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CONTACT ALLERGY TO COLOPHONY

Clinical and Experimental Studies with Emphasis on Clinical Relevance

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III. Färm G. Contact allergy to colophony and hand eczema. A follow-up study of patients with previously diagnosed contact allergy to colophony. Contact Dermatitis 1996; 34: 93-100.

IV. Gäfvert E, Färm G. Rosin (colophony) and zinc oxide in adhesive bandages. An appropriate combination for rosin-sensitive patients? Contact Dermatitis 1995; 33: 396-400.

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VI. Färm G. Repeated open application tests in patients allergic to colophony - evaluated visually and with bioengineering techniques. Acta Derm Venereol (Stockh) 1998; 78: 130-135.

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ABSTRACT

Colophony - also called rosin - is a material obtained from coniferous trees. It is used widely in many products, particularly because of its good tackifying properties. Colophony is also used in paper sizing to increase water resistance.

Colophony may cause contact allergy, and around 5% of Swedish dermatitis patients show allergic reactions to colophony at patch testing. There are many case reports of colophony in different products causing contact dermatitis. Often, however, the clinical relevance of a positive patch-test reaction to colophony is difficult to evaluate.

The principal aims of the present thesis were to study the prevalence of contact allergy to colophony and of skin disease in individuals with an occupational exposure to colophony; to study the prognosis of dermatitis in colophony-sensitive subjects, and to investigate the outcome of repeated open applications of colophony, thereby trying to elucidate the clinical relevance of contact allergy to colophony.

Employees of a tall-oil rosin (colophony) factory (*n=180*), and of an opera company where colophony was used in dancers' rosin, mascara and wig glues (*n=132*), were interviewed, examined and patch tested. 3.9% and 2% of these two groups respectively had a positive patch test to colophony. More than every fourth participant showed some kind of skin disease, but only few cases were related to work.

Eighty-three patients with previously diagnosed contact allergy to colophony were followed-up. 72% showed a positive patch-test reaction to colophony at re-testing. Around one third had hand eczema. There was no significant correlation between colophony exposure and current hand eczema.

Adhesive bandages containing colophony and zinc oxide (ZnO), colophony and mixes of colophony and ZnO, were tested in 7 colophony-sensitive subjects to see whether addition of zinc oxide inhibited elicitation of allergic dermatitis to colophony, which has been proposed. No difference in reactivity between colophony and colophony/ZnO was seen at patch testing, and there were positive patch-test reactions to all colophony-containing bandages. Thus no inhibitory effect of ZnO was shown.

Repeated open application tests were performed with cobalt chloride and colophony in sensitized guinea pigs. The animals were also patch-tested. A dose-response correlation was found with both cobalt chloride and colophony. There was a concordance between patch-test reactions and reactions at repeated open application tests, the higher the concentration of the allergen at the open test the stronger the concordance.

In 13 colophony-sensitive subjects serial dilution patch tests with colophony were performed followed by repeated open application tests using colophony of different concentrations once daily for two weeks. Reactions were assessed visually, by laser Doppler flowmetry and by measurements of transepidermal water loss. Ten subjects reacted at open applications with colophony 20%. The strength of the reaction varied greatly. A correlation between the threshold concentration at patch testing and the outcome of the repeated open application tests was found and also a dose-response relationship. Nine healthy controls did not react to repeated open applications with colophony. The measurements of transepidermal water loss and bloodflow was of no additional use to visual assessment when evaluating repeated open application test reactions with colophony.

INTRODUCTION

That some substances can cause dermatitis when the skin is exposed, has long been known anecdotally (1). Some 200 years ago the etiology of dermatitis was already being discussed, but not until the past few decades have we understood some of the underlying mechanisms of contact allergy (2). Jadahsson, a hundred years ago, presented the first "patch test" for investigating contact dermatitis, although he himself did not call it patch testing at the time (3). As colophony is a material that has been used since ancient times, skin problems caused by colophony must most probably have been known for a long time too.

Colophony, what is it?

Colophony is a naturally occurring material, obtained from different species of coniferous tree (family Pinaceae). There are two major ways of producing colophony. The most common, world-wide, is by processing it from oleoresins (exudates) tapped from living trees - gum rosin. The other way is by distillation of tall oil, a byproduct of the paper pulp industry - tall-oil rosin. In this thesis the term "colophony" will be used for both types when not specified, although in some literature, particularly American, the term "rosin" is generally used.

China, Latin American countries and Portugal are great producers of colophony of the gum rosin type. No gum rosin is produced in Sweden or the other Scandinavian countries. Sweden, however, is a great producer of tall-oil rosin, as are e.g. Finland, the USA and countries of the former USSR (4-7).

Chemical composition

Colophony is a complex mixture of resin acids (about 90%) and neutral substances (10%). Its composition varies with the species from which it is obtained and also depending on recovery processes and storage. The acids - both in gum rosin and tall-oil rosin - are dipertenoid acids mainly of two types, abietic (Fig 1) and pimaric. Tall-oil rosin contains less abietic acid and more dehydroabietic acid than gum rosin does. It also contains some modified resin acids. The neutral substances of gum rosin consist mainly of aldehydes and alcohols of the corresponding resin acids while the neutral substances of tall-oil rosin consist mainly of fatty esters and hydrocarbons formed from sterols. Colophony is easily oxidized by air. Abietic acid is more susceptible to oxidation than dehydroabietic acid is. In technical products oxidation is usually not desired since it makes colophony brittle and dark in colour. Today most colophony is, for technical reasons, modified in different ways through reaction with various chemicals (5-8).

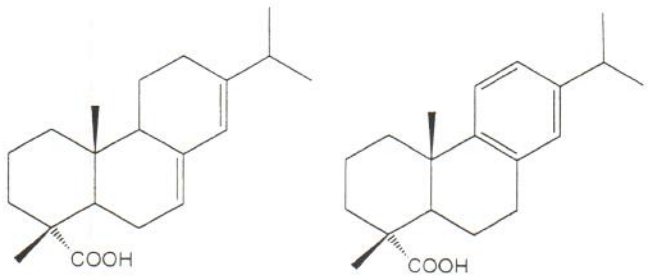

Fig 1. Abietic acid (left) and dehydroabietic acid (right).

Use

The total world-wide production of colophony is about one million tons per year. Around one third goes to the paper industry, where it is used to increase the water resistance of paper (paper size). Another third is used for production of adhesives and printing ink and the rest for other purposes (4). Colophony has three main technical properties; it has good tackifying qualities, it can be used as an emulsifier and it has acid properties without causing corrosion. It is therefore used in a vast number of different products (4-7, 9-12). Gum rosin and tall-oil rosin are often interchangeable, despite the difference in composition (4-6).

In Sweden colophony is found in about 170 different products - 45 for consumer use, apart from cosmetics (13). The extent to which it is used in cosmetics is not known since, so far, cosmetics are very seldom labelled with ingredients. Some common products generally containing colophony are listed in Table 1. Paper may contain added modified colophony in order to enhance its quality. In paper made from mechanical pulp colophony components are incorporated depending on the production process (14).

