen

Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

A study by our research group in Malmö in 2006 showed that 35 individuals referred for investigation on suspicion of photodermatitis due to ketoprofen all showed positive test reactions to ketoprofen on PPT [143], and the pattern of reactions implied photocontact allergy rather than phototoxicity or photoirritancy. In two cases contact allergic reactions were observed on the control side, but these were weaker than the reactions on the irradiated side, and the suspicion was raised of these being simply a weaker sign of photocontact allergy because of some unintentional UV exposure [143].

The study also included extended PPT of the individuals with photocontact allergy to ketoprofen, conducted using the Scandinavian photopatch test series[91] with the addition of fenofibrate, benzophenone-3, benzophenone-4, and benzophenone-10. There was a strong overrepresentation of simultaneous photocontact allergies to fenofibrate, fentichlor, benzophenone-3, benzophenone-10, 3,3′,4′,5-tetrachlorosalicylanilide, promethazine hydrochloride, chlorpromazine hydrochloride, and bithionol[143](Figure 16).

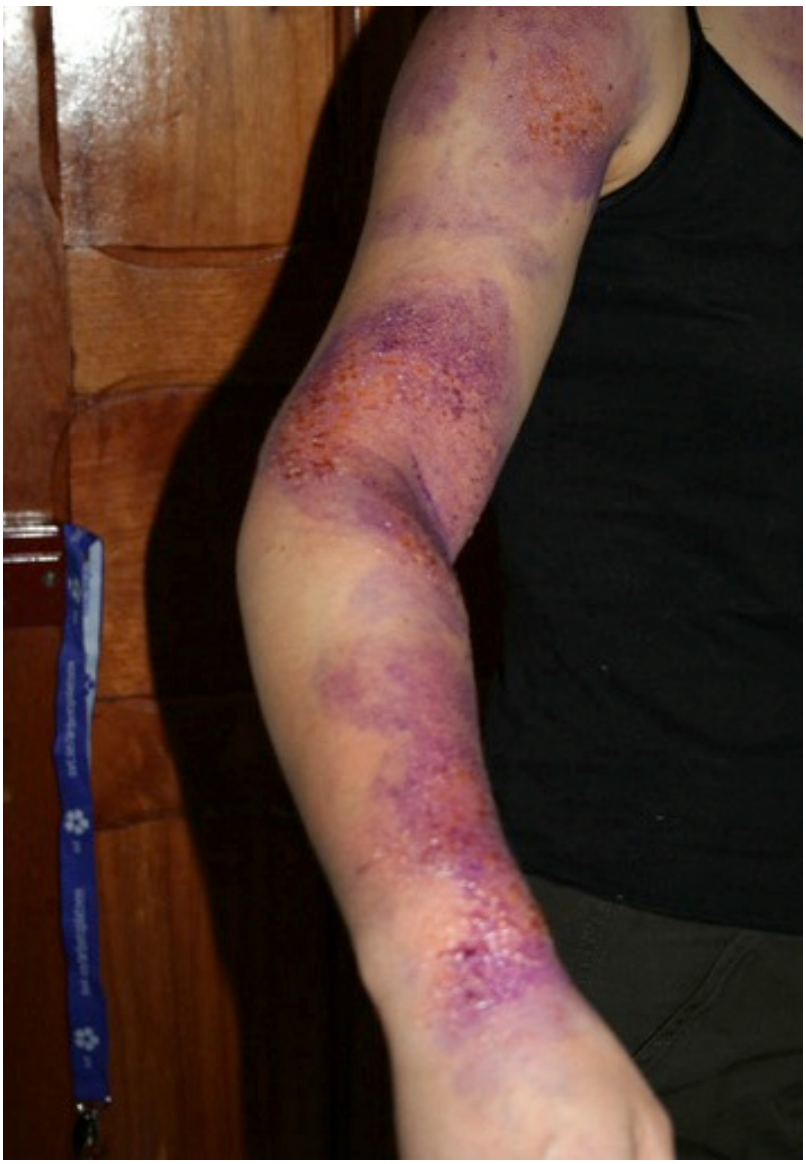


Figure 16. A case of severe photoallergic contact dermatitis due to sunscreen containing a benzophenone in a patient with photocontact allergy to ketoprofen

Photo courtesy of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö

Cross-reactions between structurally similar molecules have been described [145,146], including cross-reactions between ketoprofen and other structurally similar NSAIDs [147]. One study reported an overrepresentation of simultaneous photocontact allergic reactions between ketoprofen and fenofibrate, oxybenzone and unsubstituted benzophenone, but no simultaneous reactions were noted between ketoprofen and other arylpropionic acid derivatives without a benzophenone moiety [147]. However, the presence of structural similarities does not explain the overrepresentation of photocontact allergy to fentichlor, chlorpromazine, bithionol, tetrachlorosalicylanilide and promethazine described by Hindsén et al [143]. Similar results have been published previously [148,149](Figure 17).

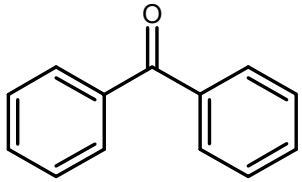
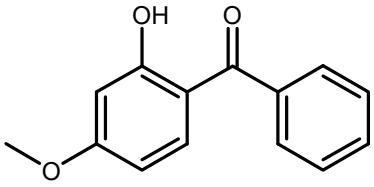
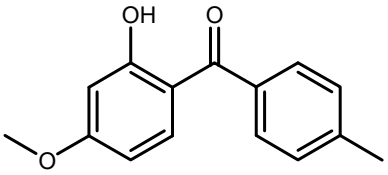
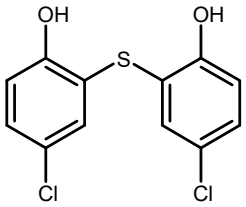
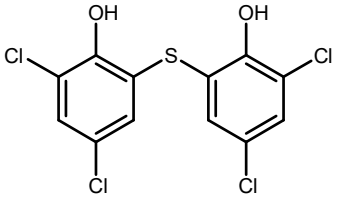
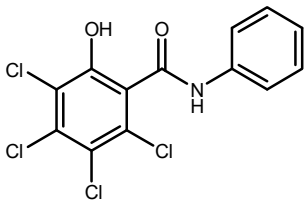
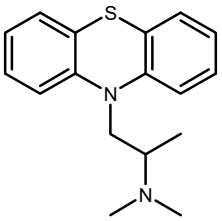
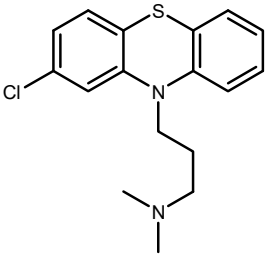
Benzophenone CAS: 119-61-9 MW: 182	
Benzophenone-3 CAS: 131-57-7 MW: 228	
Benzophenone-10 CAS: 1641-17-4 MW: 242	
Fenticlor CAS: 97-24-5 MW: 287	
Bithionol CAS: 97-18-7 MW: 356	
Tetrachlorosalicylanilide CAS: 7426-07-5 MW: 351	
Promethazine CAS: 60-87-7 MW: 284	
Chlorpromazine CAS: 50-53-3 MW: 319	

Figure 17. Chemical structure of the photosensitizers overrepresented at PPT in patients with photocontact allergy to ketoprofen

Table created by Erik Zimerson

Photocontact and contact allergy to the sunscreen agent octocrylene have been discovered to be over-represented in individuals with photocontact allergy to ketoprofen in the recent decades[138,150-153]. A Belgian study 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 degradation [154]. As ketoprofen is a substituted benzophenone, cross-reactivity due to benzophenone residues in octocrylene may be a reasonable explanation for the simultaneous photocontact allergic reactions.

Contact allergy to octocrylene has not been seen until recently but has now been described in individuals with photocontact allergy to ketoprofen, mostly in the paediatric population[150,155]. The prevalence appears to be much lower than for photocontact allergy [152]

Over time, other sensitizers have begun to be described as overrepresented in those with photocontact allergy to ketoprofen. High rates of simultaneous contact allergy to *Myroxylon pereirae* and fragrance mix I (FM I) have been reported by various research groups[139,143,156,157]. Cinnamal and cinnamic alcohol are two of the constituents of FM I. While cinnamal as a contact sensitizer is more predominant in the dermatitis population, the shift towards higher rates of contact allergy to cinnamic alcohol occurs in individuals with photocontact allergy to ketoprofen[90,156,158].

As the diagnostic PPT is well established in our clinic, we have had a relatively large number of patients referred to us on suspicion of PhACD from ketoprofen. Since 2009, ketoprofen has been included in the routine photopatch test series in our clinic. Many of these patients are patch tested with the baseline photopatch test series as well, which gives us an unique opportunity to analyse the general pattern of reactivity in our patients with photoallergy to ketoprofen.

Aims

As simultaneous contact allergies in patients with photocontact allergy to ketoprofen have become a rather common finding in the literature, the main aim of this thesis was to map the prevalence of these contact allergies in comparison with the control groups.

In order to achieve the main aim, the following sub-aims were formulated:

- To investigate the possibility of simplifying the procedure of PPT with ketoprofen
- To compare the prevalence of contact allergy to FM I and its components between patients with photocontact allergy to ketoprofen and controls from the dermatitis population and the general population
- To explore whether the clinical observation of contact allergy to some oxidized terpenes being more prevalent in individuals with photocontact allergy to ketoprofen holds true
- To investigate the prevalence of contact allergy to components of our baseline test series in individuals with photocontact allergy to ketoprofen, and to compare the findings with the dermatitis population and the general population.

Materials and methods

Photopatch test preparations

Study I

The original ketoprofen substance used in Study I came from Sigma-Aldrich, Stockholm, Sweden. Ethanol 99.5% v/v came from Kemetyl AB, Haninge, Sweden. Petrolatum (vaselinum album), USP/NF, was provided by Apoteket AB, Gothenburg, Sweden. A stock preparation of ketoprofen in petrolatum at 10.0% w/w was further diluted to desired concentrations with petrolatum (2.5% and 1.0% w/w). Solutions of ketoprofen in ethanol in the concentrations 2.5%, 1.0%, 0.1%, 0.01%, 0.001%, and 0.0001% w/v were used for PPT.

Study II

The ketoprofen test preparation used in Study II was initially made at the Department of Occupational and Environmental Dermatology in Malmö. Ketoprofen (Sigma-Aldrich) was used to make a solution in ethanol 99.5% (Kemetyl AB) at 1.0% w/v and a petrolatum (vaselinum album, Apoteket AB) preparation at 1.0% w/w. Ketoprofen in ethanol was used for PPT in 6 patients and the petrolatum preparation was used in 277 patients as a part of an extended Scandinavian photopatch test series (Chemotechnique Diagnostics, Vellinge, Sweden)[89,91](Table 4), or from 2013 as a part of the European photopatch test series (Chemotechnique Diagnostics)(Table 5).

Study III

Study III used the Scandinavian photopatch test series [91], with the addition of ketoprofen, and the European baseline photopatch test series [82,90](since 2008), both purchased from Chemotechnique Diagnostics, were used. Seven patients were photopatch tested exclusively with ketoprofen from one of the abovementioned series. For the substances in series, concentrations, and vehicles, see Tables 4 and 5.

Study IV

Study IV used the original ketoprofen substance from Sigma-Aldrich, ethanol 99.5% v/v provided by Kemetyl AB, and petrolatum (snow white quality E) from Apoteket Produktion & Laboratorier, Gothenburg, Sweden.

Between 1999 and 2008, the Scandinavian photopatch test series [91] with the addition of ketoprofen was used for PPT (Table 4). Ketoprofen-containing gels 2.5% (Siduro®, Ipex; Orudis®, Aventis Pharma; Zon®, Antula) were used as is. A stock preparation of ketoprofen in petrolatum at 10.0% w/w was further diluted to desired concentrations with petrolatum (2.5% and 1.0% w/w). Additionally, a ketoprofen solution in ethanol at 1.0% w/v was made. All these test preparations were made at the laboratory of the Malmö department. Ketoprofen in ethanol was photopatch tested in 6 patients and the petrolatum preparation was tested in 277 patients as part of an extended Scandinavian photopatch test series (Chemotechnique Diagnostics)[91]. From 2009 on, the European baseline photopatch test series was used[90], with ketoprofen 1% in petrolatum being tested as a part of the series (Table 5). The European photopatch test series was purchased from Chemotechnique Diagnostics.

Patch test preparations

Study II

PT in Study II was performed with the Swedish baseline series (Chemotechnique Diagnostics) (Table 9) and an extended Malmö baseline series (Table 10), both of which include FM I. The 8 FM I components were tested separately in those positive to FM I on PT with the Swedish baseline series. The fragrance baseline series has been part of the extended Malmö baseline series since 2009 and includes components of both FM I and FM II (Table 9).

Table 9. The Swedish baseline series (Chemotechnique Diagnostics)

Substance	Concentration and vehicle
Potassium dichromate	0.5% pet
P-phenylenediamine (PPD)	1% pet
Thiuram mix	1% pet
Neomycin sulphate	20% pet
Cobalt(II)chloride hexahydrate	1% pet
Quaternium-15	1% pet
Nickel(II)sulphate hexahydrate	5% pet
Quinoline mix	6% pet
Colophonium	20% pet
Paraben mix	16% pet
Black rubber mix	0.6% pet
Sesquiterpene lactone mix	0.1% pet
Mercapto mix	3.5% pet
Epoxy resin, bisphenol A	1% pet
Myroxylon pereirae	25% pet
4-tert-Butylphenolformaldehyde resin (PTBP-F-R)	1% pet
Fragrance mix II	14% pet
Formaldehyde	2% aq
Fragrance mix I	8% pet
Phenol formaldehyde resin (PFR2)	1% pet
Diazolidinyl urea	2% aq
Methylisothiazolinone+methylchloroisothiazolinone	0.215% aq
Amerchol L-101	50% pet
Caine mix II	10% pet
Lichen acid mix	0.3% pet
Tixocortol-21-pivalate	0.1% pet
Textile dye mix	6.6% pet
Budesonide	0.01% pet
Methyldibromo glutaronitrile	0.5% pet

Table 10. The components of the fragrance baseline series as a part of the the extended Malmö baseline series

Nr	Test substance	Concentration and vehicle
1	Cinnamal	1% pet
2	Cinnamyl alcohol	2% pet
3	Hydroxycitronellal	2% pet
4	Amyl cinnamal	2% pet
5	Geraniol	2% pet
6	Eugenol	2% pet
7	Isoeugenol	2% pet
8	Evernia prunastri (oak moss absolute)	2% pet
9	Sorbitan sesquioleate	20% pet
10	Myroxylon Pereirae	25% pet
11	Citral	2% pet
12	Farnesol	5% pet
13	Citronellol	1% pet
14	Hexyl cinnamal	10% pet
15	Coumarine	5% pet
16	Lyral	5% pet
17	Fragrance mix I	8% pet
18	Fragrance mix II	14% pet

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) at the Department of Occupational and Environmental Dermatology in Malmö. All components of FM I 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. The extended baseline series also contained a preparation of the emulsifying agent used in FM I, sorbitan sesquioleate 20% w/w in petrolatum.

Study III

Petrolatum test preparations with various concentrations of oxidized (ox.) linalool and ox. limonene (Table 11 and 12) were used for patch testing in study III.

Table 11. Preparations of oxidized linalool tested in ketoprofen-photoallergic patients (KP) and in dermatitis patients without a diagnosed photocontact allergy to ketoprofen (D).

Concentration (%w/w of linalool hydroperoxides)	0.8% [a]		1% [a]		1% [b]		Any preparation	
Group	KP	D	KP	D	KP	D	KP	D
Number tested	2	325	17	1221	10	2544	29	4021

[a] Test preparations from the Department of Dermatochemistry, University of Gothenburg
[b] Test preparations from Chemotechnique Diagnostics Vellinge, Sweden

Table 12. Preparations of oxidized limonene tested in ketoprofen-photoallergic patients (KP) and in dermatitis patients without a diagnosed photocontact allergy to ketoprofen (D).