The amount of unmodified colophony in different products varies - from 20% or more in some adhesives, paints and soldering fluxes for electronic assemblies to small traces in products containing mainly modified colophony (13, 15). The amounts are seldom declared on the package. However, all products in the EU containing 1% colophony or more should now be labelled with an allergy warning (16).

Table 1. Some common products which often contain colophony (5, 9, 10).

plaster and adhesive bandages	polishes
mascara	soldering fluxes
depilatory waxes	insulating tapes
some dental materials	paints and lacquers
glues, adhesives and tapes	cooling fluids
wood and gum from pine trees	printing inks
dancers' and string players' rosin	paper

Allergenicity

Allergic contact dermatitis from colophony in adhesives has been known for more than 50 years (9, 17) and colophony was first used in a standard series of patch testing for contact allergy in 1939 (18).

The main allergenic compounds in colophony are oxidized acids of the abietic type (19-23). Tall-oil rosin has a lower allergenic activity than colophony of the gum rosin type (20, 24-25), partly due to a lower content of abietic acid (20). With modification the allergenicity is often altered and new allergens may develop (25-32). There is, however, some unmodified colophony left in the final product (30). The allergenicity of colophony and of the different constituents of unmodified and modified colophony has been thoroughly studied by Karlberg, Gäfvert and coworkers (19-20, 24, 26, 28-40) and by Hausen et al. (21-22, 25, 27, 41-43) but others have also studied the allergenicity of unmodified colophony (23, 44).

It has been suggested that zinc oxide may have an inhibitory effect on the elicitation of allergic contact dermatitis due to colophony in colophony-sensitive subjects. One hypothesis is that this could be due to the formation of salts, so-called zinc resinates (45).

Methods for detecting colophony components

Components of colophony may be detected by gas chromatography or high-performance liquid chromatography (HPLC) (46-47). Ehrin and Karlberg have developed an HPLC method detecting abietic and dehydroabietic acid in different products (15). The method can detect abietic acid in concentrations of 0.001%, which corresponds to an amount of unmodified colophony (gum rosin) of about 0.003%.

Contact allergy to colophony

Prevalence

The prevalence of contact allergy to colophony in the general Swedish population is not known. In an Italian patch-test study of 539 healthy young men, only one showed a positive test reaction to colophony (48). In a Danish study, 0.7% of a general population of 576 individuals 15-69 years old were patch-test positive to colophony (49). The corresponding figures of that study for nickel sulphate were 6.7% and for fragrance mix and balsam of Peru 1.1% each.

The prevalence of positive patch-test reactions to colophony among dermatitis patients varies in different reports from around 2 to 6% (50-55). In the reports differentiating between men and women there is generally a female dominance. In a Swedish multicentre study of 3690 dermatitis patients patch-tested between September 1991 and February 1993, the prevalence of positive patch-test reactions to colophony was 5.2% (5.9% among the women and 4.1% among the men) (personal communication, Prof. T. Fischer, National Institute for Working Life, Solna, Sweden). A positive patch-test reaction to colophony is often accompanied by concomitant reactions, particularly to balsam of Peru and fragrance mix (24, 56-57).

Sources of sensitization

Adhesive plasters and bandages have probably been an important cause of contact sensitization. Nowadays colophony in adhesives is often - at least to some degree - replaced by acrylates (17). Since colophony is present in many products to which skin exposure is inevitable (Table 1) probably many different products are responsible for sensitization.

Case reports

There are numerous reports on allergic contact dermatitis caused by colophony in various products. Most are, however, case reports concerning one or a few cases only and little is known of how often contact allergy to colophony influences e.g. the health in different occupations.

Contact dermatitis from occupational exposure to colophony

Colophony may be encountered in many different jobs (58). In the electronics industry contact allergy to colophony in soldering flux is well-known (59-62). Not infrequently the dermatitis is due to airborne exposure and located to the face (59).

Since many paints, lacquers and glues contain colophony, employees in the paint industry may suffer from dermatitis due to colophony allergy. Reports of contact allergy to these products are sparse, though (63). Employees in the manufacturing industry may experience dermatitis due to colophony-containing cooling fluids (64-65). Grattan et al. reported a higher frequency of positive patch-test reactions to colophony in males with a suspected cutting-fluid dermatitis than among subjects tested for non-occupational eczema (66). Insulating tapes may cause occupational contact dermatitis due to colophony in the tape (67). Printing ink contains resins of different kinds, sometimes also modified colophony. Abieto-formo-phenolic resin in printing ink is reported to have caused allergic contact dermatitis in a newspaper man (68).

Paper pulp may contain colophony, and colophony in paper has been reported to cause - or worsen - contact dermatitis due to colophony (14, 69-71). Only one report on occupational dermatitis in the paper industry was found (72). It showed that two subjects (of 274 workers) had positive patch-test reactions to paper size - containing unmodified and modified colophony - but none was positive to colophony from the standard series.

Individuals occupied in different forms of woodwork are exposed to colophony in pine sawdust and may develop hand dermatitis as well as dermatitis due to airborne allergen exposure (73), a problem sometimes met also in individuals using sawdust and wood wool for other purposes (74-76). String players' rosin consists of unmodified colophony, and contact allergy to colophony in string players has been known for 60 years or more (77-78). Dancers also use unmodified colophony to avoid slipping on the floor – dancers' rosin. Searches revealed no reports of contact dermatitis to this rosin in dancers, but one in a dancer's masseur (79).

Other causes of allergic contact dermatitis from colophony

Colophony and derivatives are important ingredients in many medical plasters and adhesives and such adhesives might cause contact allergy to colophony and allergic contact dermatitis (10, 80-82). Other products used for skin treatment may also contain colophony in amounts large enough to cause allergic contact dermatitis, at least in individuals previously sensitized, e.g. hydrocolloid dressings (83) wart removers (84-87), Bavarian tiger balm (88), and traditional Chinese medicine materials (89-90).

Depilatory waxes have been reported as a cause of allergic contact dermatitis from colophony (91). Cosmetics sometimes contain colophony and there are reports of eye shadows (92), rouge (93), lip preparations (94-95) and mascara (96-98) causing colophony contact dermatitis. Bindi are applied by Indian women to the forehead. Recently the adhesive of bindi has been reported to cause allergic contact dermatitis due to colophony derivatives (99).

Shoe dermatitis can be caused by colophony in glues (100-101).

Some garden pine trees may cause contact dermatitis in colophony-sensitive gardeners due to some allergenic material in common with colophony (102-104).

Other less common causes of allergic contact dermatitis from colophony are chewing gum (105), banknote paper (106) and paper-based surgical clothing (107). Allergic contact dermatitis on hands and face has been reported from colophony added to beeswax (108) and

from spectacle frame polish (109). Airborne contact dermatitis from colophony in a linoleum floor, floor polish and paper dust has recently been reported (110).

Some dental materials contain colophony and have reportedly caused stomatitis (111) and even lichen planus in the mouth (112).