Concentration (% w/w of limonene hydroperoxides)	0.3% [a]		0.3% [b]		Any preparation	
Group	KP	D	KP	D	KP	D
Number tested	14	1292	10	2547	24	3797

[a] Test preparations from the Department of Dermatochemistry, University of Gothenburg
[b] Test preparations from Chemotechnique Diagnostics Vellinge, Sweden

Initially the patients were patch tested with 4% and 6% ox. linalool containing 0.8% and 1% of linalool hydroperoxides, respectively [50,159,160], and with 3% ox. limonene containing 0.3% limonene hydroperoxides [159,161,162]. The test preparations were made at the Department of Dermatochemistry, Gothenburg University. From 2012 onwards, commercial patch test preparations of 0.3% limonene hydroperoxides and 1% linalool hydroperoxides from Chemotechnique Diagnostics were used (Tables 11 and 12).

Study IV

The Swedish baseline patch test series is tested in Malmö with minor modifications, for example Amerchol l 101 is tested at 100% instead of 50%. Additionally, many other sensitizers are tested in an extended baseline series containing representatives of metals, preservatives, fragrance materials, plastics, dyes, and rubber chemicals. Various concentrations were tried before introducing the new preparations into the Swedish baseline series in order to find optimal test preparations of sensitizers such as formaldehyde, FM II, mercapto-mix, and methylchloroisothiazolinone-/methylisothiazolinone (MCI/MI)[163,164]. The allergens included in this study, including concentrations and vehicles, are presented in Table 13.

Table 13. Excerpt from the Malmö baseline series showing vehicles and concentrations used between 1999 and 2012.

	Component	Concentration, vehicle
1	Potassium bichromate	0.5 % pet
2	p-phenylenediamine, (PPD)	1 % pet
3	Thiuram mix	1 % pet
4	Neomycin sulphate	20 % pet
5	Cobalt chloride	0.5 % pet
6	Quaternium-15	1 % pet
7	Nickel sulphate	5 % pet
8	Quinoline mix	6 % pet
9	Colophonium	20 % pet
10	Paraben mix	16 % pet
11	Black rubber mix	0.6 % pet
12	Sesquiterpene lactone mix	0.1 % pet
13	Mercapto mix	2 % pet
14	Epoxy resin, bisphenol A	1 % pet
15	*Myroxylon pereirae*	25 % pet
16	4-tert-Butylphenolformaldehyde resin (PTBP-F-R)	1 % pet
17	Fragrance mix II (from 2006)	14 % pet
18	Formaldehyde	2 % aqua
19	Fragrance mix I	8 % pet
20	Phenol formaldehyde resin (PFR-2)	1 % pet
21	Diazolidinylurea	2 % pet
22	Methylisothiazolinone + metylchloroisothiazolinone	0.2% aqua
23	Amerchol L 101	100 % pet
24	Caine mix II	10 % pet
25	Lichen acid mix	0.3 % pet
26	Tixocortol-21-pivalate	0.1 % pet
27	Textile dye mix	6.6,% pet
28	Budesonide	0.01 % pet
29	Methyldibromo glutaronitrile	0.5 % pet
30	Methylisothiazolinone	1 % aqua
31	Thimerosal	0.1 % pet

Note: pet = petrolatum

Test preparations
Chemotechnique Diagnostics in Vellinge, Sweden, manufactured the test preparations in the baseline series reported [82].

Participants

Study I

A total of 22 patients (11 men and 11 women, mean age 49.5, range 17–68 years) with known or suspected photocontact allergy to ketoprofen participated in Study I from 2005 onwards. Patients referred to our clinic with suspected photocontact allergy to ketoprofen, but not yet photopatch tested, were automatically included (11 patients). Patients who were tested earlier (2000–2003) and showed positive photopatch reactions to ketoprofen were contacted and invited to participate in the study (11 patients).

Study II

In the period 2009–2018, 6846 patients (2256 males, 4590 females, mean age 46.2 years, range 4–99 years) underwent PT and/or PPT on a suspicion of ACD and/or PhACD (Figure 18). PPT was performed in 283 patients; 69 of them were only photopatch tested, while the remaining 214 were also patch tested. These 214 patients (mean age 48.2 years, range 4–90 years) had been photopatch tested with the European photopatch test series[90]. PT alone with the Swedish baseline series (Chemotechnique Diagnostics)(Table 9) or an extended Malmö baseline series (Tables 10&13) was performed in 6563 patients (2158 males and 4405 females; mean age 45.1 years, range 4–99 years).

The general population was represented by 518 volunteers (252 males, 266 females; mean age 53.17 years, range 18–74 years) living in the Malmö metropolitan area. These volunteers were tested within a European study on contact allergy in the general population, both with a baseline series and with the constituents of FM I [64,165].

A flowchart of testing schematics and the distribution of participants is presented in Figure 18.

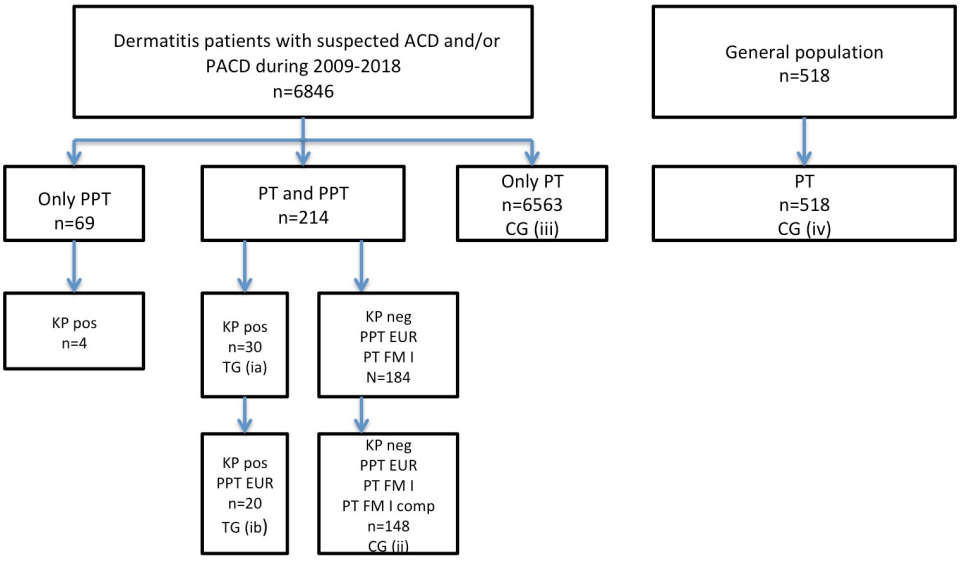


Figure 18. Flowchart demonstrating the process of selecting study patients and controls.
ACD = allergic contact dermatitis, CG = control group, FM I comp = components of fragrance mix I, KP = ketoprofen, PhACD = photoallergic contact dermatitis, PPT = photopatch testing, PPT EUR = European photopatch test series, PT = patch testing, TG = target group.

Study III

Between 2005 and 2015, 4050 patients (1426 males and 2624 females), were patch tested because of a suspected ACD, using the Swedish baseline patch test series (Chemotechnique Diagnostics) to which ox. linalool was provisionally added. Similarly, 3821 patients (1349 males and 2472 females) were patch tested between 2004 and 2015 because of a suspected ACD, using the Swedish baseline patch test series (Chemotechnique Diagnostics) to which ox. limonene was provisionally added. None of the patients with a suspected ACD were patch or photopatch tested with ketoprofen. During the periods of routine patch testing with ox. linalool and ox. limonene, 24 patients tested with both ox. linalool and ox. limonene along with 5 patients tested only with ox. linalool showed positive photopatch test reactions to ketoprofen.

Study IV

Between 1999 and 2018, 400 patients were photopatch tested with ketoprofen at the Department of Occupational and Environmental Dermatology in Malmö. Of these, 94 patients showed positive results (58 females, 36 males, mean age 44.2 years, range 15–84 years). Between 1999 and 2008 the PPT was performed on a suspicion of photoallergy to ketoprofen, while from 2009 onwards ketoprofen became a permanent part of the photopatch test series in Malmö, and the majority of patients were photopatch tested because of a suspected PhACD from sunscreen. Comparisons were made between all KP-photoallergic patients and dermatitis patients during 1999–2018. The group tested during 1999–2008 (group 1) and the

group tested during 2009–2018 (group 2) were compared to the corresponding dermatitis population tested with the baseline series during the same period of time. Further comparison was made between group 2 and the general population.

All patients who were photopatch tested with ketoprofen during 1999–2018 constituted PPT group 0. Patients who were photopatch tested during 1999–2008 constituted PPT group 1, and those tested during 2009–2018 constituted PPT group 2.

PPT group 0: 400 patients photopatch tested with ketoprofen (94 positive), including 219 also tested with the photopatch test series (25 positive).

PPT group 1: 121 patients photopatch tested with ketoprofen (60 positive), including 8 also tested with a photopatch test series (4 positive).

PPT group 2: 283 patients photopatch tested with ketoprofen (34 positive), including 211 tested with a photopatch test series where ketoprofen was included (21 positive).

Four patients were photopatch tested twice between 1999 and 2018.
Of the 94 patients with photoallergy to ketoprofen, 70 were patch tested with the baseline series as a part of the investigative procedure. These patients were further divided into groups:

KP group 0: 70 patients positive for ketoprofen and also tested with the baseline series between 1999 and 2018.

KP group 1: 41 patients positive for ketoprofen and also tested with the baseline series between 1999 and 2008.

KP group 2: 30 patients positive for ketoprofen and also tested with the baseline series between 2009 and 2018.

Controls

Study I

Controls were used to investigate whether phototoxicity could explain positive results. Twenty dermatitis patients investigated for a suspected ACD with PT were simultaneously photopatch tested with ketoprofen at 2.5% and 1% w/w in petrolatum with 1h occlusion, followed by 5 J/cm² UVA irradiation.

Study II

Three groups of controls were used according to Figure 18. One group consisted of the dermatitis patients who were both patch and photopatch tested (n=214) in the period 2009-2018. This group was used as both a target group (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 (Figure 18) (n=148). All 148 were patch tested with FM I and also with its constituents (control group (ii)). Another group) (iii) consisted of the dermatitis patients who were only patch tested and not photopatch tested in the period 2009-2018 (n=6563). The third group (iv) included 518 volunteers, representing the general population, living in the Malmö metropolitan area. 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 (Figure 18). The methodology used and results of the patch testing have been published previously [64,165]. Study II used the results of the testing with petrolatum preparations of FM I and its components. 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.

Study III

Between 2005 and 2015 a total of 4050 patients (1426 male, 2624 female) were patch tested because of a suspected ACD with the Swedish baseline patch test series in which ox. linalool was provisionally inserted. Similarly, between 2004 and 2015 a total of 3821 patients (1349 male, 2472 female) were patch tested because of a suspected ACD with the Swedish baseline patch test series in which ox. limonene was provisionally inserted.

Study IV

Patients who were patch tested during 1999–2018 with our baseline series, but not photopatch tested with ketoprofen, served as controls. These controls were divided into three groups to match the time frame of testing with ketoprofen. The total group (patients patch tested during 1999–2018) formed CPT group 0, those tested during 1999–2008 formed CPT group 1, and those tested during 2009–2018 formed CPT group 2. Individuals from the general population who were tested in an earlier study [64] served as separate controls, and constituted the GPCPT group. The number range in each group is explained by the different number of patients tested with each individual constituent of the baseline series.

CPT group 0: n=6622–12 221

CPT group 1: n=1706–6425

CPT group 2: n=2471–6820

GPCPT group: n=518

The exact numbers of controls tested with each constituent of the baseline series are given in Table 24.

Photopatch testing

Light source (Studies I–IV)

The light source used in all studies was a UV440DT IP20 luminare (ESSHÅ Elagentur AB, Värnamo, Sweden) equipped with four Philips PL-L 36W UVA tubes (Philips AB, Sundsvall, Sweden). A Delcomp UV meter (PUVA Combi Light, Leuven, Belgium) was used to ensure that the right UVA dose was given.

Study I

All 22 patients in Study I were photopatch tested with ketoprofen using both 24h and 1h occlusion. Patches with test preparations were placed on the back in 22 cases and on the upper arm in 11 cases. The PPT was performed using Finn chambers of 8mm diameter secured with Scanpor® tape. The test sites were irradiated with 5 J/cm^2 of broadband UVA. Two different approaches were used depending on whether photocontact allergy to ketoprofen had already been shown or was only suspected.

Patients in **group 1** (with suspected photocontact allergy to ketoprofen) were tested with our standard photopatch test series together with ketoprofen in serial dilutions (referred to as "standard patches 24h") attached to the left side of the back, and ketoprofen patches alone (referred to as "study patches 1h") attached to the left upper arm. Identical sets of "standard" and "study" patches were applied to the right side of the back and to the right upper arm to serve as non-irradiated controls.

Patients in **group 2** (with known photocontact allergy to ketoprofen) were tested with two sets of patches containing serial dilutions of ketoprofen in ethanol applied to the right side of the upper back ("standard patches 24h") and to the left side of the upper back ("study patches 1h") respectively. Single patches with the highest tested concentration of ketoprofen for each respective group (1.0% w/v for "standard patches 24h" and 2.5% w/v for "study patches 1h") were attached to the back below to serve as non-irradiated controls. Patients who had earlier shown strong positive reactions to ketoprofen were not tested with 1.0%.

A standardized protocol was used to evaluate the results. A plain erythema was interpreted as a doubtful reaction, erythema with a slight infiltration as + reaction, erythema with few papules as ++ reaction, and erythema with many papules or with vesicles as +++ reaction. Concentrations and vehicles are given in Table 14, and the procedure is summarised in Tables 14 and 15.

Table 14. Overview of the photopatch testing procedure with 1h and 24 occlusion including control patches.

	Group 1 (patients with suspected photocontact allergy)				**Group 2 (patients with known photocontact allergy)**			
	Study patches 1h	Standard patches 24h [a]	Control patches 1h	Control patches 24h	Study patches 1h	Standard patches 24h [b]	Control patches 1h	Control patches 24h
Location	Left side of the upper back [c]	Left upper arm [d]	Right side of the upper back [c]	Right upper arm [d]	Left side of the upper back [d]	Right side of the upper back [d]	Left side of the back [d]	Right side of the back [d]
Concentration of ketoprofen	2.5% 1.0% w/w	1.0%, 0.1%, 0.01%, 0.001% 0.0001% w/v	2.5% 1.0% w/w	1.0%, 0.1%, 0.01%, 0.001% 0.0001% w/v	2.5%, 1.0%, 0.1%, 0.01% 0.001% w/v	1.0%, 0.1%, 0.01%, 0.001% 0.0001% w/v	2.5% w/v	1.0% w/v
Occlusion time	1h	24h	1h	24h	1h	24h	1h	24h
UVA irradiation	yes	yes	no	no	yes	yes	no	no

[a] Standard patches consisting of photopatch test series including ketoprofen in serial dilutions
[b] Standard patches consisting of ketoprofen in serial dilutions only
[c] Ketoprofen preparations in petrolatum
[d] Ketoprofen preparations in ethanol

Table 15. Comparison of the standard and shortened procedure for photopatch testing with ketoprofen occluded for 24h and 1h, respectively.

"24h"-procedure (standard)	"1h"-procedure
Day 0: Application of the patches	Day 0: Application of the patches, their removal and UVA-irradiation of the test site
Day 1: Removal of the patches. UVA-Irradiation of the test site	
Day 3: Reading	Reading

Study II

Photopatch testing was conducted according to standard procedure [89,91]. The testing was performed using duplicate Finn chambers of 8 mm diameter (SmartPractice®, Phoenix, Arizona, USA) secured to the back with Scanpor tape (Norgesplaster A/S, Vennesla, Norway). Identical 20 mg petrolatum preparations [86] or identical 15 µl ethanol solutions [88] were used on each side. The test preparations were applied to the chambers immediately before the application on the back [166]. Occlusion time was 24h [89,91]. One side was immediately covered with black cloth after the removal of the test strips to avoid any unintentional UV irradiation, while the other side was irradiated with 5 J/cm² of broadband UVA (see above). Reading was performed on D3 according to the ICDRG classification [82,90,96].

Study III

Study III used the Scandinavian photopatch test series[91] with the addition of ketoprofen, and from 2008 onwards the European baseline photopatch test series[90], both purchased from Chemotechnique Diagnostics. Seven patients were photopatch tested exclusively with ketoprofen from one of the abovementioned series. Finn chambers of 8 mm diameter were mounted on Scanpor tape, loaded with 20 mg of the petrolatum preparations[86], and then secured on the upper back in duplicate as parallel columns. The patches remained under occlusion for 24h[89,90] and were then removed with a minimum of light exposure and with one side covered immediately with black cloth. The other side was irradiated with 5 J/cm² of broadband UVA (PUVA4000, Photochemotherapy, Herbert Waldmann, Werk für Lichttechnik, Germany). Reading was performed on D3 according to the ICDRG classification[82,96].

Study IV

Finn chambers of 8 mm diameter were mounted on Scanpor tape (Norgesplaster A/S, Oslo, Norway), loaded with 20 mg of the petrolatum preparations [86] and 15 µl of the liquid preparations [88], secured on the upper back in duplicate as parallel columns, and occluded for 24h [89,91]. After removal with a minimum of light exposure, one side was immediately covered with black cloth. The other side was irradiated with 5 J/cm² of broadband UVA (PUVA4000, Photochemotherapy, Herbert Waldmann, Werk für Lichttechnik, Germany). The number of readings of PPT was different in the two groups. For PPT group 1 (tested during 1999–2008) one reading was performed on day D3 according to the ICDRG classification[89,91,96], while for PPT group 2 (tested during 2009–2018) two readings were performed on D3 and D7 (personal communication with Magnus Bruze, 2021).

Patch testing

Study II

Finn chambers of 8 mm diameter were loaded with 20 mg petrolatum preparations of the test substances [86] immediately before application to the back using Scanpor tape [166]. The patches were removed after 48h and the reading was performed twice, on D3 or D4 and on D7. Scoring was performed according to the ICDRG classification [82,96].

Study III

Finn chambers of 8 mm diameter were loaded with 20 mg of petrolatum test preparations [86] with various concentrations of ox. linalool (Table 11) and ox. limonene (Table 12) were applied on small (diameter 8 mm) Finn Chambers, mounted on Scanpor® tape, and immediately applied to the back of the patient [167]. The patches remained under occlusion on the back for 48h. Test readings took place on D3 or D4 and on D7 according to the ICDRG classification [82,96].

Study IV

Finn chambers of 8 mm diameter were loaded with 20 mg of the petrolatum preparations[86] and 15 µl of the liquid preparations[88] before being applied to the back of the patient. Volatile sensitizers such as fragrance materials and formaldehyde were added to the chambers immediately before application to the patient[166,167]. The patches remained under occlusion on the back for 48 h. Test readings took place on D3 or D4 and on D7, according to the ICDRG classification [82,96].
The 518 participants representing a random sample of the Malmö metropolitan area were patch tested using panels 1–3 of the T.R.U.E. test standard series, containing 29 allergens[64]. They were also patch tested with petrolatum preparations of sesquiterpene lactone mix, FM I, and FM II. Finn chambers of 8 mm diameter were loaded with 20 mg of the petrolatum preparations[86] and applied to the participants' backs. The fragrance preparations were applied to the chambers immediately before the application on the back[166,167]. The patch tests were read only once on D3.

Chemical investigations

Study II

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS technique uses a combination of gas chromatography and mass spectrometry, in order to identify and quantify a substance (Figure 19). The *gas chromatograph* separates the different components of a mixture. An inert heated carrier gas (often nitrogen) carries the vapour of the substance to be analysed through a 30 m long glass column containing a stationary phase. The column is

heated, often from 70°C to 300°C during the time of the analyses that usually takes 0.5-1h. Different substances travel through the column with different speed depending on the temperature and their ability to dissolve in the stationary phase, which efficiently separates substances in complex mixtures. When entering the mass spectrometer the substance is bombarded with a stream of high-energy electrons and is decomposed into different charged fragments. The mass and amount of each fragment is recorded. The pattern of the fragments, the mass spectrum, is then used as a fingerprint of the substance. The mass spectrum can be used to search for the identity of the substance in a database of mass spectra. Such databases can include mass spectra of > 300 000 substances.

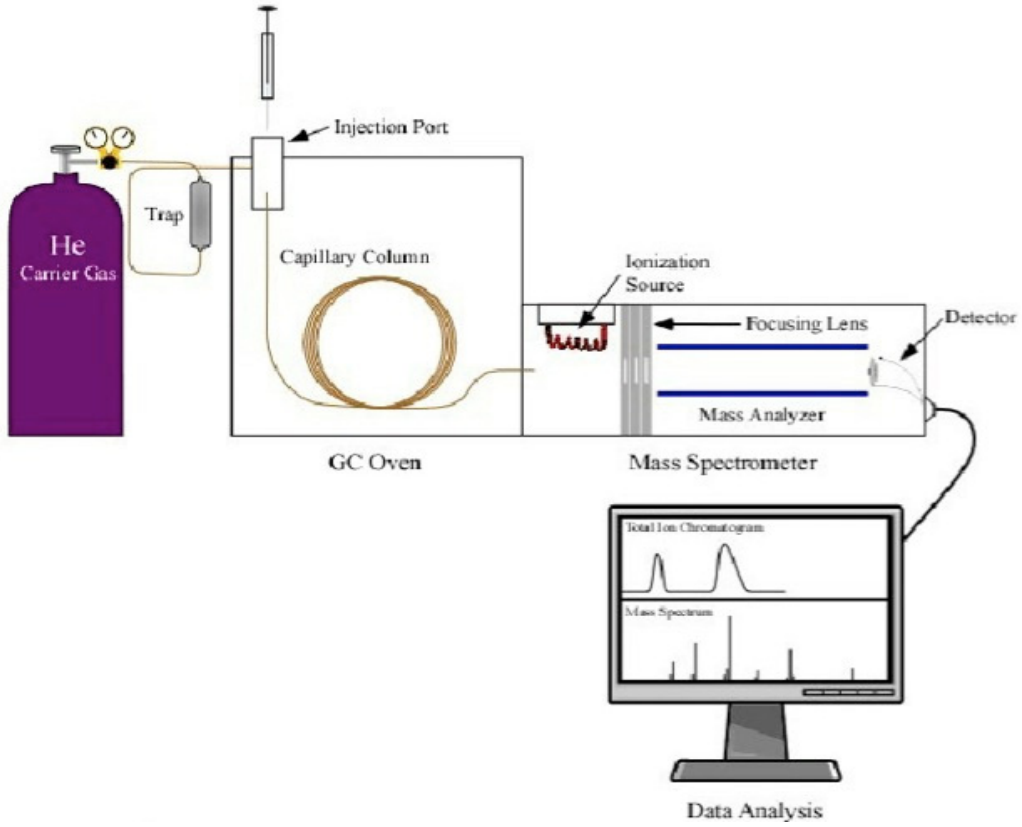


Figure 19. The schematic overview of gas chromatography-mass spectrometry.

Illustration: Applications of Chromatography Hyphenated Techniques in the Field of Lignin Pyrolysis - Scientific Figure. https://www.intechopen.com/chapters/31212 [accessed 3 Aug, 2021] [168]. Creative Commons Attribution 3.0 License, https://creativecommons.org/licenses/by/3.0/

GC-MS analysis in the Study II

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 by 8˚C per min^{-1} to a final temperature of 300˚C and remained 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 already been analysed before their expiration date, when the question raised in this study was first asked. Preparations of cinnamal and cinnamyl alcohol tested in the period 2009-2012, which were prepared at our department, were also analysed. First, 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.

Data recording

Data retrieved from Daluk, a restricted-access computer-based system containing the records of patient testing, including age, sex, occupation, results of PT and PPT and the interpretation, was used in studies II, III, and IV.

The volunteers who participated in Study I were selected using Daluk.

Ethics

Study I was approved by the Regional Ethical Review Board, Lund, Sweden (ref: 356/2006), and agreement to participate was obtained from every participant. Information about the study was provided both in person and in writing. Studies II, III, and IV were approved by the Regional Ethical Review Board, Lund, Sweden (ref: 2020/02190). When patients are patch tested and/or photopatch tested, they are informed that their data may be used for comparisons on a group level, and approval is mandatory if the patients' data are stored in the computer system.

Statistics

Study I

The hypothesis of Study I was that there would be a total concordance between the results of PPT with 1h occlusion compared to 24h occlusion. However, it was not possible to statistically prove this correlation. To achieve a confidence interval of 0.83–1.0, a total of 20 patients had to be tested with both methods (occlusion for 1h and 24h, respectively) and positive concordant results demonstrated. A two-sided Fisher's test was used to compare the number of positive reactions in test patients and in controls.

Study II

Pairwise comparisons using Fisher's exact test (two-sided) were performed between the target groups with photocontact allergy to ketoprofen and the control groups regarding contact allergy rates to FM I and its constituents (Tables 17-19). 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) (Figure 18). Dermatitis patients with photocontact allergy to ketoprofen who had been patch and photopatch tested with the European photopatch test series (target group ib) were compared with the dermatitis patients without photocontact allergy to ketoprofen who had been patch and photopatch tested with the European photopatch test series (control group ii) (Figure 18). McNemar's binominal exact test (two-tailed) was used to compare the distribution of test reactions to the chemically closely related substances cinnamyl alcohol and cinnamal, as well as eugenol and isoeugenol, within the ketoprofen photocontact allergy groups (target groups ia and ib; Figure 18) and the control groups (ii) and (iii). 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; Figure 18) and the control group (iii) (Table 20). A p-value <0.05 was regarded as statistically significant.

Study III

Fisher's exact test (two-sided) was used to compare the frequency of contact allergy to ox. linalool in routinely patch tested dermatitis patients with the frequency of contact allergy to ox. linalool in patients photoallergic to ketoprofen. The same comparison was made for ox. limonene. Fisher's exact test (two-sided) was also used to compare the number of simultaneous reactions to ox. linalool and ox. limonene in the routinely patch tested dermatitis patients and those photoallergic to ketoprofen based on the total number of individuals tested within the respective group and also on the basis of those within the respective groups who reacted to ox.

linalool and/or ox. limonene. The Mann-Whitney U-test was used to compare the intensities (+, ++ or +++) of patch test reactions to ox. linalool and ox. limonene between individuals with photocontact allergy to ketoprofen and routinely tested dermatitis patients. A p-value < 0.05 was considered significant.

Study IV

The prevalence rates of contact allergy in the target groups of patients with photocontact allergy to ketoprofen were compared to those in the respective control groups using Fisher's exact test (two-tailed) or a chi square test (two-sided). Odds ratios (ORs) with standard errors and 95% confidence intervals were calculated according to Altman[169].

Results

Study I

There was a complete concordance between the results of previous PPT and the re-testing of the patients with known photocontact allergy to ketoprofen, meaning that all patients positive on earlier PPT showed positive results at re-testing during Study I (Table 16). The morphology and evaluation of the results were consistent on both testing occasions.

Table 16. Results of the photopatch testing with ketoprofen using 1h and 24h occlusion in 22 individuals. Results are presented only for the irradiated test sites, with readings 3 days after the application. Non-irradiated controls gave no positive reactions and are not presented in the table.

	Ketoprofen, 24h occlusion					Ketoprofen, 1h occlusion [a]				
	1%	0.1%	0.01%	0.001%	0.0001%	2.5%	1%	0.1%	0.01%	0.001%
N1	+++	+++	+++	++	+	+++	+++	NT	NT	NT
N2	+++	+++	-	-	-	+++	+++	NT	NT	NT
N3	+++	+++	+++	-	-	+++	+++	NT	NT	NT
N4	+++	+++	+++	-	-	+++	+++	NT	NT	NT
N5	+++	+++	++	-	-	+++	+++	NT	NT	NT
N6	+	+	+	-	-	++	+	NT	NT	NT
N7	+++	+++	+	-	-	+++	+++	NT	NT	NT
N8	+++	++	++	++	-	+++	+++	NT	NT	NT
N9	+++	+++	+	-	-	+++	+++	NT	NT	NT
N10	+++	+++	++	-	-	+++	+++	NT	NT	NT
N11	+++	+++	++	-	-	+++	+++	NT	NT	NT
N12	NT	+++	++	-	-	+++	+++	+++	+++	+++
N13	NT	+++	+	-	-	+++	+++	+++	+	-
N14	NT	(+)	(+)	-	-	+++	+++	+	-	-
N15	NT	+++	-	-	-	+++	++	(+)	-	-
N16	NT	+	++	-	-	++	+	-	-	-
N17	NT	++	-	-	-	+++	+++	++	-	-
N18	NT	+	-	-	-	+++	+++	++	(+)	-
N19	NT	+++	++	-	-	+++	+++	+++	++	-
N20	NT	++	-	-	-	+++	+++	(+)	-	-
N21	+++	+	-	-	-	+	(+)	-	-	-
N22	(+)	-	-	-	-	++	++	-	-	-

[a] Patients N1-11 (group 1, suspected photocontact allegy) are tested using ethanol as vehicle and only two dilutions of ketoprofen , applied to the upper arm when tested with 1h occlusion.

Patients N12-22 (group 2, known photocontact allegy) are tested with ketoprofen dilutions in ethanol for 24 h occlusion and with ketoprofen dilutions in petrolatum for 1h occlusion.

Of the 22 patients tested, 20 showed positive test results on the site tested with the standard method using 24h occlusion. The remaining two, who showed doubtful reactions (Table 16), both had a known photocontact allergy to ketoprofen; one had previously reacted with +++ for ketoprofen 1.0% w/v and another patient had reacted with + for the same concentration. All of the patients positive to ketoprofen at standard PPT, as well as the two patients with doubtful reactions, were positive at PPT with 1h occlusion. None of the 20 dermatitis patients who served as controls were positive ($p<0.001$).

With 1h occlusion, the strength of the reactions was +++ in 17 cases, ++ in 2 cases, and + in 2 cases when tested with 1.0% ketoprofen; the remaining one patient showed a doubtful reaction. In both groups (24h and 1h) there were positive reactions down to 0.001%, and in one case in the 24h group a positive reaction was seen at 0.0001% w/v dilution of ketoprofen.

Among the 13 patients who were tested with 1.0% ketoprofen with both 24h and 1h occlusion, 10 +++ reactions were present after 24h occlusion and 9 +++ reactions were present after 1h occlusion. In one case, a +++ reaction in the 24h group became a doubtful (+) reaction when occlusion was 1h. Conversely, one doubtful reaction with 24h testing turned to a ++ reaction with the 1h protocol.

Of the 12 patients who were tested with 0.1% ketoprofen with both 24h and 1h occlusion, three of the reactions that were positive in the 24h testing turned negative in the 1h testing, while two +++ and one ++ reaction from the 24h testing became doubtful in the 1h testing. Three patients showed a trend towards stronger reactions in the 1h test for this concentration.

Study II

The results of the PPT and PT were based on two readings on D3/D4 and D7 in dermatitis patients, and on one reading on D3[64] for the general population.

Target group ia versus control group iii, Figure 18 and Table 17

In the period 2009–2018, 283 patients were photopatch tested with ketoprofen (Figure 18). Photocontact allergy was registered in 34 patients (12.0%). Of the 214 patients who were both patch and photopatch tested, photocontact allergy to ketoprofen was diagnosed in 30 (target group ia, Figure 18). Simultaneous contact allergy to FM I was noted in 16/30 (53.3%) (Table 17). Among dermatitis patients who were patch tested with FM I but not photopatch tested (control group iii, Figure 18 and Table 17), contact allergy was noted in 438/6563 patients (6.7%) (16/30 versus 438/6563, $p<0.001$)

The comparison of contact allergy rates to the individual components of FM I between the group with photocontact allergy to ketoprofen (target group ia) and the dermatitis group who were patch tested by not photopatch tested (control group iii)

is shown in Table 17. Rates of contact allergy to cinnamyl alcohol (p<0.001), cinnamal (p=0.0041), eugenol (p<0.001), and isoeugenol (p=0.028) were statistically significantly higher in the target group (patients with photocontact allergy to ketoprofen).