Contact allergy to colophony and light reactions
Many patients with chronic actinic dermatitis show contact allergy, particularly to substances originating from plants (sesquiterpenes, fragrances, balsam of Peru and colophony) (113-114). Contact allergy is mostly diagnosed prior to light sensitivity. The significance of the association between chronic actinic dermatitis and contact sensitivity is unknown (114).

Other health effects of colophony

Colophony causes occupational asthma (115-116). Whether this is due to a type I allergy or to irritation of the respiratory tract is not fully investigated (116). Colophony may also cause urticaria (117).

Diagnostic testing in contact allergy

Patch testing

Contact allergy is generally diagnosed by patch testing. At patch testing, test substances are applied on the skin of the back of the patient, under occlusion in patches, generally for 48 hours (118). The patch-test sites are "read" - the skin is examined for eczematous reactions - generally after 2-4 days. The time of reading varies, by tradition, in different countries. In Sweden the tests have generally been read three days after application. It has been shown, though, that positive patch-test reactions might be missed if tests are read after 3 days only (119-121) and there is now a recommendation from the Swedish Contact Dermatitis Research Group to evaluate patch test results twice (personal communication, Prof. JE Wahlberg).

At patch testing with colophony the gum rosin type is generally used. The patch-test concentration has varied between 10 and 60% over the years (18, 34, 122-123), but for the past 10 years, 20% in petrolatum has been the standard for patch testing in Sweden. It has been suggested to test also with tall-oil rosin, isolated colophony allergens and modified colophony when contact allergy to colophony is suspected and a standard patch test is negative (39).

Contact allergy, once induced, is often considered to be life-long (124). There are however studies that show loss of patch-test reactions in individuals re-tested after some years (125-131).

Open tests/Use tests

Open tests, i.e. tests where the allergen is applied to the skin without occlusion, are mostly used for confirmation of a positive patch test to ingredients in a consumer's product. Hannuksela and Salo introduced the Repeated Open Application Test (ROAT), an open test where the allergen is applied to the same skin site twice daily for seven days (132).

A use test, like the ROAT, often imitates the everyday exposure to an allergen better than the patch test does. So far the ROAT has been performed mostly with formulated products, but recently studies where ROATs have been performed also with allergens have been published (133-134).

Assessment of patch-test reactions

In clinical practice the patch test reaction is assessed visually and with the fingertips only. According to international recommendations (118) a patch test is judged positive when there is erythema and infiltration. Since a weak erythema may be difficult to observe, particularly in dark skin, and infiltration may be judged differently by different investigators, objective methods for assessing patch-test reactions would be desirable. Several objective, non-invasive methods have been developed during recent years e.g: measurement of skin capacitance, skin conductance and transepidermal water loss (TEWL). The skin's surface colour can also be quantified and skin thickness may be measured with high frequency ultrasound (135). Skin blood flow can be quantified with laser Doppler flowmetry (135, 136). Recently skin impedance has been used in some experimental studies (137) and laser Doppler perfusion imaging has been used in evaluating skin irritation as well as contact sensitization (138). So far these bioengineering methods have been used mostly in experimental studies concerning skin irritation, but sometimes also in assessing allergic patch test reactions (139-142). Only those methods used in the studies forming this thesis will be further presented here.

Laser Doppler flowmetry

Laser Doppler flowmetry is based on the Doppler principle that moving red blood cells, when hit by a light of a certain frequency, will reflect it with a shifted frequency. The instrument used for flowmetry will give relative, dimensionless values of the blood flow, which will increase with increased microcirculation in the skin (143). An allergic patch-test reaction causes inflammation in the skin, which leads to enhanced microcirculation that can thus be measured with this method. The instrument may be more sensitive than the naked eye (144). Guidelines for laser Doppler flowmetry have been presented by the Standardization group of the European Society of Contact Dermatitis (ESCD) (145).

Measuring transepidermal water loss (TEWL)

TEWL refers to the total amount of water vapour loss through the skin, including sweat. Where sweating is kept low, the water loss reflects the barrier function of the epidermis. TEWL is measured using an evaporimeter, which estimates the water vapour pressure immediately above the surface of the skin (146). The method is sensitive to changes in the environment and subject, and guidelines from the Stadardization group of the ESCD have been published (147). Skin involved in dermatitis will show a disturbed barrier function.

Testing in experimental animals

Guinea pigs are the experimental animals of choice, when studying contact allergy. Generally guinea pig studies are performed to evaluate whether a substance has allergenic properties. There are several methods of sensitizing guinea pigs. Two are the guinea pig maximization test (GPMT) (148), and Freund's complete adjuvant test (FCAT) (149). The animals are sensitized with intradermal injections of the allergen with addition of Freund's complete adjuvant to enhance the response and also in the GPMT by topical application of the allergen.

The animals are patch tested with the allergen after three weeks - the challenge - to see how many animals have been sensitized compared to a sham-treated control group.

In a recent study (150), guinea pigs were sensitized with cobalt chloride, a known allergenic substance, and challenge was done not only with patch testing but also with repeated open application tests (ROATs), where the allergen was applied to the skin once daily for seven days. It proved possible to perform ROATs in guinea pigs, and there was a dose-response relationship at the ROATs.

AIMS OF THE STUDY

The overall aim of the present thesis was to elucidate the clinical relevance of contact allergy to colophony by:

studying the prevalance of contact allergy to colophony and skin disease in individuals with occupational exposure to colophony;

studying the prognosis of dermatitis, particularly hand eczema, in individuals with contact allergy to colophony;

studying whether the addition of zinc oxide to colophony has an inhibitory effect on the elicitation of allergic contact dermatitis;

developing further an open test method for contact allergy which reflects better than the patch test the clinical relevance of contact allergy and

comparing the results of colophony patch tests with the outcome of an open test, using colophony.

SUBJECTS, MATERIALS AND METHODS

Subjects (I, II, III, IV, VI)

(I). One hundred and eighty individuals - 134 men and 46 women - participated in the study. These included 163 of all 180 current employees (91%) plus 17 of 35 invited former employees, of the only Swedish factory for production of colophony (tall-oil rosin). About 1/4 of the subjects were employed in administrative work, while the others, to some degree, were exposed to the tall-oil rosin produced. Half of the participants had been with the company 10 years or more.

(II). One hundred and thirty-two individuals - 79 singers, 37 dancers and 16 make-up artists (80%, 52% and 100% respectively of total in each working group, 76 women, 56 men, mean age 38) - of an opera company participated. Mean time in the job was 14 years.

(III). In this study, 83 individuals took part (64 women, 19 men, mean age 48). All of them had shown a positive patch-test reaction to colophony at patch testing at the Karolinska Hospital, 9-13 years earlier. The participants were still living in Stockholm and were not more than 65 years old at the time of the present investigation. 103 subjects were identified, 20 refused participation. The reason for previous patch testing was hand dermatitis in 64 of the 83 subjects.

(IV). Seven individuals, previous patients at the Department of Occupational Dermatology, Karolinska Hospital, with known contact allergy to colophony, were patch tested with colophony in different preparations in this experimental study.