Table 17. Comparison of patch test results for contact allergy to fragrance mix I (FM I) and its components in the period 2009–2018 in two groups of dermatitis patients. Of the 6781 patients who were patch tested, 6563 patients were only patch tested (control group iii, Figure 18) while 214 patients were also photopatch tested. Photocontact allergy to ketoprofen (KP) was diagnosed in 30 of the 214 dermatitis patients (target group ia, Figure 18)

	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	<0.001	16 (8-33)
Cinnamyl alcohol	30	7	23.3	6563	44	0.7	<0.001	45 (18-111)
Cinnamal	30	3	10.0	6563	69	1.05	0.0041	11 (3-35)
Hydroxycitronellal	30	1	3.3	6563	32	0.5	0.14	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	<0.001	77 (30-194)
Isoeugenol	30	2	6.7	6563	56	0.9	0.028	8 (2-36)
Evernia prunastri extract [a]	30	2	6.7	6308¶	117	1.9	0.108	4 (0.9-16)
Sorbitan sesquioleate	30	0	0.0	6563	24	0.4	1	0 (0, NaN)

Note: NaN = data not calculable, pos = positive

[a] 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.

Target group ib versus control group ii, Fig. 18 and Table 18

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, Figure 18) are presented in Table 18. A statistically significant overrepresentation of contact allergy was seen for FM I (p<0.001), cinnamyl alcohol (p<0.001), and eugenol (p=0.0077) in the patients with photocontact allergy to ketoprofen.

Table 18. Comparison of patch test results for contact allergy to fragrance mix I (FM I) and its components in the period 2009–2018 in 168 patch tested dermatitis patients who were 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, Figure 18).

	Patients with ketoprofen photocontact allergy n=20			Patients without ketoprofen photocontact allergy n=148			p-value	Odds ratio (95% CI)
	No tested	No pos	% pos	No tested	No pos	% pos		
FM I	20	11	55.0	148	25	16.9	<0.001	6 (2-16)
Cinnamyl alcohol	20	7	35.0	148	7	4.7	<0.001	11 (3-36)
Cinnamal	20	2	10.0	148	9	6.1	0.62	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	0.0077	9 (2-40)
Isoeugenol	20	1	5.0	148	2	1.4	0.32	4 (0.3-45)
Evernia prunastri extract	20	2	10.0	148	10	6.8	0.64	2 (0.3-8)
Sorbitan sesquioleate	20	0	0.0	148	1	0.7	1	0 (0, NaN)

Note: NaN = data not calculable, pos = positive.

Target group ia versus control group iv, Fig. 18 and Table 19

The comparison between the group with photocontact allergy to ketoprofen (target group ia) and the group of volunteers from the general population who were patch tested but not photopatch tested (control group iv, Figure 18) regarding rates of contact allergy to FM I and its individual components is shown in Table 19. Rates of contact allergy to FM I (<0.001), cinnamyl alcohol (p<0.001), cinnamal (p=0.0027), and eugenol (p<0.001) were statistically significantly higher in the target group with photocontact allergy to ketoprofen.

Table 19. Comparison of patch test results for allergy to fragrance mix I (FM I) and its components in patients photoallergic to ketoprofen (KP) (target group ia, Figure 18) and the general population in Malmö, Sweden (control group iv, Figure 18) 165. Data are 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 (95% CI)
	No tested	No positive	% positive	No tested	No positive	% positive		
FM I	30	16	53.5	518	13	2.5	<0.001	44 (18-110)
Cinnamyl alcohol	30	7	23.3	518	2	0.4	<0.001	79 (16-399)
Cinnamal	30	3	10.0	518	3	0.6	0.0027	19 (4-99)
Hydroxycitronellal	30	1	3.3	518	1	0.2	0.107	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	<0.001	129 (15-1117)
Isoeugenol	30	1	3.3	518	1	0.2	0.107	18 (1-292)
Evernia prunastri 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)

Note: NaN = data not calculable.

Pairs of chemically related substances

When comparing the results of PT with the two pairs of chemically closely related ingredients of FM I (cinnamal/cinnamic alcohol and eugenol/isoeugenol) within the photopatch tested group with photocontact allergy to ketoprofen and the patch tested dermatitis group, the rate of contact allergy to cinnamyl alcohol was slightly but not significantly higher than the rate of contact allergy to cinnamal (p=0.3) in the ketoprofen group, and significantly lower (p=0.023) in the dermatitis group. Similarly, the rate of contact allergy to eugenol was numerically but not statistically higher than the rate of contact allergy to isoeugenol (p=0.15) in the ketoprofen group, while the dermatitis group showed a significantly higher prevalence of contact allergy to isoeugenol (p=0.0012).

Given the small size of the ketoprofen group, we also compared 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 vs. 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=0.038 for cinnamic substances and p=0.019 for eugenol/isoeugenol) (Table 20).

Table 20. Distribution and comparison of positive patch test reactions to pairs of chemically related substances (cinnamyl alcohol/cinnamal and eugenol/isoeugenol) in 30 patients with photocontact allergy to ketoprofen (KP) 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		

Among the patients who 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 those to cinnamal and isoeugenol, respectively, but the difference was not significant in those with photocontact allergy to ketoprofen. 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.

Chemical analyses

GC-MS analysis of the patch test preparations of cinnamal and cinnamyl alcohol confirmed that the substance labels on the respective syringes corresponded to their contents. We detected cinnamal as a contaminant in all the investigated preparations of cinnamyl alcohol, corresponding to approximately 14% of the total weight of cinnamyl alcohol. No contamination of cinnamyl alcohol was detected in the cinnamal preparations (detection limit <0.03%).

Study III

Study III included only the results of the testing with components of FM I. The contact allergy rates and p-values for the comparisons made are presented in Tables 21 and 22.

Table 21. Reactions to preparations of oxidized linalool in ketoprofen-photoallergic patients (K) and in dermatitis patients without a diagnosed photocontact allergy to ketoprofen (D).

Period	02/2005–11/2005		07/2006–01/2008, 04/2010–11/2010		07/2012–12/2015			
Concentration (%w/w of linalool hydroperoxides)	0.8% a		1% a		1% b		Any preparation	
Group	K	D	K	D	K	D	K	D
no. tested	2	325	17	1221	10	2544	29	4021
no. positive	1	9	11	43	7	139	19	190
no. + reactions	1	6	3	28	5	106	-	-
no. ++ reactions	0	3	5	13	1	23	-	-
no. +++ reactions	0	0	3	2	1	10	-	-
p-value (Fisher's exact test, positive vs. negative)	0.060		<0.001		<0.001		<0.001	
p-value (Mann-Whitney)	>0.3		0.010		>0.3		-	

[a] Test preparations from the Department of Dermatochemistry, University of Gothenburg.
[b] Test preparations from Chemotechnique Diagnostics Vellinge, Sweden.

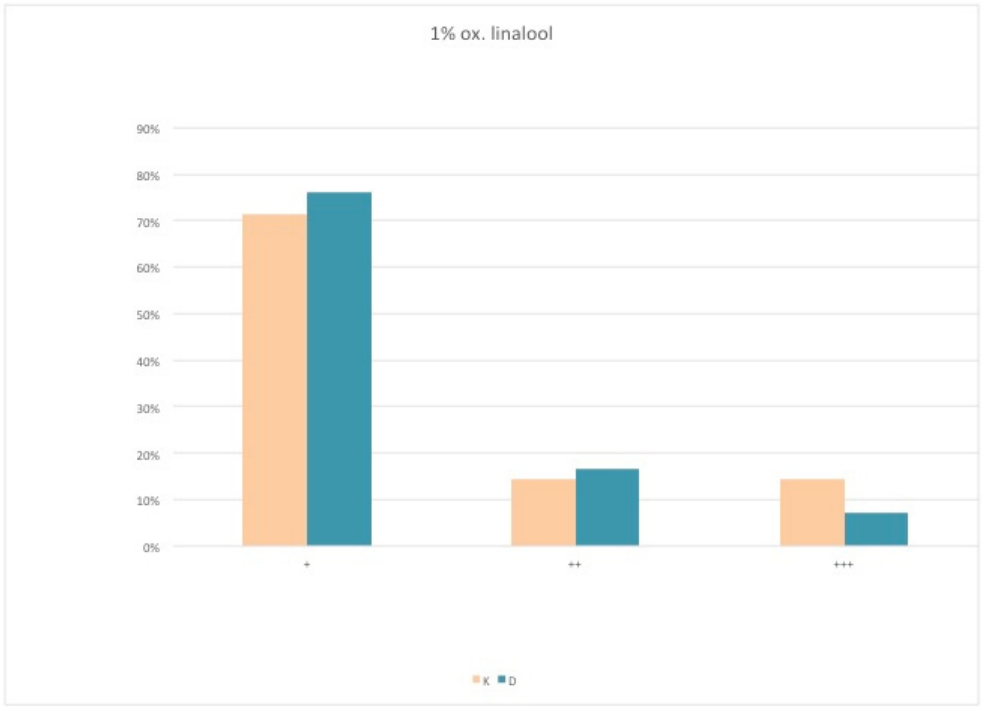
Table 22. Reactions to preparations of oxidized limonene in ketoprofen-photoallergic patients (K) and in dermatitis patients without a diagnosed photocontact allergy to ketoprofen (D).

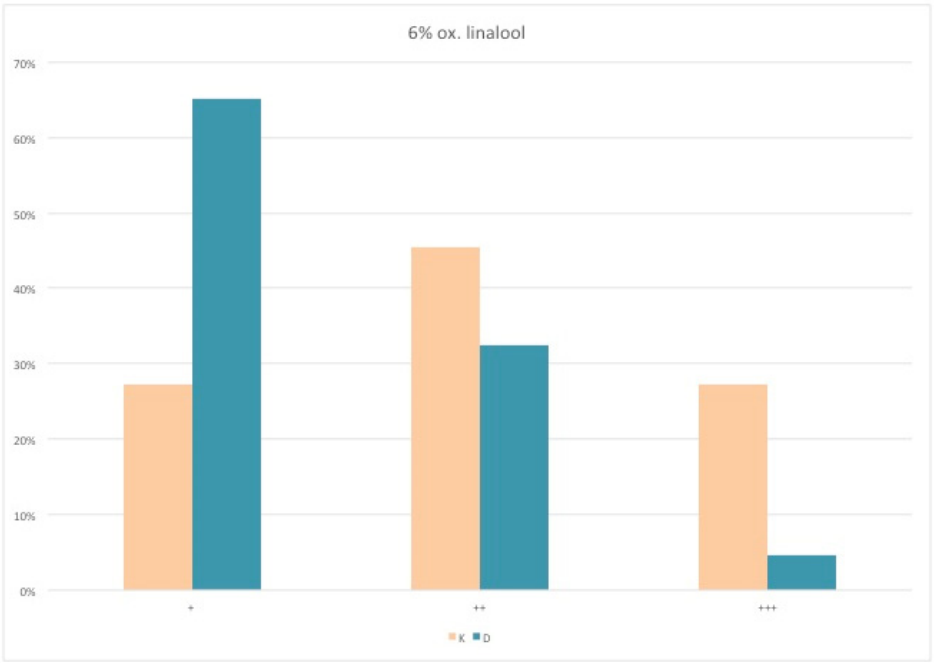
Period	09/2004–11/2005, 07/2006–01/2007, 04/2010–11/2010		07/2012–12/2015			
Concentration (% w/w of limonene hydroperoxides)	0.3% a		0.3% b		Any preparation	
Group	K	D	K	D	K	D
no. tested	14	1292	10	2547	24	3797
no. positive	4	30	6	83	10	111
no. + reactions	1	16	3	61	-	-
no. ++ reactions	2	10	2	15	-	-
no. +++ reactions	1	4	1	7	-	-
p-value (Fisher's exact test, pos vs neg)	<0.001		<0.001		<0.001	
p-value (Mann-Whitney)	0.25		0.30		-	

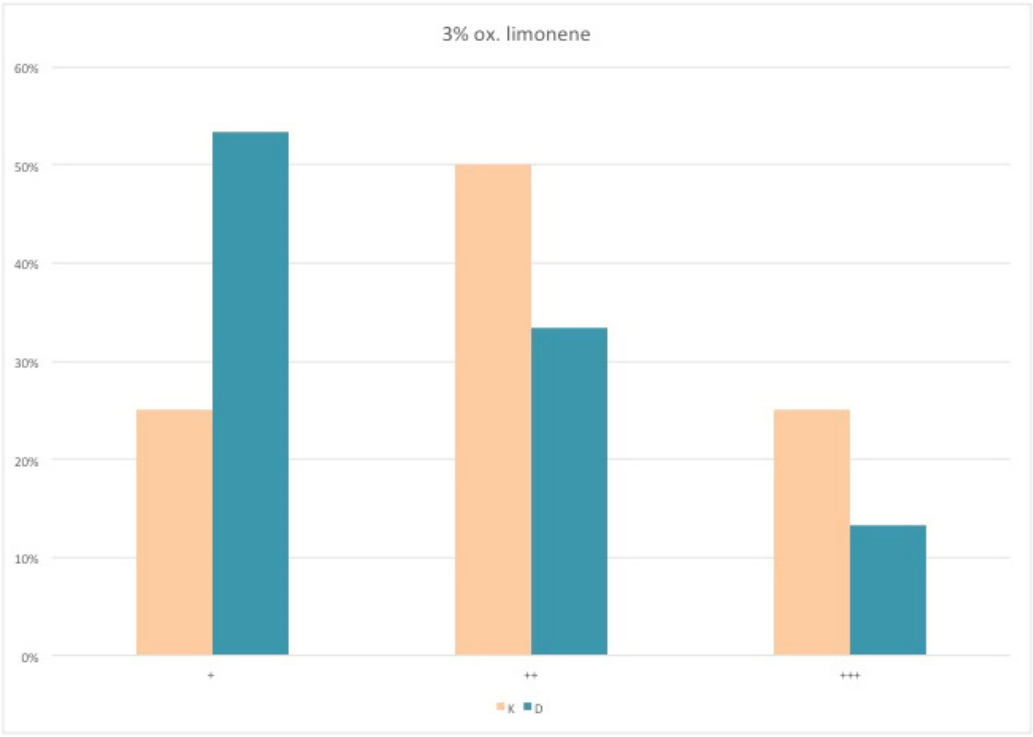
[a] Test preparations from the Department of Dermatochemistry, University of Gothenburg.
[b] Test preparations from Chemotechnique Diagnostics Vellinge, Sweden.

Of the 4021 patients without a known contact allergy to ketoprofen who were patch tested with ox. linalool, 190 (4.7%) tested positive. The corresponding numbers for ox. limonene were 3797 patients and 111 positive reactions (2.9%). A total of 19 contact allergic reactions to ox. linalool was noted in the 29 patients (65.5%) who were diagnosed with photocontact allergy to ketoprofen during the test period ($p<0.0001$). The corresponding figures for ox. limonene were 10 positive reactions in the 24 individuals (41.7%) with photocontact allergy to ketoprofen ($p<0.0001$). The distribution of degrees of patch test reactivities to ox. linalool and ox. limonene in the group of 24 individuals with photocontact allergy to ketoprofen who were simultaneously patch tested with both ox. linalool and ox. limonene is presented in

Tables 21 and 22. In general, the patients with photocontact allergy to ketoprofen showed more intense reactions to both ox. limonene and ox. linalool than the dermatitis patients. However, a statistically significant difference in the degree of reactivity was only found for one of the preparations of ox. linalool (6%) tested in the period 2006–2010 (p=0.010; Mann-Whitney U test, two-sided) (Figure 20).