(VI). In this study 13 colophony-sensitive individuals and 9 healthy volunteers, as controls, were included. The colophony-sensitive subjects were chosen among previous patients at the Department of Occupational Dermatology at Karolinska Hospital, who during the previous 6 years had shown a positive patch-test reaction to colophony. Most controls were recruited among hospital staff and chosen to match the colophony-sensitive individuals in gender and age. The participants had no personal history of atopic dermatitis.

Interview and clinical examination (I, II, III)

Interviews concerning history of skin disease, type of work, etc were performed with the help of questionnaires. In one study (I) the questionnaire was sent to the subjects prior to the interview. The skin of the hands, arms, face, chest and back, as well as other parts of the body, if affected by skin disease, was examined for dermatitis or other skin disease.

(III). Hand dermatitis, present at the clinical examination, was evaluated according to a scoring system in which fingers, palms, backs of the hands and wrists were examined separately. Each of these 4 sites scored 1, if there were only patches of dry skin with possibly 1 or 2 fissures. If most of the examined part was involved with dry skin, a score of 2 was given. When there were also other clinical signs of eczema, scores were doubled to 2 and 4 respectively, depending on the extent of involvement. The scores were then summed with a possible maximum of 16. The participants were divided into groups by score: (i) no visible

hand dermatitis; (ii) mild hand dermatitis, score 1-4; (iii) moderate hand dermatitis, score 5-8 and (iv) severe hand dermatitis, score ≥ 9.

All interviews and clinical examinations were performed by the author.

Patch testing in humans (I, II, III, IV, VI)

Patch testing in humans was performed by the internationally accepted method (118) using Finn Chambers® (Epitest Ltd Oy, Finland) and Scanpor® tape (Norgesplaster A/S, Norway). Application time was 2 days and reading was done 3 days after application. Criteria for a + reaction were redness and infiltration, for a ++ reaction redness, infiltration and papules while a +++ reaction also included vesicles.

Test substances

Standard allergens (I, II, III)

Slightly modified European standard series (Chemotechnique Diagnostics, Malmö, Sweden) including colophony 20% in petrolatum (pet.), were used.

Additional test substances (I, II, IV, VI)

Portuguese gum rosin (colophony) used in the different studies was obtained from Socer, Lisbon, Portugal.

(I). Portuguese gum rosin, tall-oil rosin (Beviros 90®), glycerol-esterified tall-oil rosin, pentaerythritol-esterified tall-oil rosin, maleic-modified rosin esterified with glycerol and polymerized tall-oil rosin (Bergvik Kemi AB, Sandarne, Sweden) were all used in tests 20% in pet. Two main components of modified rosin - maleopimaric acid and glyceryl triabietate - were synthesized in our laboratory and tested 0.6% in pet. and 8% in pet., respectively.

(II). All participants were tested with dancers' rosin (20% in pet.) and with 20 different products - adhesives for wigs and eyelashes, facial and eye makeup, powder, lip gloss - regularly used for makeup at the time of the investigation. Mascara was tested 75% in pet. and a black eye makeup 10% in pet. The other products were tested "as is". Make-up artists were tested with 5 additional hair care products "as is". The test material was obtained from the opera company.

(I, II). Test substances or products where the irritant potential was not known were tested in healthy controls prior to the study.

(IV). Portuguese gum rosin (colophony) was methyl-esterified. (Previous studies have shown that there was no significant difference in the eliciting capacity of colophony components with methyl-esterification (35-36)). Six different commercial adhesive bandages declared to contain colophony and zinc oxide (ZnO) and one bandage declared not to contain colophony (as control), were bought from the local pharmacy. Patch testing was performed with unmodified gum rosin (10% in pet.), a mixture of unmodified gum rosin plus ZnO (10%+10% in pet.), methyl-esterified gum rosin (10% pet.) and a mixture of methyl-esterified gum rosin plus ZnO (10%+10% in pet.) (Table 6). Pieces of bandage, 1 cm^2, were tested "as is". Acetone extracts of the bandages were also tested.

(VI). Colophony-sensitive subjects were patch tested with a serial dilution of colophony (Portuguese gum rosin) 20%, 10%, 1%, 0.1% and 0.01% in a vehicle of acetone/arachis oil 1:1 (w/w). They were also tested with the vehicle. Controls were tested with colophony 20% in pet. (Chemotechnique Diagnostics, Malmö, Sweden). No filter paper discs were used in the Finn Chambers to avoid the risk of colophony adhering to the filter paper (14). Test substances were applied in a random order and read blindly.

Acetone/arachis oil 1:1 (w/w) was chosen as vehicle after clinical trials with several mixtures of oils and solvents. It was tested in healthy controls prior to the study and no reactions were found.

Induction of contact allergy in guinea pigs (V)

The experiments were carried out in female albino Dunkin Hartley guinea pigs (Sahlin, Malmö, Sweden). Their average weight was 300 g when induction began.

The guinea pig maximization test (GPMT) method (148) was used to induce contact allergy to cobalt chloride ($CoCl_2$). $CoCl_2$ 1% (w/w) in distilled water and in Freund's complete adjuvant (FCA) was used for intradermal induction on day 0 and the slightly irritant concentration 5% in pet. for topical induction on day 7. The control animals were treated in the same way (FCA, pet., occlusion with Elastoplast, etc.) except that the allergen was omitted.

To induce allergy to colophony, the Freund's complete adjuvant test (FCAT) was used (149). 5% colophony (w/w) in FCA was used for induction (3 injections) while the control animals received 3 injections with FCA without colophony.

Patch testing in guinea pigs (V)

Guinea pigs were patch tested using Finn chambers (diameter 8 mm, Epitest Ltd, Finland) on the clipped flanks, according to the original protocol (148), on day 21 prior to ROATs in some experiments ("pre-ROAT") and in some also/or after ROATs ("post-ROAT"). Test concentrations of $CoCl_2$ were 0.3% in pet. and in some experiments also a serial dilution test 0.3, 0.1, 0.03, 0.01 and 0.003% (w/w) in pet. Colophony-induced animals were tested with colophony 10, 3, 1, 0.3 and 0.1% (w/w) in pet. as well as a vehicle control. Approximately 15 µl of the test preparations was used for each test. Rotation of test sites (3 per flank) and blind readings were used.

Repeated open application tests (ROAT)s in guinea pigs (V)

Two circular areas with a diameter of 30 mm, were demarcated with ink on the back of the animals. Test sites were clipped prior to readings and treatments. Treatments were carried out once daily on days 35-41 after induction (treatment days 0-6) and readings also on day 42. Readings were done prior to treatments and a positive ROAT was defined when a confluent erythema was obtained. Treatments were carried out for each animal with two different substances (one for each test site), in general one concentration of the allergen and the vehicle. Cobalt-induced animals (and controls) were treated with $CoCl_2$ in a 10% dimetyl sulfoxide aqueous solution. Concentrations were 0.1, 0.01, 0.005 and 0.001% (w/w), and approximately

100 µl was used for each site. Some animals were treated with sodium lauryl sulphate 0.1% (w/w). Test substances were gently rubbed into the skin with cotton-wool-tipped applicators. Colophony-induced animals and corresponding controls were treated in the same way, but with with colophony in acetone/arachis oil (3:1 w/w) 1.0, 0.1 or 0.01%. 50µl of the colophony preparation was applied to the test site with a micropipette.

ROATs in humans (VI)

The ventral aspect of the subject's forearms was used for ROATs. Round test sites, three per arm, with a diameter of 20 mm, were marked and 5 µl of the test substances was applied to these sites with a pipette, spread over the area with a glass rod and left to dry. Applications were performed once daily Monday to Friday the first week and Monday to Thursday the second week. Three concentrations of colophony in acetone/arachis oil 1:1 (w/w): 10%, 1% and 0.1% were tested. The vehicle was also tested, as was a 25% aqueous solution of sodium lauryl sulphate (SLS) which was included as a positive control for irritancy (151, 152). One test site was left untreated as a control site. The test substances were applied in random order by an assistant, blinded to the investigator. Test sites were assessed daily (Monday to Friday) by the investigator visually and using two bioengineering techniques. This was done prior to application of test substances and also the day after the last application. A ROAT reaction was considered positive when there were five or more maculae or papules within the test area, or when a homogenous erythema covered the test area.

A pilot study involving five colophony-sensitive individuals preceded the main study. This pilot study included patch tests with colophony in acetone/arachis oil parallel with colophony in pet. as well as ROATs with colophony.

Chemical analyses (IV)

The content of abietic acid in adhesive bandages was analysed using reversed-phase HPLC, ad modum Ehrin & Karlberg (15). From the samples investigated, colophony compounds were extracted with acetone in an ultrasonic bath for 15 min. After evaporation of acetone the extracts were stored at 5°C under nitrogen. The extracts of the bandages were injected in concentrations of 0.3-10 mg/ml in mobile phase.

To examine whether zinc resinate formed in the mixture of colophony and ZnO, colophony and colophony + ZnO in pet. were analysed with Fourier-transform infra-red spectroscopy (FT-IR). The samples were analysed between potassium bromide (KBr) plates.

Bioengineering methods (VI)

Laser Doppler bloodflow and transepidermal water loss (TEWL) were measured in all six test sites of all 22 subjects undergoing the ROATs. Measurements were performed daily (Monday to Friday) for 2 weeks, by the author, prior to application of test substances.

Laser Doppler flowmetry (LDF)

Laser Doppler flowmetry was performed according to guidelines of the ESCD Standardization Group (145), using the apparatus, Periflux (Perimed, PF1d, Stockholm,

Sweden). An intergrating probe with a probe holder, PF 107, was used. In this probe a bundle of seven different fibres with a fibre separation of 0.25 mm is used to guide laser light to the tissue. Blood flow - in arbitrary units - was recorded on a pen recorder. Measuring time was 2-3 minutes depending on when stability was reached.

Measuring transepidermal water loss (TEWL)

TEWL from test sites was measured using an evaporimeter, EP1 (Servo Med., Stockholm, Sweden). Data were recorded using the Evaporimeter applications software package (EVM) on a personal computer. The TEWL was measured during 60 seconds and the value in g/m^2h recorded was the mean of the last 15 seconds. Two recordings were performed from each test site and the value presented the mean of those two measurements. The measurments were performed according to ESCD guidelines (147) and prior to laser Doppler flowmetry.

Statistics (II, III)

Confidence intervals for the differences between proportions were calculated using the normal distribution (153).

RESULTS

Clinical examination and course of dermatitis (I, II, III)

(I, II). Forty-eight of 180 subjects from the tall-oil rosin factory and 39/132 from the opera company had some kind of skin disease at examination. The diagnoses and the numbers are presented in Table 2. Most cases were mild.

Table 2. Number of cases of skin disease present on examination in employees at a tall-oil rosin factory and of an opera company.

Diagnosis	Tall-oil rosin factory *(n=180)*	Opera company *(n=132)*
hand eczema, all types	10	7
seborrhoeic dermatitis[a)]	12	4
atopic dermatitis[b)]	1	1
facial dermatitis, other than seborrhoeic or atopic	3	6
dermatitis on the body, other than seborrhoeic or atopic	10	5
psoriasis	3	2
acne	1	6
rosacea	-	3
miscellaneous	12	6
all diagnoses	52[c)]	40[d)]

[a)]On face, scalp and/or body. [b)]On face, extremities and/or body. [c)]In 48 individuals.
[d)]In 39 individuals.

(III). On examination 41 of 83 former patients had some degree of hand dermatitis (some also dermatitis of other locations). Hand dermatitis was scored as mild in 24 cases, as moderate in 10 and severe in 7 cases (Table 5). Numbers of participants and locations of dermatitis at onset and at present examination are presented in Table 3. Among those subjects where hand dermatitis was the reason for previous patch testing, 59% had current hand dermatitis. Among the others, 16% had hand dermatitis at the present examination. The difference between these groups is statistically significant ($p<0.001$).

Table 3. Numbers of participants and locations of dermatitis at onset and at follow-up examination.

	Location (no. participants=83)			
Period	hands[a)]	face[b)]	other than hands or face	no dermatitis
at onset	64	6	12	1[c)]
at follow-up examination	41	1	5	36[d)]

[a)] Hands only or in combination with other locations. [b)] Face only or in combination with other locations, except hands. [c)] 1 individual reported no dermatitis, but had been patch tested due to unclear systemic symptoms. [d)] 1 individual had ongoing psoriasis, 1 had paronychia, 34 had no skin lesions.

Twenty-six of 83 former patients, when followed-up after approximately 10 years, reported a chronic course of hand dermatitis, 31/83 reported longer periods healed than with dermatitis, and another 26 reported that they had had no hand dermatitis during the follow-up period.

Patch testing in humans (I, II, III, IV, VI)

Standard series (I, II, III)

(I, II). The results of patch testing with the standard series, in employees of the tall-oil rosin factory and the opera company, are presented in Table 4.

There were four positive patch-test reactions to colophony among present employees of the factory and three among former employees. Among the opera company two singers and one dancer showed positive patch test reactions to colophony.

Table 4. Number of positive patch test reactions to substances of the standard series, tested in employees at a tall-oil rosin factory and of an opera company.

Test substance [a)] Conc. in pet.	Tall-oil rosin factory *(n=180)*	Opera company *(n=132)*
nickel sulphate 5%	10	16
balsam of Peru 25%	5	10
fragrance mix 8%	4	9
potassium dichromate 0.5%	6	5
colophony 20%	7	3
4-phenylenediamine base 1%	3	2
cobalt chloride 0.5% or 1%	1	4
caine mix 3%	3	not tested
Kathon CG 200 ppm (in water)	1	2
epoxy resin 1%	0	3
lanolin 100% or wool alcohols 30%	2	0
benzocaine 5%	2	0
formaldehyde 1%	0	2
thiuram mix 1%	0	2

[a)] Results presented for substances with ≥ 2 pos. reactions.