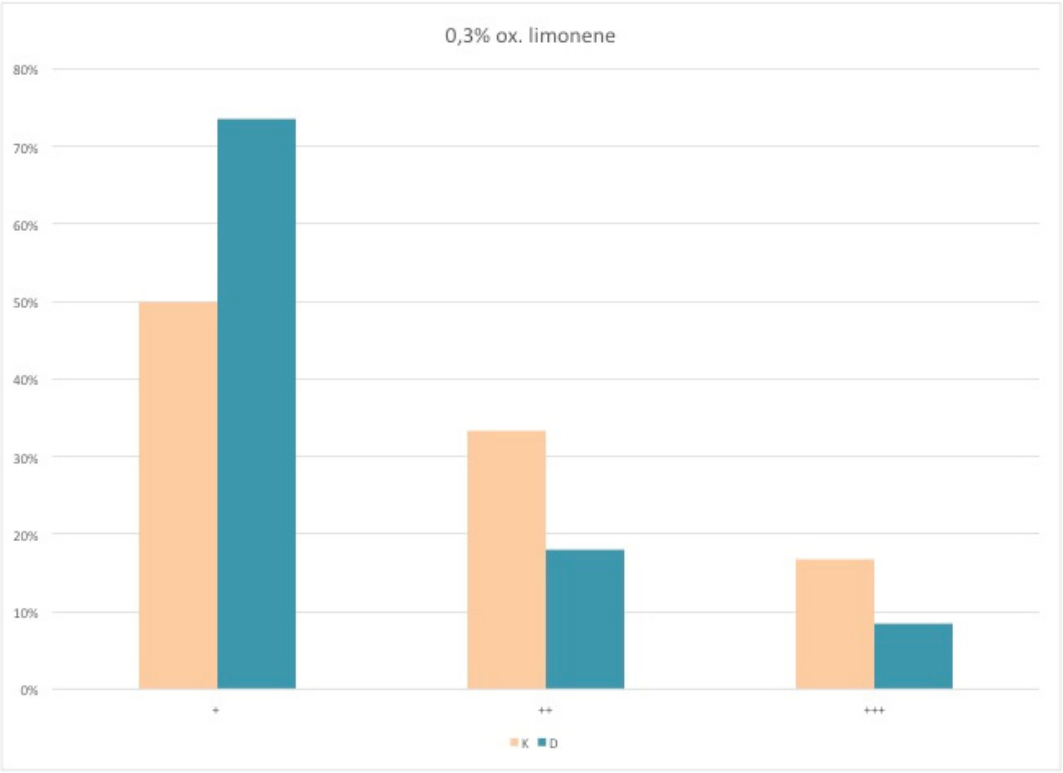


Figure 20. Distribution of positive reactions to ox. linalool and ox. limonene with regard to degree of reactivity (% of total number of positive reactions scored as +, ++, and +++) in patients with photocontact allergy to ketoprofen (K) and in dermatitis patients (D).

There was a high degree of concomitant reactions to ox. linalool and ox. limonene in the ketoprofen-allergic patients. In this group, 9 of 24 patients tested with both ox. linalool and ox. limonene tested positively to both (Table 20). The corresponding figures for the routinely patch tested dermatitis patients were 51 of 3502 ($p<0.0001$). The same comparison between the two patient groups based on those within the respective group reacting positively to ox. linalool and/or ox. limonene also resulted in a significant difference (9 of 16 versus 51 of 226; $p=0.0054$) (Table 23).

Table 23. Patients tested simultaneously with oxidized limonene and oxidized linalool. Number of positive reactions to oxidized linalool and/or oxidized limonene in ketoprofen-photoallergic patients (KP) and in dermatitis patients without a diagnosed photocontact allergy to ketoprofen (D)

Test preparation	KP	D
ox. linalool and ox. limonene	9 (38%)	51(1.5%)
only ox. linalool	6 (25%)	120 (3.4%)
only ox. limonene	1 (4.2%)	55 (1.6%)

Study IV

The contact allergy rates for the sensitizers in our baseline series for the ketoprofen-photoallergic patients and the controls as well as the p-values of the pair-wise comparison of contact allergy rates between the two groups are shown in Tables 24-27. Only the statistically significant results are presented below.

The total ketoprofen group **(KP group 0)** in comparison to **CPT group 0** showed a significant overrepresentation of contact allergy to the following sensitizers in the baseline series: FM I (432.3% vs. 6.6%, $p<0.001$, OR: 11, 95% CI: 7–17), *Myroxylon pereirae* resin (47.9% vs. 6.6%, $p<0.001$, OR: 14, 95% CI: 9–22), black rubber mix (7.2% vs. 0.6%, $p<0.001$, OR: 13, 95% CI: 5–33), para tertiary butylphenol formaldehyde resin (PTBP-F-R) (11.4% vs. 1.0%, $p<0.001$, OR: 13, 95% CI: 6–29), phenol formaldehyde resin 2 (PFR-2) (32.9% vs. 0.9%, $p<0.001$, OR: 68, 95% CI: 39–116), budesonide (7.2% vs. 0.9%, $p<0.001$, OR: 9, 95% CI: 4–24), and FM II (10.2% vs. 3.0%, $p=0.015$, OR: 4, 95% CI: 2–10) (Table 24).

Table 24. Numbers of positive test reactions to the components of the baseline series and several additionally tested substances, 1999–2018. Comparison between ketoprofen-allergic patients (KP) and dermatitis patients (Derm).

Tested substance	KP-pos, N° tested	KP pos, N° pos	% pos	Derm, N° tested	Derm, N° pos	% pos	p-value	Odds ratio (95% CI)
Potassium dichromate	70	3	4.3	12 764	505	4.0	0.76	1 (0.4-4)
PPD	63	1	1.6	12 729	293	2.3	1	1 (0.1-5)
Thiuram mix	70	0	0	12 793	183	1.4	0.63	0,0 (NaN)
Neomycin sulfate	70	1	1.4	12 793	161	1.3	0.59	1 (0.2-8)
Cobalt(II)sulfate hexahydrate	70	5	7.2	12 091	734	6.1	0.62	1 (0.5-3)
Quaternium-15	70	0	0	12 797	123	1.0	1	0,0 (NaN)
Nickel(II)sulfate hexahydrate	70	11	15.7	12 728	2361	18.4	0.65	1 (0.4-2)
Quinoline mix	70	0	0	12 792	48	0.4	1	0,0 (NaN)
Colophonium	70	4	5.7	12 758	425	3.3	0.30	2 (0.6-5)
Paraben mix	70	0	0	12 792	52	0.4	1	0,0 (NaN)
Black rubber mix	70	5	7.2	12 793	76	0.6	<0.001	13 (5-33)
Sesquiterpene lactone mix	70	0	0	12 757	98	0.8	1	0,0 (NaN)
Mercapto mix	70	0	0	12 793	40	0.3	1	0,0 (NaN)
Epoxy resin, bisphenol A	70	0	0	12 770	173	1.4	0.63	0,0 (NaN)
Myroxylon pereirae	71	34	47.9	12 777	812	6.6	<0.001	14 (9-22)
PTBP-F-R	70	8	11.4	12 791	122	1.0	<0.001	13 (6-29)
Fragrance mix II	49	5	10.2	8 322	243	3.0	0.015	4 (2-10)
Formaldehyde	70	1	1.5	12 770	474	3.7	0.52	0.4 (0.05-3)
Fragrance mix I	71	30	42.3	12 787	811	6.6	<0.001	11 (7-17)
PFR-2	70	23	32.9	12 792	92	0.9	<0.001	68(39-116)
Diazolidinyl urea	70	0	0	12 796	107	0.8	1	0,0 (NaN)
MCI/MI	56	1	1.8	11 392	511	4.5	0.52	0.4 (0.05-3)
Amerchol L-101	70	5	7.2	12 801	418	3.3	0.08	2 (0.9-6)
Caine mix II	70	1	1.4	12 792	185	1.5	1	1 (0.1-7)
Lichen acid mix	70	0	0	12 793	87	0.7	1	0,0 (NaN)
Tixocortol-21-pivalate	70	1	1.4	12 790	125	1.0	0.50	2 (0.2-11)
Textile dye mix	64	4	6.3	12496	310	2.5	0.08	3 (0.9-7)
Budesonide	70	5	7.2	12790	104	0.9	<0.001	9 (4-24)
Methyldibromo glutaronitrile	69	3	4.4	12 794	387	3.0	0.47	2 (0.5-5)
Methylisothiazolinone	50	0	0	9293	292	3.1	0.41	0,0 (NaN)
Thimerosal	61	4	6.6	8659	301	3.5	0.17	2 (0.7-5)

Note: MCI/MI=methylchloroisothiazolinone/methylisothiazolinone; NaN=not calculable; N° =number; pos=positive; PFR-2=phenol formaldehyde resin 2; PPD=p-phenylenediamine; PTBP-F-R=para tertiary butylphenol formaldehyde resin.

KP group 1 (1999–2008) in comparison to **CPT group 1** showed a significant overrepresentation of contact allergy to FM I (34.2% vs. 6.4%, p<0.001, OR: 8, 95% CI: 4–15), *Myroxylon pereirae* resin (56.1% vs. 6.4%, p<0.001, OR: 20, 95% CI: 11–37), black rubber mix (7.3% vs. 0.7%, p<0.001, OR: 11, 95% CI: 3–38), PTBP-F-R (9.8% vs. 1.2%. p<0.001, OR: 9, 95% CI: 3–26), PFR-2 (24.4% vs. 1.0%, p<0.001, OR: 37, 95% CI: 17–78), budesonide (5.0% vs. 1.0%, p=0.058, OR: 6, 95% CI: 1–23), and FM II (15.0% vs. 3.0%. p=0.021, OR: 6, 95% CI: 2–21)(Table 25).

Table 25. Numbers of positive test reactions to the components of the baseline series and several additionally tested substances, 1999–2008. Comparison between ketoprofen-photoallergic patients (KP) and dermatitis patients (Derm)

Tested substance	KP-pos, N° tested	KP pos, N° pos	% pos	Derm, N° tested	Derm, N° pos	% pos	p-value	Odd ratio (95% CI)
Potassium dichromate	41	2	4.9	6360	256	4.0	0.68	1 (0.3-5)
PPD	41	0	0	6376	118	1.8	1	0,0 (NaN)
Thiuram mix	41	0	0	6384	97	1.5	1	0,0 (NaN)
Neomycin sulfate	41	1	2.4	6384	108	1.7	0.51	2 (0.2-11)
Cobalt(II)sulfate hexahydrate	41	4	9.8	6358	408	6.4	0.34	2 (0.6-5)
Quaternium-15	41	0	0	6384	64	1.0	1	0,0 (NaN)
Nickel(II)sulfate hexahydrate	41	8	19.5	6344	1261	19.9	1	1 (0.5-2)
Quinoline mix	41	0	0	6383	30	0.5	1	0,0 (NaN)
Colophonium	41	2	4.9	6359	228	3.6	0.66	1 (0.3-6)
Paraben mix	41	0	0	6383	27	0.4	1	0,0 (NaN)
Black rubber mix	41	3	7.3	6384	44	0.7	0.003	11 (3-38)
Sesquiterpene lactone mix	41	0	0	6347	53	0.8	1	0,0 (NaN)
Mercapto mix	41	0	0	6384	25	0.4	1	0,0 (NaN)
Epoxy resin, bisphenol A	41	0	0	6368	84	1.3	1	0,0 (NaN)
Myroxylon pereirae	41	23	56.1	6380	388	6.4	<0.001	20 (11-37)
PTBP-F-R	41	4	9.8	6381	75	1.2	<0.001	9 (3-26)
Fragrance mix II	20	3	15.0	1686	49	3.0	0.021	6 (2-21)
Formaldehyde	41	0	0	6364	208	3.3	0.64	0,0 (NaN)
Fragrance mix I	41	14	34.2	6383	397	6.4	<0.001	8 (4-15)
PFR-2	41	10	24.4	6382	56	1.0	<0.001	37 (17-78)
Diazolidinyl urea	41	0	0	6384	60	0.9	1	0,0 (NaN)
MCI/MI	27	1	3.7	4862	145	3.0	0.56	1 (0.2-9)
Amerchol L-101	41	3	7.3	6384	157	2.5	0.08	3(0.9-10)
Caine mix II	41	0	0	6384	88	1.4	1	0,0 (NaN)
Lichen acid mix	41	0	0	6384	53	0.8	1	0,0 (NaN)
Tixocortol-21-pivalate	41	0	0	6381	59	0.9	1	0,0 (NaN)
Textile dye mix	38	1	2.6	6064	106	1.8	0.49	2 (0.2-11)
Budesonide	41	2	5.0	6382	59	1.0	0.058	6 (1-23)
Methyldibromo glutaronitrile	40	2	7.7	6384	240	3.8	0.66	1 (0.3-6)
Methylisothiazolinone	26	0	0	3271	33	1.0	1	0,0 (NaN)
Thimerosal	41	4	9.8	6382	236	3.7	0.066	3 (0.99-8)

Note: MCI/MI=methylchloroisothiazolinone/methylisothiazolinone; NaN=not calculable; N° = number; pos = positive; PFR-2=phenol formaldehyde resin 2; PPD=p-phenylenediamine; PTBP-F-R=para tertiary butylphenol formaldehyde resin.

KP group 2 (2009-2018) in comparison to **CPT group 2** showed a significant overrepresentation of contact allergy to FM I (54.8% vs. 6.6%, p<0.001, OR: 18, 95% CI: 9–37), *Myroxylon pereirae* resin (38.7% vs. 6.6%, p<0.001, OR: 9, 95% CI: 5–19), black rubber mix (10.0% vs. 0.5%, p<0.001, OR: 24, 95% CI: 7–81), PTBP-F-R (16.7% vs. 0.9%, p<0.001, OR: 25, 95% CI: 9–68), PFR-2 (46.7% vs. 0.8%, p<0.001, OR: 151, 95% CI: 69–331), budesonide (13.3% vs. 0.8%, p<0.001, OR: 20, 95% CI: 7–60), and FM II (10.0% vs. 2.9%, p=0.054, OR: 4, 95% CI: 1–13)(Table 26).

Table 26. Numbers of positive test reactions to the components of the baseline series and several additionally tested substances, 2009–2018. Comparison between ketoprofen-photoallergic patients (KP) and dermatitis patients (Derm).

Tested substance	KP pos, No tested	KP pos, No pos	% pos	Derm tested	Derm pos	% pos	p-value	Odds ratio (95% CI)
Potassium dichromate	30	2	6.7	6773	258	3.8	0.32	2 (0.4-8)
PPD	30	1	3.3	6715	183	2.7	0.57	1 (0.2-9)
Thiuram mix	30	0	0	6782	89	1.3	1	0,0 (NaN)
Neomycin sulfate	30	0	0	6782	59	0.9	1	0,0 (NaN)
Cobalt(II)sulfate hexahydrate	30	1	3.3	6770	345	5.1	1	1 (0.1-5)
Quaternium-15	30	0	0	6786	61	0.9	1	0,0 (NaN)
Nickel(II)sulfate hexahydrate	30	3	10.0	6748	1163	17.2	0.47	1 (0.2-2)
Quinoline mix	30	0	0	6782	19	0.3	1	0,0 (NaN)
Paraben mix	30	0	0	6782	25	0.4	1	0,0 (NaN)
Black rubber mix	30	3	10.0	6782	32	0.5	<0.001	24 (7-81)
Sesquiterpene lactone mix	30	0	0	6781	51	0.8	1	0,0 (NaN)
Mercapto mix	30	0	0	6781	15	0.2	1	0,0 (NaN)
Epoxy resin, bisphenol A	30	0	0	6772	98	1.5	1	0,0 (NaN)
Myroxylon pereirae	31	12	38.7	6766	437	6.6	<0.001	9 (5-19)
PTBP-F-R	30	5	16.7	6781	54	0.9	<0.001	25 (9-68)
Fragrance mix II	30	3	10.0	6776	193	2.9	0.054	4 (1-13)
Formaldehyde	30	1	3.3	6776	273	4.0	1	1 (0.1-6)
Colophonium	30	2	6.7	6769	212	3.2	0.25	2 (0.5-9)
Fragrance mix I	31	17	54.8	6772	431	6.6	<0.001	18 (9-37)
PFR-2	30	14	46.7	6782	39	0.8	<0.001	151 (69-331)
Diazolidinyl urea	30	0	0	6785	48	0.7	0.19	0,0 (NaN)
MCI/MI	30	1	3.3	6783	379	5.6	1	0.6 (0.1-4)
Amerchol L-101	30	3	10.0	6790	262	3.9	0.11	3 (0.8-9)
Caine mix II	30	1	3.3	6781	99	1.5	0.36	2 (0.3-17)
Lichen acid mix	30	0	0	6782	35	0.5	1	0,0 (NaN)
Tixocortol-21-pivalate	30	1	3.3	6782	69	1.0	0.27	3 (0.5-25)
Textile dye mix	30	4	13.3	6783	208	3.1	0.013	5 (2-14)
Budesonide	30	4	13.3	6781	50	0.8	<0.001	20 (7-60)
Methyldibromo glutaronitrile	30	1	3.3	6782	151	2.2	0.49	2 (0.2-11)
Methylisothiazolinone	24	0	0	6014	312	5.2	0.63	0,0 (NaN)
Thimerosal	21	1	3.3	2450	69	2.8	0.46	2 (0.2-13)

Note: MCI/MI=methylchloroisothiazolinone/methylisothiazolinone; NaN=not calculable; Nº = number; pos = positive; PFR-2=phenol formaldehyde resin 2; PPD=p-phenylenediamine; PTBP-F-R=para tertiary butylphenol formaldehyde resin.