(III). Sixty of the 83 previously diagnosed colophony-sensitive patients showed a positive patch-test reaction to colophony at the re-testing. In 12 cases this was a solitary positive patch-test reaction. Forty-eight subjects had one or more concomitant positive patch-test reactions. The most common reactions were to nickel (34 cases) and fragrance mix and/or balsam of Peru (34 cases). Results of patch testing in relation to current hand dermatitis are presented in Table 5.

Table 5. Results of patch testing related to findings on examination in 83 individuals in whom contact allergy to colophony was diagnosed 9-13 years previously.

Result of current patch testing	Findings on examination *(n=83)*			
	no hand dermatitis *(n=42)*	mild hand dermatitis *(n=24)*	moderate hand dermatitis *(n=10)*	severe hand dermatitis *(n=7)*
Positive to colophony only *(n=12)*	5	5	0	2
Positive to colophony and other substances *(n=48)*	23	15	5	5
Negative to colophony, positive to other substances *(n=17)*	9	4	4	0
Patch-test negative *(n=6)*	5	0	1	0

Additional test substances (I, II, IV, VI)

(I). One of the seven colophony-sensitive subjects at the factory, a former employee, also reacted to tall-oil rosin. There were no reactions to the modified rosins.

(II). According to lists of ingredients or chemical analyses, only four of the tested products from the opera company contained colophony. Those subjects reacting to colophony at patch testing also reacted to some colophony-containing product at work. In addition, one subject, with a negative test to colophony, reacted to three colophony-containing products. The dancer reacting to colophony had a doubtful reaction to her dancers' rosin only. There were few patch test reactions to the other products tested.

(IV). The result of patch testing with the different colophony preparations in seven colophony-sensitive subjects is presented in Table 6. Five of the seven adhesive bandages (both "as is" and as extracts) gave positive reactions in all subjects, but one (no. 7, Table 6). There were no positive reactions to the two other bandages - one declared not to contain colophony and one declared to contain modified colophony.

Table 6. Results of patch testing [a)] colophony-sensitive patients with various mixes of colophony and zinc oxide.

Test material, conc. in pet.	Patient no.						
	1	2	3	4	5	6	7
gum rosin, 10%	++	+++	+++	+++	++	+++	++
mix of gum rosin and zinc oxide, 10%/10%	++	+++	+++	+++	++	+++	?+
methyl-esterified gum rosin, 10%	+++	+++	+++	++	+	++	-
mix of methyl-esterified gum rosin and zinc oxide, 10%/10%	++	+++	+++	++	+	++	?+
zinc oxide, 10%	-	-	-	-	-	-	-
petrolatum	-	-	-	-	-	-	-

[a)] - = neg; ?+ = doubtful, +,++,+++ = pos reaction

(VI). The results of the serial dilution patch testing with colophony in 13 colophony-sensitive subjects prior to ROATs are presented together with the results of the ROATs in Table 8, below.

Chemical analyses

Abietic acid was detected in all but one of the bandages declared to contain ZnO and colophony. No abietic acid was detected in the bandage labelled as containing no colophony. The detection limit of abietic acid in the extract was 10 ppm.

Unmodified gum rosin in pet. showed a strong peak at 1710 cm^{-1} due to C=O in -COOH of the resin acids in FT-IR. The sample containing unmodified gum rosin and ZnO showed the same strong peak at 1710 cm^{-1}. No C=O peaks indicating zinc salt formation were observed. Also the "fingerprint" regions of the two samples were identical.

Skin disease - relation to work and colophony exposure (I, II, III)

Most, but not all, colophony-sensitive subjects had noticed intolerance of adhesive plaster according to the interview.

(I). No case of ongoing skin disease among the factory workers could with certainty be established as occupational in origin, and few of the employees suspected that their work had influenced previous or ongoing skin disease. None of the former employees studied stated that they had left the company due to skin problems.

(II). One make-up artist had an ongoing hand eczema that she related to work. Seventy-five of 132 subjects had a history of intolerance to at least one cosmetic, another eight had suffered from intolerance to adhesives or a colophony-containing wax at work, while 49/132 had no history of such intolerance. Among those 75 with a history of cosmetic intolerance, 17 (23%) had a positive patch-test reaction to perfumes (fragrance mix, balsam of Peru, lichen acid mix or *d*-limonene) and/or preservatives (parabens, formaldehyde, Quaternium 15 or Kathon CG). Among those 49 subjects with *no* such history, only one (2%) had a positive patch-test reaction to balsam of Peru. The difference is statistically significant.

(III). Exposure to colophony in previous and current jobs in the former patients was difficult to estimate. Colophony exposure at work was estimated from job descriptions in 12/83. Twenty-four other subjects reported paperwork while in 47 no particular occupational colophony exposure was suspected. Some degree of hand dermatitis was seen, on examination, in 22/36 (61%) with probable colophony or paper exposure, and in 19/47 (40%) with no such exposure. Fifteen subjects had changed jobs due to dermatitis or contact allergy to colophony. Seven of these were healed on examination, among them three women with previous soldering jobs. Previous and current exposure to colophony during leisure was estimated in 14 subjects, of whom one reported symptoms from such exposure.

The cause of initial sensitization to colophony was suspected by the subject to be soldering flux in three cases, plaster or adhesive bandages in seven and a massage cream in one. One subject suspected that sawdust had caused his allergy to colophony.

Patch-testing in guinea pigs (V)

Thresholds of sensitivity after induction

In two series of $CoCl_2$-induced animals *(n=30 and 27, respectively)* and in the colophony-induced animals *(n=28)* the thresholds of sensitivity at serial dilution tests were determined. Results are presented in Table 7.

Table 7. Thresholds of sensitivity at serial dilution tests in guinea pigs induced with $CoCl_2$ (GPMT method) or colophony (FCAT method). The patches were applied on day 21 - prior to the ROATs. Scoring: + = patchy erythema; ++ = confluent erythema.

	$CoCl_2$			Colophony	
72 h reading	Patch test conc. %	Series I *(n=30)*	Series II *(n=27)*	Patch test conc. %	*(n=28)*
Neg.	0.3	3	0	10	0
+	0.3	5	0	10	1
++	0.3	4	7	10	1
++	0.1	8	4	3	10
++	0.03	7	8	1	12
++	0.01	2	7	0.3	3
++	0.003	1	1	0.1	1

ROATs in guinea pigs (V)

Dose-response relationship

$CoCl_2$

Twenty-seven animals, patch-test positive to $CoCl_2$ were divided by sensitivity threshold (Table 7) into three comparable groups and treated (ROAT) with various concentrations (0.1, 0.01 or 0.001%) of $CoCl_2$. All nine animals treated with 0.1%, 7/9 treated with 0.01% and 3/9 treated with 0.001% reacted at ROAT. The reactivity at the treated sites appeared earlier with 0.1% than with 0.001% $CoCl_2$.

Colophony

Twenty-eight colophony-sensitive animals (Table 7) were divided into three comparable groups and treated (ROAT) with 1%, 0.1% and 0.01% colophony solutions respectively. All guinea pigs treated with 1% and 0.1% reacted and 6/9 treated with 0.01%. The reactivity appeared earlier at the sites treated with 1% than at those treated with 0.1% and 0.01% (Fig 2).

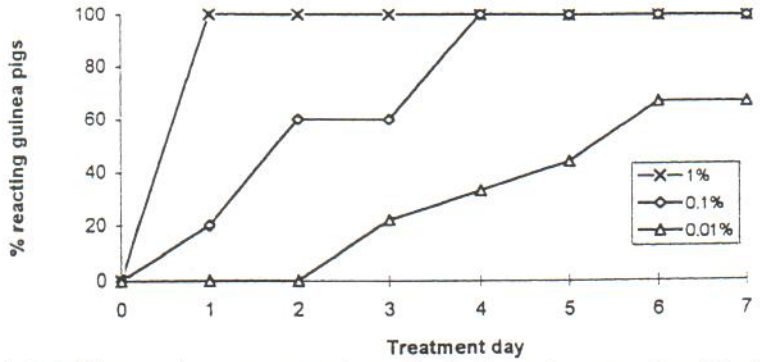

Fig. 2. Reactivity in ROATs at various concentrations of colophony in acetone/arachis oil (3:1) in guinea pigs induced according to Freund's complete adjuvant test and demonstrating a positive reaction at patch testing prior to the ROAT.

Relationship to patch test results ("pre-ROAT")

The concordance between patch-test reactions and ROATs (defined as a positive patch-test reaction to, at least, the strongest concentration of the allergen at the "pre-ROAT" patch testing, and positive ROAT or a negative "pre-ROAT patch test" combined with a negative ROAT) was concentration-dependent. The concordance was e.g. 88% (14/16) in animals treated at ROAT with 0.1% CoCl2; and 47% (9/19) among those treated with 0.001%. In colophony-sensitive animals the concordance was 100% (9/9 and 10/10 respectively) in animals treated with 1% and 0.1% colophony and 67% (6/9) in those treated with 0.01%.

Reactions in controls

ROAT reactions were found in 7/28 test sites when sham-treated guinea pigs were treated with the highest concentration of $CoCl_2$ (0.1%) for seven days. With 0.01% $CoCl_2$ the corresponding figure was 3/46, and there was no reaction at the sites treated with 0.001%. With colophony there was a weak erythema on Day 4 in one sham-treated animal at treatment with colophony 1%, but the animal became negative despite continued daily treatments.

ROATs in humans (VI)

Visual evaluation

The results of the ROATs in colophony-sensitive subjects are presented in Table 8. Half the subjects with a threshold concentration of 10%, when patch-tested with a serial dilution of colophony, had a positive ROAT with 20% colophony. With lower thresholds (1 or 0.1%) *all* reacted at ROATs with 20% colophony. There were no ROAT reactions to the vehicle and no ROAT reactions to colophony among the colophony-negative control subjects.

Table 8. Results of serial dilution patch test with colophony in acetone/arachis oil 1:1 (w/w) (test concentrations: 20, 10, 1, 0.1, 0.01%) and repeated open application tests (ROATs) with colophony (20%, 1% and 0.1%) in 13 colophony-sensitive subjects.

Subject			Patch-test reaction to colophony 20%	Threshold for a pos. patch-test reaction		First day[a] for a pos. ROAT-reaction to		
no.	gender	age	score	conc.%	score	20%	1%	0.1%
1	F	47	++	10	++	-	-	-
2	F	61	+	10[b]	+	2	-	-
3	F	25	++	1	+	3	-	-
4	F	66	+	10[c]	+	2[d]	3	-
5	F	53	+	10	+	-	-	-
6	M	48	++	1	+	3	-	-
7	F	52	+++	10	+++	7	-	-
8	M	31	+++	1	++	2[d]	4	-
9	F	47	+++	0.1	+	3	4	-
10	M	52	++	0.1	+	2[d]	-	-
11	F	18	++	10	+	-	-	-
12	F	66	+++	1	++	2[d]	-	-
13	F	38	+++	0.1	+++	1[d]	1[d]	4[e]

[a]Day 0=day of first examination *and* first application of test substances (after examination). Day 1=day of the second examination *and* second application etc. No examinations or applications were performed on Days 5 or 6 (Saturday, Sunday). [b]Patch-test positive also to 0.1%, but negative to 1%. [c]Suntanned back. [d]Treatment for application was Day 0-4. [e]ROAT-positive on Day 4. Reactions had disappeared on Day 7, but reappeared on Day 11.

The ROAT reactions, in colophony-treated areas, started as small scattered red macules or papules which gradually became more numerous with continued application of test substances. In 5 subjects (Table 8) the reaction to 20% colophony turned into a manifest eczema and the applications were stopped after 1-5 treatments. One subject (no. 13, Table 8) developed an itchy eczema spreading outside the test area after one application of 20% colophony solution and after 2 applications of 1% colophony solution.

Twelve of 22 subjects reacted at ROAT with SLS 25%. The first day for a visible reaction varied from Day 2 to Day 11. The SLS reactions differed morphologically from colophony reactions and here a homogenous redness was seen, covering the whole test area but not outside.

Evaluation using bioengineering methods (VI)

Laser Doppler flowmetry (LDF)

The median blood flow values for the non-treated and vehicle-treated sites in all 22 participants were stable throughout the experimental period. A 100% or greater rise in the value was considered meaningful and was seen in most test sites with a visually positive ROAT reaction. In all cases but one, the rise was noted on the same day, or after the reaction was noticed visually.

Measurement of transepidermal water loss (TEWL)

Also concerning TEWL the median values for all subjects of the non-treated and vehicle-treated sites were stable over time. A 50% or greater increase in TEWL was here considered meaningful. In five test sites, all treated with 20% colophony, there was such an increase in TEWL. In all cases the rise came after the visual reaction, in four not until the second week of treatment and in some after applications had stopped. Most subjects, some with no visible positive ROAT reaction, showed an increase in TEWL after application of SLS 25%. In some subjects the rise came before the visible reaction.

GENERAL DISCUSSION

Colophony is encountered frequently in our society because of its use in many products. With a prevalence of positive patch-test reactions in dermatitis patients of around 5%, colophony is, in Sweden, one of the five most common causes of contact allergy. Yet the relevance of contact allergy to colophony for, e.g., the development of hand eczema is often difficult to establish. Leaving the hand eczema patient with a list of products to avoid, based on the results of patch testing, may considerably influence his or her daily life, but may not always result in healing of the eczema. This thesis has tried to cast some light on the clinical relevance of contact allergy to colophony.