KP group 2 in comparison to the **GPPT group** showed a significant overrepresentation of contact allergy to FM I (53.5% vs. 2.5%, p<0.001, OR 44 (18-110)); black rubber mix (10.0% vs. 0.4%, p=0.0014, OR 29 (5-179)); PTBP-FR (16.7% vs. 0.2%, p<0.001, OR 104 (12-919)); budesonide (13.3% vs. 0.4%, p<0.001, OR 40 (7-227)); and FM II (10.0% vs. 1.7%, p=0.023, OR 6 (2-25))(Table 27).

Table 27. Numbers of positive test reactions to the components of the baseline series and several additionally tested substances, 2009–2018. Comparison between ketoprofen-photoallergic patients (KP) and the general population (GP).

Tested substance	KP pos, Nº tested	KP pos, No pos	% pos	GP, No tested	GP, Nº pos	% pos	p-value	Odds ratio (95% CI)
Potassium dichromate	30	1	3.3	518	1	0.2	0.107	18 (1-292)
PPD	30	1	3.3	518	6	1.2	0.33	3 (0.4-25)
Thiuram mix	30	0	0	518	1	0.2	1	0,0 (NaN)
Neomycin sulfate	30	0	0	518	4	0.8	1	0,0 (NaN)
Cobalt(II)sulfate hexahydrate	30	1	3.3	518	6	1.2	0.33	3 (0.4-25)
Quaternium-15	30	0	0	518	2	0.4	1	0,0 (NaN)
Nickel(II)sulfate hexahydrate	30	3	10.0	518	42	8.1	0.73	1 (0.4-4)
Quinoline mix	30	0	0	518	1	0.2	1	0,0 (NaN)
Colophonium	30	2	6.7	518	4	0.8	0.055	9 (1-52)
Paraben mix	30	0	0	518	0	0	1	0,0 (NaN)
Black rubber mix	30	3	10.0	518	2	0.4	0.0014	29 (5-179)
Sesquiterpene lactone mix	30	0	0	518	0	0	1	0,0 (NaN)
Mercapto mix	30	0	0	518	1	0.2	1	0,0 (NaN)
Epoxy resin, bisphenol A	30	0	0	518	5	1.0	1	0,0 (NaN)
PTBP-F-R	30	5	16.7	518	1	0.2	<0.001	104 (12-919)
Fragrance mix II	30	3	10.0	518	9	1.7	0.023	6 (2-25)
Formaldehyde	30	1	3.3	518	3	0.6	0.202	6 (0.6-59)
Fragrance mix I	30	16	53.5	518	13	2.5	<0.001	44 (18-110)
MCI/MI	30	1	3.3	518	3	0.6	0.202	6 (0.6-59)
Amerchol L-101	30	3	10.0	518	0	0	<0.001	0,0 (NaN)
Caine mix II	30	1	3.3	518	1	0.2	0.106	18 (1-292)
Tixocortol-21-pivalate	30	1	3.3	518	2	0.4	0.16	9 (0.8-101)
Budesonide	30	4	13.3	518	2	0.4	<0.001	40 (7-227)

Note: MCI/MI=methylchloroisothiazolinone/methylisothiazolinone; NaN=not calculable; Nº=number; pos=positive; PPD=p-phenylenediamine; PTBP-F-R=para tertiary butylphenol formaldehyde resin.

Discussion

The impetus for this research dates back to the early 2000s, when the first reports started to raise awareness of adverse reactions to topical ketoprofen formulations. Our research team was created in 2005, initially with the aim of contributing knowledge about the prevalence of these adverse reactions. However, it proved to be virtually impossible to speak about photocontact allergy to ketoprofen without mentioning the prevalence of simultaneous reactions to other chemicals. The Department of Occupational and Environmental Dermatology in Malmö conducts around 800 patch and photopatch tests every year, which provides a large research platform. At first, simultaneous photoallergic reactions were shown to be overrepresented in those with photocontact allergy to ketoprofen[144]. Later, other research groups also started to report an overrepresentation of contact allergy in the same group.

Contact allergy is one of the preventable causes of chronic or relapsing skin problems. In 2007, a review of contact sensitization rates in the general population found that the median prevalence of contact allergy to at least one allergen was around 20% (range: 12.5–40.6%), based on data from multiple studies conducted in different countries between 1966 and 2007[170]. Race, age, and geographic origin of the individual studies included in the review did not appear to influence the result on a significant level (Table 28).

Table 28. Studies on contact allergy in the general population conducted between 1966 and 2007 [170]

Author	Year of publication	Country	Population	*n*	Age (years)	Allergens used for patch testing	Patch test reading performed at day	Positive reaction to at least 1 allergen; total (%)	Most common allergens
Forsbeck	1966	Sweden	Relatives to patients with allergic contact dermatitis	93	> 10	Standard series	—	24.7	Nickel, fragrance mix, and Balsam of Peru/formaldehyde/ *p*-phenylendiamine procaine
Sipos	1967	Hungary	Subjects with intact skin	659	—	[b]	3	13.7	$HgCl_2$, formaldehyde, and nickel/PPD
Forsbeck	1968	Sweden	Twins	202	43–82	Standard series	3	15.8	Nickel, chromium, and methyl thiuram disulfide
Magnusson	1979	Sweden	Patients awaiting hip surgery	274	Mean = 65	Standard series	3	22	Nickel, Balsam of Peru, and formaldehyde
Weston	1986	USA	Children	314	½–18	Standard series	3	20.3	Neomycin, nickel, and chromium
Seidenari	1990	Italy	Cadets	593	18–28	Standard and textile series	3	12.5	Thimerosal, nickel, and $HgCl_2$
Barros	1991	Portugal	School-children	562	5–14	Standard series	2	13.3	Neomycin, thimerosal, and *p*-tertiary-butylphenol-formaldehyde
Nielsen.	1992	Denmark	General population	567	15–69	TRUE-tests	2	15.2	Nickel, thimerosal cobalt/Balsam of Peru
Dotterud	1994	Norway	Schoolchildren	424	7–12	Epiquick test	2	23.3	Nickel, cobalt, and MCI/MI
Nielsen	1998	Denmark	General population	469	15–41	TRUE-tests	2	18,6	Nickel, fragrance mix, and thimerosal
Bruckner	2000	USA	Infants	85	½–5	TRUE-tests	4 and 5	24.5	Nickel, thimerosal, and MCI/MI
Mortz	2001	Denmark	School-children	1146	12–16	TRUE-tests	3[c]	15.2	Nickel, fragrance mix, and thimerosal/colophony/cobalt
Schäfer	2001	Germany	General population	1141	28–78	Standard series	3	28	Nickel, fragrance mix, and thimerosal
Bryld[d]	2003	Denmark	Twins	627	20–44	TRUE-tests	3	21.4	Thimerosal, nickel, and colophony/fragrance mix
Basketter	2004	Thailand	General population	1178–2545	18–55	[e]	2	—	Nickel, PPD, and chromium
Dotterud	2007	Norway	General population	1236	18–69	TRUE-tests	3	26.3	Nickel, cobalt, and thimerosal

[b] $HgCl_2$, formaldehyde, nickel, chromium, novocaine, PPD, turpentine, and lanoline.
[c] 40 children were not read on D3 but rather on D2, D4, or D7. Some were also read by parents who had been previously instructed.
[d] Calculations made on individuals without hand eczema.
[e] Nickel, Fragrance, formaldehyde, PPD, MCI/MI, colophonium, and chromate.
Table by Thyssen et al., shortened and adapted from Thyssen JP, Linneberg A, Menné T, Duus Johansen J. The epidemiology of contact allergy in the general population--prevalence and main findings. Contact Dermatitis. 2007 Nov;57(5):287-99. https://doi.org/10.1111/j.1600-0536.2007.01220.x Permission obtained from John Wiley and Sons. Licence number 5124681376024

The prevalence data above include individual studies from Germany [171,172], Denmark [173] and Norway [174], among others. A more recent large multi-centre study on the prevalence of contact allergy in the general population, published in 2015, presented sensitization rates for five European countries: Sweden, the Netherlands, Germany, Italy, and Portugal. In total, 27% of the volunteers had at least one positive reaction to an allergen of the European baseline series. The highest prevalence was found for nickel, thimerosal, cobalt, FM I, FM II, hydroxyisohexyl 3-cyclohexene carboxaldehyde, PTBP-F-R, and para-phenylenediamine[64].

The prevalence of some form of contact dermatitis is expected to be higher in the dermatitis population, due to the fact that this group is defined by the presence of an inflammatory skin condition. In a Spanish study, at least one positive PT result was found in 55% of the tested dermatitis patients. Contact allergy as an explanation for dermatitis was estimated to be present in 28.2% of cases[175], with irritant contact dermatitis responsible for 20.1%, PhACD for 2.2%, and phototoxic contact dermatitis for 1.2%% of positive reactions[175].

Epidemiological data for photocontact allergy in the general population are scarce[176], possibly due to the relative rarity of photocontact allergy, the degree of awareness of the risk that a reaction is photo-induced, and the fact that only a limited number of centres perform PPT. Most studies on the topic present data on individuals photopatch tested on indication of photodermatitis[177-180]. In a study from New Zealand, seventy dermatitis patients were photopatch tested over a 12-year period. Of the 58 patients tested with a photopatch test series, 10% had a positive reaction. The most common diagnosis after the PPT was endogenous dermatitis (54%), followed by ACD (21%), PhACD (9%), and chronic actinic dermatitis (4%)[179].

Different photoallergens have been described as prevalent, depending on the area the study originates from. A study from the USA evaluated 76 dermatitis patients and detected 69 positive photopatch and 45 positive patch test reactions in 30 and 23 patients, respectively. Sunscreens contributed to 23.2% of these, antimicrobial agents to 23.2% (60% of which were due to fentichlor), medications to 20.3%, fragrances to 13%, plants and plant derivatives to 11.6%, and pesticides to 8.7%[180]. In a British study, 1155 dermatitis patients were investigated with PPT using sunscreen chemicals in addition to suspected topical products. A total of 130 patients (11.3%) had allergic reactions. Photocontact allergy was detected in 51 (4.4%), contact allergy in 64 (5.5%), and combined photocontact and contact allergy in 15 (1.3%). The most common photoinduced reactions were to benzophenone-3 (21%)[177].

A multicentre photopatch test study from Germany, Switzerland, and Austria evaluated two test periods. In the first period, 2859 positive test reactions in 1129 dermatitis patients were evaluated, and 28.6% were assessed as plain contact reactions, 71.4% as photoinduced reactions, and 3.8% as photoallergic. In the

second period, 1415 positive test reactions were observed in 1261 dermatitis patients, and 28.7% were assessed as plain contact reactions, 71.3% as photoinduced reactions, and 8.1% as photoallergic reactions. In both test periods, the leading photoallergens were NSAIDs, disinfectants, and phenothiazines. The use of computer-assisted reaction pattern analysis in the second test period led to a notably reduced number of non-relevant positive test reactions per patient. In contrast, the percentage of photoallergic reactions increased significantly from 3.8% to 8.1% of all positive test reactions[181].

In a study from Spain, 224 dermatitis patients were photopatch tested and 39.3% showed one or more positive tests, 71% of which were considered clinically relevant. The most prevalent allergens were NSAIDs, especially ketoprofen (43 patients), followed by benzydamine (7 patients) and etofenamate (5 patients). The authors stated, among other things, that ketoprofen was the most frequent photoallergen in Spain[182]. A Portuguese study on 83 dermatitis patients reported that 43.3% had at least one positive reaction on photopatch testing. The main relevant reactions were to benzophenone-3, benzophenone-4, promethazine, and chlorpromazine; 26.7% had a relevant positive PPT to benzydamine from a topical gel or oral solution, and 6.7% to ketoprofen[178].

Which photoallergen (or indeed which contact allergen) is found to be the most prevalent would depend on the availability of different substances on the market in the studied area as well as the risk of exposure at the workplace and/or during leisure time in that area. Ketoprofen is described as a common photoallergen in countries where topical preparations with ketoprofen are, or were, readily available[141,143,178,182]. Conversely, countries where ketoprofen has not been distributed as a topical NSAID, such as the USA, report different photosensitizing agents, for example sunscreens [180]. Although a topical formulation of ketoprofen is distributed in the United Kingdom, neither the multicentre study mentioned above [177] nor the two other British studies on the prevalence of photocontact allergy in the UK [79,183] show the data on photocontact allergy to this photosensitizer, the reason being that ketoprofen was at the time for the studies not included in the PPTs the study results were based upon. This illustrates the fact that the content of the photopatch test series may vary between the countries, or even between testing facilities, and may change over time, which influence epidemiological data. With the introduction of ketoprofen to the European Photopatch test series [90] there is now a greater chance to detect photocontact allergy to ketoprofen, provided that PPT is conducted and the series is used.

The prevalence data on contact allergy in the studies cannot be compared to the prevalence data on photocontact allergy, as the indications for PT versus PPT differ in terms of primary evaluation of the patients' history, and partly in terms of the possibility to perform PPT, thus leading the clinician towards one method before the other. In order for the comparison to be made, the chosen patient group needs to undergo both photopatch and patch testing during a given time frame. As described

in the chapter on "Ketoprofen as an allergen", many such studies have been conducted[90,139,143,156-158], showing an overrepresentation of certain contact allergens in the ketoprofen-photosensitized population.

The main goal of this thesis was to describe the epidemiology of photocontact allergy to ketoprofen and simultaneous contact allergy to other substances, specifically fragrances and the components of the baseline patch test series used at the Department of Occupational and Environmental Dermatology in Malmö.

Study I

The standard PPT procedure requires that the allergen is occluded for 24h [89,91] or 48h[89] prior to the irradiation. According to a study from 2006, the 48h protocol might be more sensitive[184]. Both occlusion protocols are used, but the 24h protocol is the standard in our clinic in Malmö. Apart from the occlusion time, the dose of the sensitizer and the PPT technique, including the choice of the source and dose of UV radiation, may influence the results[93,94,184-186]. The permeability of the skin barrier, the site of application, and the pharmacokinetics of the sensitizer all influence the number of molecules entering the epidermis and hence the concentration in the epidermis over time. Thus, a longer occlusion time is crucial for the slow-release substances. For fast-release substances, long occlusion time may in theory mean that a certain amount of the sensitizer has left the epidermis, and therefore that the sensitivity of the test is jeopardized. However, much remains unknown about photoallergen formation, meaning that the occlusion time (among other parameters) is set empirically, and the most reliable protocol is selected after studying several empirically chosen protocols.

In this study we wanted to challenge the existing practice in order to see if reliable PPT results can be achieved by modifying test parameters. Our primary goal was to simplify the process of PPT for ketoprofen, both for the patient and for the testing unit. The advantages of the proposed approach can be seen in Table 29, which demonstrates the number of times the patient needs to visit the testing unit for PPT according to the standard 24h versus the experimental 1h procedure.

Table 29. Comparison of the conventional protocol for ketoprofen photopatch testing in Sweden and Europe with an experimental protocol using 1h occlusion. X=conventional testing; Y=testing using 1h occlusion.

Photopatch testing				
	Day			
	0	1	2	3
Application	X/Y			
UVA irradiation Sweden 5 J/cm^2 Europe 1-10 J/cm^2 1h study	Y	X	X	
Reading				X/Y

The results are promising, since we could confirm that the shortened occlusion time produced comparable PPT results with no need to change either the concentration of the test substance or the UV dose. The best concordance between the two groups (24h vs. 1h) was present for the concentration of 1.0%, although the vehicle was different. This makes 1.0% the concentration of choice, though the role of the vehicle is not completely clear. According to our experience, testing with both vehicles gives equivalent results. Interestingly, one patient showed a negative/doubtful reaction on PPT with 24h occlusion, but a ++ positive reaction to the same dose on testing with 1h occlusion. Theoretically, reactions can depend on phototoxicity. However, the presence of negative controls, the pattern of the reactions in the dilution series, and the morphology of the reactions speak strongly against this. A phototoxic reaction would likely have been present in the other controls upon 1h exposure, but this was not observed, confirming the assumption of photoallergic reaction.

Topically applied ketoprofen is reported to give the same tissue concentration of the active substance at the place of application as can be achieved by oral intake. However, the plasma levels post application are estimated to be approximately 60 times lower for the topical preparation, although the inter-individual variation is large. The bioavailability of topical ketoprofen is about 5% compared to orally administered ketoprofen [187].

Ketoprofen reaches its maximum serum concentration (C_{max}) in the first hour of administration if taken orally, and after six hours with topical application[188]. The maximum flux (J_{max}) is defined as the mass or number of molecules moving through a certain area during a given period of time. For ketoprofen, J_{max} is reported as 0.75

μg/cm/h[189]. A comparison of absorption versus elimination of a drug may indicate the level of drug deposition in the tissue. In the case of ketoprofen, the absorption would be continuous and extremely slow, with the dermis serving as its reservoir[190]. Clinically, this has been found to be true, as ketoprofen residues can be detected in the epidermis several weeks after the exposure[137]. The primary assumption that a slow-release substance would need longer occlusion time does not seem to hold true in the case of ketoprofen. As ketoprofen accumulates in the epidermis, its slow release into the bloodstream may mean that the higher concentration remains at the site of action for a longer period of time, but a short occlusion time does not seem to jeopardize the test results.

We are aware that it is not possible to adjust the photopatch test procedure to each individual component in the photopatch test series solely on the basis of our study with 1h occlusion for ketoprofen. The standard approach that follows the guidelines is needed in clinical praxis. However, in those rare situations when ketoprofen is the only allergen needed to be tested, or perhaps if testing with ketoprofen is a part of a research study, testing with 1h occlusion presents clear advantages. Further studies are needed to examine whether a similar approach can be used for other components of the photopatch test series. To compensate for possibly fewer molecules of the photosensitizer, adjustments can be made in the doses of irradiation and/or chemical. Despite the limitations of the method at present, it is in our opinion crucial to emphasize that even well-functioning, empirically proven methods should be questioned in order to find other possible ways of achieving the same results.

Study II

FM I is often used as one of the test preparations of the baseline PT series and is responsible for a significantly higher number of positive patch test reactions in patients with photocontact allergy to ketoprofen[138,139,143,156,157,191] as compared to dermatitis patients. FM I is not an uncommon cause of contact allergy, with an estimated prevalence of 2.6–3.5% in the general population [165,192] and 6–10% in dermatitis patients [87,193-197]. When our data on the ketoprofen-photoallergic patients were analysed, the prevalence for FM I was as high as 53.3%.

An overrepresentation of positive reactions to cinnamal and cinnamyl alcohol in patients with photocontact allergy to ketoprofen has been described by several research groups [144,156,191]. When patch testing ketoprofen-photoallergic patients with constituents of FM I, we have also noticed a significant overrepresentation of contact allergy not only to cinnamal and cinnamyl alcohol, but also to isoeugenol and eugenol, compared to the both the dermatitis population and the general population[165]. When the search was narrowed to only the photopatch tested patients with and without photocontact allergy to ketoprofen, the contact allergy rates to FM I, cinnamyl alcohol, and eugenol remained significantly higher in the ketoprofen group compared to the two control populations, while there were no significant

differences in contact allergy rates to cinnamal and isoeugenol. Moreover, the pattern of allergic contact reactions within the pairs of chemically related fragrance material (eugenol/isoeugenol and cinnamal/cinnamyl alcohol) differed between the ketoprofen group and the control groups. Cinnamyl alcohol gave significantly more positive reactions than cinnamal in those with photocontact allergy to ketoprofen compared to the dermatitis population. Similar results have been reported by other research groups [139,148,156,198-200]. When examining the contact allergy rates for eugenol and isoeugenol, we found that the rate of contact allergy to eugenol was numerically but not statistically higher than that to isoeugenol (p=0.15) in the ketoprofen group, while the situation was reversed in the dermatitis group, with a significantly higher prevalence of contact allergy to isoeugenol (p =0.0012).

A review of sensitization rates for 162 fragrance compounds among dermatitis patients and the general population[201] reported rates of 0.3%–9% vs. 0.8% (dermatitis vs. general population) for cinnamal, 0.14%–11.2% vs. 0.3% for cinnamyl alcohol, 0.3%–3.4% vs. 0.2% for eugenol, and 1.0%–4.5% vs. 0.7% for isoeugenol[201]. In general, cinnamal is considered a more frequent sensitizer than cinnamyl alcohol in a dermatitis population[197,202,203]. Study II also found a slight but non-significant predominance of contact allergy to cinnamal versus cinnamyl alcohol in the dermatitis population, with rates of 1.05% and 0.7% respectively.

While the overrepresentation of simultaneous contact allergy to cinnamal and cinnamyl alcohol has been described previously, at the time of writing this author is not aware of any report on the higher prevalence of contact allergy to either eugenol or isoeugenol among patients with photocontact allergy to ketoprofen. In a earlier study from Belgium, 1/18 tested patients with photocontact allergy to ketoprofen showed positive contact allergic reaction to isoeugenol, and 4/18 to eugenol on PT with the components of FM I. The slight shift towards eugenol giving more positive reactions than isoeugenol, although probably non-significant, follows the same pattern as described in our study. No data were given for a control population, and so it is not possible to draw any conclusions[138].

As both eugenol and isoeugenol are widely available on the market, exposure to these should be taken into consideration as a possible explanation for high sensitization rates. Both are used as constituents of perfumes and skin care products and are found in a number of plants and etheric oils. Additionally, eugenol is a common additive to endosealers in odontology. The market share for eugenol is larger than for isoeugenol (1–4% vs. <1%)[203], which indicates higher possible exposure to eugenol. On the other hand, isoeugenol is considered a stronger sensitizer than eugenol on the basis of the sensitization exposure quotient, which is used for comparing the relative frequency of sensitization with the relative frequency of use/labelling[203]. Sensitisation rates appear to be going up for isoeugenol at present, while remaining constant for eugenol[204]. No obvious simultaneous exposure to eugenol/isoeugenol and ketoprofen has been reported, and

the ketoprofen preparations available on the Swedish market do not contain either of these fragrance materials.

Cinnamal and cinnamyl alcohol both occur naturally in a variety of fruits and spices and are widely used in perfumes and skin care products[205,206]. The market share for cinnamal is 4.3% and that for cinnamyl alcohol is 3.7%[203]. Cinnamal is considered an extreme skin sensitizer, and cinnamyl alcohol a weak one[207]. A decreasing trend in positive test reactions to cinnamal has been described[204,208], possibly explained by lowered test concentration and changes in the use profile of the chemical. Regarding the test concentration, cinnamal is tested at 1% both in FM I and as a separate component, while the test concentrations of cinnamyl alcohol are 1% and 2% respectively. A significant difference between the results of testing with 1% and 2% cinnamal has been observed[209], but the consensus has been that PT with cinnamal at 2% results in too many irritant reactions. While the reported difference in the number of positive test reactions to cinnamal and cinnamyl alcohol may in theory be explained by the difference in test concentrations, it does not explain the difference in the outcome of PT of the patients with photocontact allergy to ketoprofen and the control groups, where cinnamyl alcohol produced significantly more positive reactions than cinnamal in those with photocontact allergy to ketoprofen. No obvious simultaneous exposure to ketoprofen and cinnamal or cinnamyl alcohol has been reported.

Another possibility that needed to be ruled out concerning a possible overrepresentation of contact allergy to cinnamyl alcohol in those with photocontact allergy to ketoprofen was the risk of labelling error, as an overrepresentation of contact allergy to cinnamal rather than cinnamyl alcohol has also been reported[157]. Wrongly labelled test preparations, as well as impurities, have been reported by research groups in the past[210-212]. GC-MS was used to analyse the test preparations of cinnamal and cinnamyl alcohol in Study II, and the results confirmed that the labelling was correct but some impurities existed. Although there was a high concentration of cinnamal in the cinnamyl alcohol preparation, this contamination could not explain the overrepresentation of contact allergy to cinnamyl alcohol in our patients with photocontact allergy to ketoprofen.

As the overrepresentation of contact allergy to eugenol and isoeugenol in patients with photocontact allergy to ketoprofen appears to be a relatively unknown phenomenon, no valid explanation model exists so far. In the case of cinnamal and cinnamyl alcohol, several hypotheses have been proposed.
One of these suggests that cinnamal is the “true” allergen, while cinnamyl alcohol has to be transformed to cinnamal before contact allergic reactions could occur[213], 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[214,215]. However, it has also been hypothesized that cinnamyl alcohol may be in fact a separate antigen that does not require transformation into cinnamal to become a sensitizer [191,216].

Another hypothesis is that the overrepresentation of allergic contact reactions could depend on cross-reactivity. It has been suggested that the structure of cinnamyl alcohol is similar to that of ketoprofen, based on the results of computerized conformational analysis[156]. In Study II, most of the fragrance compounds responsible for significantly higher contact allergy rates in ketoprofen-photoallergic patients were aromatic. No increase in the rate of contact allergy was demonstrated for the aliphatic compounds geraniol and hydroxycitronellal. One explanation for the higher rates of contact allergy to certain aromatic compounds could be that these compounds metabolize with the formation of allergenic end products that cross-react with ketoprofen.

The potential role of enzymes in biotransformation of cinnamic chemicals has been studied[214,217]. Cutaneous alcohol dehydrogenase and 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 lead via different metabolic pathways to interindividual differences, cross-reactivities, or co-sensitisation to different cinnamic compounds[214]. The general predisposition to type IV allergy in some individuals has been suggested as a possible explanation for the occurrence of multiple simultaneous allergies, with or without UV involvement[218]. This hypothesis, although valid, does not explain the shift in the reaction pattern towards different prevalent sensitizers in those with photocontact allergy to ketoprofen.

Finally, the metabolism/oxidation/biotransformation hypothesis has been scrutinized by multiple research groups. Epoxides of cinnamal and cinnamyl alcohol, built upon bioactivation of the fragrance compounds, have been studied regarding their sensitizing capacity, with the rate of positive PT reactions to epoxides being similar to the rates of positive reactions to cinnamal and cinnamyl alcohol. The conclusion of the study was that epoxides are not important haptens in contact allergy to cinnamon fragrances[53].

These hypotheses are not mutually exclusive, and all deserve to be further investigated. The results of Study II are based on a retrospective data analysis, which did not account for changes in exposure. A longitudinal prospective study of sensitization rates, paired with user profiles, would be one way to map the patterns of sensitization over time.

Study III

What once was only a suspicion has now been confirmed in this study. The overrepresentation of contact allergy to ox. linalool and/or ox. limonene in patients with photocontact allergy to ketoprofen was highly statistically significant, and valid for all tested preparations of ox. linalool and ox. limonene. The strength of the reactions did not differ significantly between the ketoprofen-photoallergic and

dermatitis populations except for one concentration, ox. linalool 6%, which gave stronger reactions in patients with photocontact allergy to ketoprofen.

Both linalool and limonene belong to a large and structurally diverse group of terpenes. Terpenes share a common structural unit in isoprene (C_5H_8) [219]. Pure, non-oxidized limonene and linalool are considered weak allergens, rarely causing allergic contact reactions in the dermatitis population[220,221]. Both are subjected to an abiotic activation (i.e. autooxidation) on air exposure, and a range of moderately to strongly sensitizing primary oxidation products has been detected[222-224]. Unstable oxidation products may be formed as well but are difficult to detect and quantify. A number of secondary oxidation products have been described, comprising conjugated aldehydes and allylic epoxides, some of which are regarded as important sensitizers[159,225]. Both ox. limonene and ox. linalool contain sensitizing isomeric hydroperoxides[226,227](Figure 21).

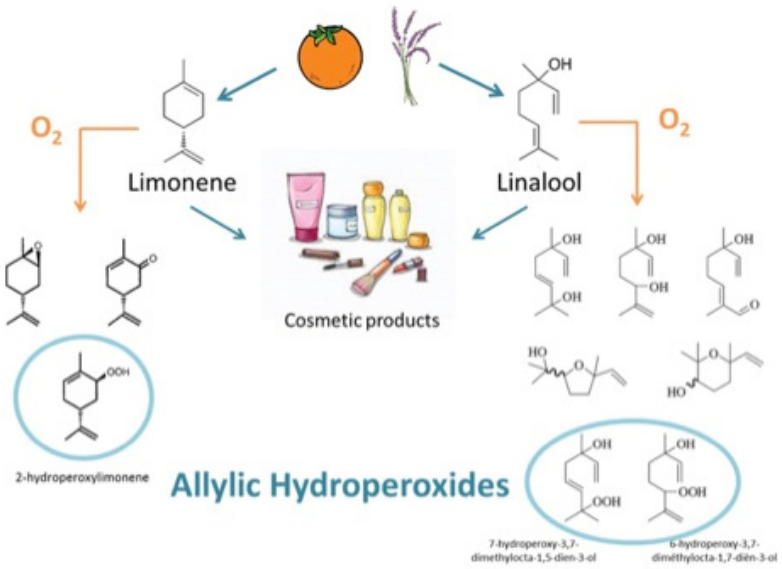


Figure 21. Limonene and linalool autoxidize on air exposure, forming highly allergenic compounds such as allylic hydroperoxides.

Illustration by Raffalli et al. Fragrance Allergens Linalool and Limonene Allylic Hydroperoxides in Skin Allergy: Mechanisms of Action Focusing on Transcription Factor Nrf2. Permission obtained from Oxford University Press. Licence number 5130700556465

Contact allergy to a chemically defined hydroperoxide is reported to be highly specific[222,223]. Individuals with photoallergy to ketoprofen present a higher rate of contact allergy to oxidized terpenes. Furthermore, concomitant positive PT reactions to both ox. terpenes are 2.5 times more common among those with photocontact allergy to ketoprofen. How can this be explained? Everyday exposure to fragrances is high, with even apparently non-scented products often containing some fragrance material[201].

Both linalool and limonene are reported to be among the most common fragrance ingredients used in consumer products, together with geraniol, citronellol, hexyl cinnamal, and hydroxyisohexyl 3-cyclohexene carboxaldehyde[219,228-231]. Furthermore, a German study identified exposure to limonene and linalool as the most common simultaneous exposure among fragrance chemicals[232]. As oxidation products are usually found in preparations with limonene and linalool, this could explain the high rates of concomitant reactions to ox. limonene and ox. linalool, but not the fact that the rates of contact allergy to these oxidized terpenes are significantly higher in the population with photocontact allergy to ketoprofen. One possible explanation could be a simultaneous exposure to ketoprofen and linalool and/or limonene. The singlet oxygen produced from excited ketoprofen may theoretically lead to a formation of autooxidation products, perhaps in higher concentration than usually present in the terpene-containing products.

A known simultaneous exposure to both ketoprofen and linalool is via Orudis® (Sanofi-Aventis AB, Bromma, Sweden), one of the most frequently used ketoprofen-containing products for topical use in Sweden. Lavender oil, which contains linalool[233,234], is one of its ingredients. However, when tested with lavender oil, patients with photocontact allergy to ketoprofen did not show positive PT reactions[144]. The probability of the photopatch tested lavender oil containing linalool hydroperoxides is high[226,235,236], which could explain some cases of co-sensitization. However, neither linalool nor limonene is present in the other two topical ketoprofen preparations distributed in Sweden (Siduro, Ipex Medical AB, Solna, Sweden; Zon®, Antula Healthcare AB, Stockholm, Sweden).

The reports on simultaneous contact allergy and photocontact allergy in ketoprofen-photoallergic patients show a clear overrepresentation of aromatic sensitizers (i.e. sensitizers possessing a benzene ring) such as benzophenones, cinnamal, and cinnamyl alcohol. Linalool and limonene, on the other hand, are terpenes and thus non-aromatic compounds. Furthermore, no aromatic compounds have been detected upon the oxidation process of linalool and limonene[222,223,237,238]. Whether such compounds are indeed being formed on autooxidation or perhaps due to enzymatic activation, but are not stable enough to be detected, is yet to be investigated.

Study IV

Study IV again found that the PT in patients with photocontact allergy to ketoprofen resulted in an overrepresentation of contact allergies to a number of contact sensitizers. Statistically significant overrepresentation was demonstrated for contact allergy to *Myroxylon pereirae* resin and FM I, which was expected based on previous reports[139,143,148,150,156,191,239]. However, the present investigation showed, with the same high statistical significance, that contact allergies to PFR-2, PTBP-F-R, and black rubber mix were also strongly overrepresented in the ketoprofen group. Moreover, we could see an overrepresentation of positive reactions to FM II and budesonide, even though the significance was somewhat lower for these two sensitizers.

Two separate groups were analysed in Study IV, based on the fact that the indications for PPT and the testing procedure had changed over the years. Between 1999 and 2008, virtually all patients photoallergic to ketoprofen were referred to our clinic on a direct suspicion of this particular photocontact allergy. Between 2009 and 2018, however, most patients with a suspected photodermatitis were tested with ketoprofen as a part of the photopatch test series. While in many cases the indication for this would include a suspicion of ketoprofen photoallergy, even more often it would include a suspicion of sunscreen allergy. Furthermore, the number of readings of PPT was different. Between 1999 and 2008, the results of PPT were read only on D3, while from 2009 to 2018 the majority of PPT results were read on D3 and D7 (personal communication with Magnus Bruze, 2021). This could theoretically mean that patients in the earlier group were those with stronger photocontact allergy to ketoprofen. In our experience, patients who are sensitized to ketoprofen tend to react already by D3, and we are not aware of any case of delayed PPT reaction to ketoprofen. The results of PT were rather consistent in both groups, showing statistical overrepresentation of the same sensitizers, seemingly independently of the indication for PPT with ketoprofen and the number of readings (Tables 25 and 26).

Except for budesonide, the patch test preparations that showed simultaneous contact allergies in Study IV were mixtures of several allergens (black rubber mix, FM I, and FM II), or were themselves complex mixtures (*Myroxylon pereirae* resin, PFR-2, PTBP-F-R). The simultaneous contact allergies are probably caused by one or more individual allergens in each of these test preparations, but our present knowledge of the chemicals in each mix does not allow us to draw any unambiguous conclusions about possible cross-reactivity with ketoprofen. Aromatic structures are present in all of the mixes, and propensity of simultaneous photocontact allergy to ketoprofen to coexist with contact or photocontact allergy to (some) aromates has been discussed earlier in this thesis.

There are no other obvious similarities between the allergens in ketoprofen and, for example, black rubber mix, apart from the fact that both ketoprofen and the

components in the mix are aromatic substances. The presence of benzene ring applies also to p-phenylenediamine, which the components of black rubber mix are derived from, but we did not see any overlap in terms of contact/photocontact allergy between p-phenylenediamine and ketoprofen (Tables 24–27). Similarly, the strongly statistically significant overrepresentation of contact allergy to budesonide did not influence the rate of contact allergy to tixocortol-17-pivalate in our ketoprofen-photoallergic cohort. It is worth asking whether or not the overrepresentation of contact allergy to certain sensitizers could be the result of simultaneous exposure.

Corticosteroids can be divided into five groups based on the so-called Coopman classification, proposed by Coopman et al. in 1989[240](Table 30).

Table 30. Classification of corticosteroids

Class	Examples	Notes
Group A	Tixocortol pivalate, hydrocortisone, prednisolone, methylprednisolone, prednisone	May cross react with group D2
Group B ('ides')	Budesonide, amcinonide, desonide, fluocinolone acetonide, fluocinonide, triamcinolone acetonide	
Group C	Betamethasone, dexamethasone, halomethasone	
Group D1 ('ates')	Betamethasone dipropionate, betamethasone 17-valerate, clobetasol propionate, fluticasone propionate, mometasone furoate	
Group D2	Hydrocortisone 17-butyrate, prednicarbate, methylprednisolone aceponate, hydrocortisone aceponate	May cross react with budesonide S-isomer and group A corticosteroids

Table by Schellenberg et al. Oral Corticosteroids in Asthma: A Review of Benefits and Risks
Canadian respiratory journal: journal of the Canadian Thoracic Society14 (suppl c):1C-16C. November 2007
DOI: 10.1155/2007/160691. License CC BY 4.0 (Creative Commons)

The majority of corticosteroids belonging to Group A is used as systemic formulations in Sweden, though hydrocortisone is used in a variety of over-the-counter topical formulations, either as a pure corticosteroid preparation or in combination with antimycotics.

In Group B, budesonide is used mainly for treatment of asthma and allergic rhinitis, in the form of inhalations or nasal sprays. Fluocinolone acetonide is used as eye drops but is also prescribed as a topical steroid preparation for treatment of inflammatory skin conditions. Triamcinolone acetonide is prescribed as part of a combination preparation with corticosteroid and antimycotics.

The most common topical steroids prescribed by physicians used for treatment of skin conditions in Sweden include bethametasone, mometasone furoate, clobetasone propionate, and clobetasone butyrate. These belong mainly to Group C and D1.

Considering that both Group A (tixocortol-17-pivalate as a marker at PT) and Group B (budesonide as a marker at PT) are represented on the Swedish market as treatment options for inflammatory skin conditions, is difficult to evaluate their exposure without a study specifically designed for this purpose. It is, however, possible that patients with strong photoallergic contact reactions to ketoprofen would need a more potent topical corticosteroid than those sold over the counter, and thus would be exposed to corticosteroids belonging to Groups C, D, and perhaps B.

The high rates of simultaneous contact allergy to PFR-2 and PTBP-F-R are difficult to explain as being dependent on exposure. Contact allergy to both phenol formaldehyde resins is uncommon in the dermatitis population, and sensitization appears to mainly be of an occupational nature, occurring in cobblers, car manufacturers, and those involved in the production of materials such as laminate, wood glue and varnish, casting sand, or mineral wool[241]. Some non-occupational sensitization may occur upon contact with adhesives that contain the resin but considering that the estimated prevalence of contact allergy in our dermatitis population was around 1% for PTBP-F-R and 0.9% for PFR-2, these sensitizers are not likely to be a common source of non-occupational sensitization. It is therefore very difficult to explain the high rates of contact allergy to PTBP-F-R (11.4%; 9.8–16.7%) and even more strikingly to PFR-2 (32.9%; 24.4–46.7%) in patients with photocontact allergy to ketoprofen. These simultaneous contact allergies are probably caused by one or more allergens in each of these test preparations. Photoinduced transformation of ketoprofen may result in the formation of allergens with a chemical structure similar to that of the allergens responsible for simultaneous contact allergies.

As shown in our previous work, contact allergy to some of the components of FM I is overrepresented in patients with photocontact allergy to ketoprofen. *Evernia prunastri* is one of the main sensitizers in FM I [195]. The *Evernia prunastri* used for testing is a lichen extract containing several substances, such as evernic acid, atranorin, atranol and chloroatranol among others. Both atranol and chloroatranol, considered strong allergens[90,242], possess a benzene ring, thus displaying structural similarities with many other constituents of FM I.

Our present knowledge about the allergens, present in black rubber mix and fragrance mixes, and about the main allergens in *Myroxylon pereirae* resin, PFR-2, and PTBP-F-R, does not provide us with an obvious common chemical structure for either these sensitizers or for possible metabolites. Instead, the differences seem to be greater than the similarities.

Not enough data are available to hypothesize the relevance of these simultaneous contact reactions. Further investigations are needed in order to assess the possible impact these allergies may have on the quality of life in the sensitized individuals. However, based on the results of Study IV, we are convinced that patients with a positive ketoprofen photopatch test need to be not only photopatch tested with the photopatch test series, but also patch tested with the baseline patch test series, including the sensitizers presented in this study.

General aspects

This thesis is based on 4 studies, all including individuals with photocontact allergy to ketoprofen and various control groups. One study is a clinical trial, while 3 studies are retrospective, epidemiological studies. Like virtually any study, these studies have limitations and strengths.

Limitations

The data on the medical history of the participants were not consistently available in the register. However, virtually all individuals with photocontact allergy have had dermatitis with present or past clinical relevance. On the other hand, it is impossible to draw definite conclusions on possible relevant co-existing factors.
The sample size of our ketoprofen-photoallergic cohort was considerably smaller than that of the dermatitis cohort. The necessity of e.g. the Bonferroni correction was briefly considered, but rejected because the significance level of our main findings was considered sufficient. The possibility of some additional statistically significant associations may therefore exist.

When comparing different groups of volunteers/patients statistically significant differences may be identified. Such differences were found in Studies II, III, and IV. In this situation it is customary to look for confounding factors. Age, gender, and atopic dermatitis/atopic constitution are the most common confounding factors in this type of studies. The higher age the more contact allergy is detected. This has been reported for most sensitizers including fragrance materials, both from the Malmö department [243] and other clinics [192]. In the three retrospective studies (II, III, and IV), the mean ages of the patients with photocontact allergy to ketoprofen were similar to the mean ages of the respective control material of patch tested dermatitis patients but lower than the mean age of the controls from the general population. Contact allergy to most sensitizers including fragrances is more common in females than in males, which has been reported from the Malmö department and other

clinics[192,243]. There were similar gender distributions in the 3 studies among the patients with photocontact allergy to ketoprofen as to the dermatitis population used for the comparison in the respective study. In the controls from the general population the gender distribution was virtually equal. Atopic dermatitis, finally, was less common in the patients with photocontact allergy to ketoprofen as to the various control materials from the dermatitis population in the three studies.

Considering all the data above concerning mean ages, gender distribution and presence of atopic dermatitis in the ketoprofen patients and dermatitis patients, it seems highly unlikely that a multivariable analysis would have resulted in statistically weaker associations. The same conclusion can be drawn concerning the patients with photocontact allergy to ketoprofen and the controls from the general population. The mean age and the percentage of males were higher in the general population.

Strengths

One of the main strengths of Studies II, III and IV is the size of the control groups and the possibility to explore relationships between large numbers of tested substances over long periods of time. Another strength is the fact that photopatch testing is readily available in our clinic, and that most patients with a suspected photodermatitis are subjected to testing with the photopatch test series; moreover, many of these are also patch tested with the baseline series as a part of the investigative procedure.

The fragrance materials patch tested as FM I and FM II as well as individually in Studies III and IV in the patients with photocontact allergy to ketoprofen and in the dermatitis patients originated from one and the same batch for each fragrance material. These batches were also used for the patch testing in the general population.

Both patch testing and photopatch testing were performed by experienced technicians and assistant nurses. Defined amounts of petrolatum test preparations (40 mg/cm^2) were applied on the test chambers.
All patch and photopatch test readings were done by experienced dermatologists.

Main hypotheses to explain concomitant contact allergies in individuals with photocontact allergy to ketoprofen

- *Cross-reactivity.* Photosensitizing properties of ketoprofen are thought to be mediated by the presence of a diaryl ketone chromophore [244], which is also present in other benzophenone-derivatives. This could possibly explain the simultaneous photocontact reactions to the benzophenones, but possibly also to other aromates. However, not all sensitizers overrepresented on PT during our research possess a benzene ring. For instance, ox. limonene and ox. linalool are terpenes, thus being chemically non-related to ketoprofen. To date, there are no known mutual haptens involved in sensitization

towards ketoprofen and these oxidized terpenes. The residues of unsubstituted benzophenone har recently been found in raw octocrylene material as well as 28 octocrylene-containing products [154]. Cross-reactivity due to benzophenone residues is therefore a reasonable explanation to the prevalence of simultaneous photocontact allergic reactions to ketoprofen and octocrylene.

- *Simultaneous exposure*
- *Oxidative transformation* of chemically non-related substances in the presence of ketoprofen
- The role of *skin enzymes* in the formation of allergens.
- *Ex vivo/in vivo formation of photoadducts.*

In conclusion, this thesis presents a large retrospective analysis of the prevalence of simultaneous contact allergies in individuals photosensitized to ketoprofen. We do not have a definite explanation for this phenomenon. In many cases, the mechanism of simultaneous contact allergy remains unclear, and most possible explanations are of a speculative nature. This material presents a solid basis for future investigations of the phenomenon of simultaneous allergic reactions, and we sincerely hope that many research groups will find it helpful in their quest for the answers.

The future

Two clinical trials have not made it into this thesis. Although they were approved by the Regional Ethical Review Board and are currently ongoing, they have remained underpowered due to the COVID-19 pandemic restrictions. The aim of these studies is to explore the possible reverse relationship between contact allergy to some substances and photocontact allergy to ketoprofen. Patients with known positive patch test reactions to FM I, *Myroxylone pereirae*, PTBP-F-R or PFR-2, but without a history of photodermatitis and/or use of topical ketoprofen preparations, are being photopatch tested with ketoprofen in serial dilutions in order to estimate whether any simultaneous reaction occurs. At the same time, we aim to gain a broader knowledge of the exact components of some complex allergen mixes, responsible for contact allergy, by patch testing each patient with the series of allergens, known to be present in the mix/complete mixture the patient has a known contact allergy to. Currently, our biggest wish is for the end of the global pandemic that has caused so much death and suffering. Afterwards, we look forward to completing the two studies and sharing the knowledge obtained from them.

Apart from clinical trials and epidemiological studies, much has been done to understand the chemistry and immunology behind the phenomenon of simultaneous

contact allergies. However, much future work remains. Some ideas for future research include:

- Clinical trials directed towards the identification of possible reverse relationships between simultaneous photocontact allergy to ketoprofen and contact allergy to other sensitizers identified as being overrepresented in those with photocontact allergy to ketoprofen. Two such studies are currently being conducted by our research group.
- A prospective longitudinal epidemiological study on the rates of sensitization, paired with user profiles and current market availability of the respective sensitizers.
- Research into the clinical relevance of the sensitizers identified as being responsible for high rates of simultaneous contact allergy in those with photocontact allergy to ketoprofen. Most of the contact sensitizers, which were overrepresented in our findings are common, and the risk of clinically relevant contact sensitization to these would be significantly higher in the group with photocontact allergy to ketoprofen, warranting special attention.
- Investigation of the autooxidation, enzymatic transformation, and possible metabolic pathways of ketoprofen and the simultaneously overrepresented contact sensitizers.
- *In vivo* chemical study of the phototransformation of ketoprofen in human skin during an active PhACD, which can be simulated with PPT.

Clinical implications today

Since we started to focus on photocontact allergy to ketoprofen in 2005, our research group has gained a lot of experience and knowledge in the area. Most of the results of the investigations have been or soon will be published in the literature [144,245-248], but there is also ongoing research as mentioned in the previous section (The future). However, our experience and results of the investigations presented in this thesis demonstrate that there are things that should be implemented in the clinics already today.

- Dermatologists should perform PPT more frequently than what is done today. There are three major clinical situations when this testing should be performed. (i) When an eczematous dermatitis is located on UV-exposed areas and there is systemic exposure to possible, photosensitive drugs/chemicals/food items; (ii) When there is neither history, or signs of an endogenous dermatitis, nor any relevant contact allergies detected at patch testing, but an eczematous dermatitis located to areas such as the face and neck which are "constantly" exposed to UV radiation; (iii) When a

patch test is negative to a topically applied product (including ingredients) strongly suspected to be the cause of the dermatitis on a skin area that can be UV-irradiated.

- Whenever a photocontact allergy has been identified at photopatch testing, additional testing should be performed with a baseline patch test series.
- When contact allergy has been established at patch testing with any of following: fragrance mix I, *Myroxolon pereirae*, cinnamic alcohol, cinnamal, eugenol, isoeugenol, oxidized limonene, oxidized linalool, black rubber mix, budesonide, para-tertiary butylphenol-formaldehyde resin, and phenol-formaldehyde resin, additional photopatch testing with the European photopatch test series should be considered, particularly when no clinical relevance can be associated with these strongly over-represented contact allergies in individuals with photocontact allergy to ketoprofen.

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