Prevalence of colophony sensitization in occupationally exposed subjects

The prevalence of contact allergy to colophony, diagnosed by patch testing, was 3.9% (7/180) among present and former employees at the tall-oil rosin factory (I). Among the *present* employees only 4/163 (2.5%) reacted to colophony of the standard series and none to tall-oil rosin or to the modified rosins tested. Since there were no reactions to tall-oil rosin, one might speculate whether the sensitization could have occurred from other sources of colophony, e.g. plasters, and not from tall-oil rosin at work. In that case, the amount of allergenic substances in tall-oil rosin and in modified rosins may have been too low to elicit a patch test reaction in the sensitized subjects, since tall-oil rosin has a lower allergenic activity than gum rosin of the standard series (20, 24-25) and modifications of colophony alter its allergenicity (25, 29-30). Three of seventeen (18%) former employees were patch-test positive to colophony and one also to tall-oil rosin. Whether this difference in prevalence between present and former employees was due to chance only is uncertain, since the number of former employees invited was low, and only half of them participated. A higher prevalence among former employees than among present would indicate a healthy-worker effect (154). None of the interviewed former employees stated that they had left the job at the factory due to skin problems, though.

The reason why not more employees were sensitized to tall-oil rosin despite - in many cases - daily exposure for many years, may be partly that most of the tall-oil rosin handled was freshly produced and thus contained fewer oxidized resin acids, which are the main allergenic compounds of colophony of all types (19-20) and partly that much of it was in modified form.

Three of 132 employees of the opera company (II) showed a positive patch-test reaction to colophony. If the subject who was negative to colophony but positive to colophony-containing products is included, there was a prevalence of contact allergy to colophony of 3%. At the time of the investigation few products in use contained colophony and the exposure to colophony among the artists, apart from the dancers, might therefore not have been much higher than exposure in the female population in general. Female dancers, especially, are from a rather early age, exposed to colophony from their dancers' rosin. It is therefore surprising that there is no previous report on dancers being sensitized to their rosin. In this study, the colophony-positive dancer had only a weak patch-test reaction to rosin, not fulfilling the criteria for a positive reaction. She had, however, been patch-tested before with a positive reaction also to the rosin. The content of allergenic substances in the rosin might vary depending on its origin and storage (5, 8).

Proper evaluation of the prevalences of colophony sensitivity found in the two studies (I, II) is not possible since the prevalence in the Swedish population is not known. In a Danish study of a general population, 0.7% showed a positive patch-test reaction to colophony (49). The method used in that study differs, however, so much from the present one that they cannot really be compared. In the Danish study ready-to-use patch tests were used, the patients applied the tests themselves and the reading was performed two days after application, one hour after removal of the patches. As is shown for many substances, also for colophony, positive patch test reactions may turn up later than after 48 hours (55, 119, 155) and positive reactions might therefore have been missed.

An epidemiological study of the prevalence of contact allergy and dermatitis in the general population would be most useful.

Results of re-testing in patients with previously diagnosed contact allergy to colophony

Only 60 of 83 subjects with positive patch-test reactions to colophony, when tested previously, showed a positive patch test reaction to colophony at the re-testing (III). Another seven had a doubtful reaction. Even if it might be assumed that the doubtful reactions did in fact reflect contact allergy to colophony, since colophony 20% is not known to cause irritation (34, 123), 16 of the previously positive colophony reactions (19%) were lost. Other follow-up studies where patch testing was performed have also shown loss of previously positive reactions e.g. to chromate (126, 131) and to nickel sulphate (125, 128). Other studies have shown that there is not a total concordance between two patch tests even performed simultaneously (156-157). There might be also other reasons, apart from a true loss of contact sensitivity to colophony, why so many positive reactions were lost. A second test reading some days later might have revealed more reactions (119-121). At the previous testing several nurses were involved in applying the tests and several dermatologists with different experience did the patch-test reading, and the risk of mistakes and of differences in interpretation of the results must have been greater than at the re-testing where one assistant and the author performed all the patch-test applications and readings. The content of allergenic substances in the test preparation might also have varied somewhat throughout the years (5, 8).

At the follow-up patch test only 12 of the 83 subjects (14%) had colophony as a solitary positive patch-test reaction, while 48/83 (58%) were patch-test positive to colophony *and* one or more substances, the most common concomitant positive reactions being to nickel sulphate and fragrance mix and/or balsam of Peru. This tallies with findings in other studies (24, 56-57) and in our department in 1989-1996 where only 33 of 124 colophony-sensitive patients had colophony as a single positive test reaction.

Patch-test reactions to other substances

The prevalence of positive patch test reactions to nickel suphate was 5.6% among the factory employees and 12% among the opera company. The difference between these two groups is probably due to the difference in gender among the participants: 5.6% in a population dominated by men (I) and 12 % in a population where the majority were women (II) is

probably what could be expected (158-161). The prevalence of positive patch-test reactions tofragrance mix and balsam of Peru was higher among the opera company than among the factory workers (Table 4). Again the prevalence in the general population is not known. One might suspect, though, that there was a higher than normal frequency among the opera company, since most cosmetics contain perfume of some kind. Perfume allergy seemed to be involved in cosmetic intolerance, since there were more subjects with positive patch-test reactions to perfumes and/or preservatives among those with a history of cosmetic intolerance than among those with no such history. A high frequency of perfume allergy among patients with cosmetic intolerance has also been found in other studies (162-163).

Colophony sensitivity and clinical symptoms

About one fourth of the employees of the tall-oil rosin factory and one third of those of the opera company showed some kind of skin disease (Table 2). The prevalence of current skin disease in the Swedish general population is not known, but in a large questionnaire study of a Swedish population of working age the prevalence of skin disease was 25% (164). Thus in most cases mild dermatoses in 25-30% of a population - as found in the present work - is perhaps what could be expected, when one is actively looking for skin disease.

Although skin disease was common, few cases were related to work. The colophony-sensitive subjects at the factory, who were exposed to tall-oil rosin, did not report work-related dermatitis. This is probably partly due to a lower content of oxidation products - the main allergens of all types of colophony - in tall-oil rosin than in colophony of the standard patch test series (20).

Among the opera company, all colophony-sensitive subjects had experienced dermatitis from colophony-containing products. These products were all sticky materials, which were left on the skin, on the eyelashes or under occlusion (wig adhesives) for several hours. The colophony-sensitive dancer had previously seen a dermatologist for dermatitis from her dancers' rosin. At the time of the examination she was free of dermatitis. Awareness of her contact allergy and a more careful use of her rosin might have contributed to this.

Of the former patients with previously diagnosed contact allergy to colophony, half showed hand dermatitis when followed up after approximately 10 years (Table 5). Most cases were mild. In 15 of those subjects with mild hand dermatitis, the scores were given because of dry skin only. Thus there is a risk that hand dermatitis was over-diagnosed. If these 15 subjects are withdrawn from the 41 with current hand dermatitis, the overall figure of hand dermatitis on examination was 26 or 31% of participants. The proportion of subjects with dermatitiswas very similar among those with a positive patch-test reaction to colophony only, among those with concomitant patch-test reactions and among those negative to colophony but positive to other substances. Thus contact allergy to colophony did not seem of great importance for *current* hand dermatitis. This might, however, be an effect of avoiding skin contact with colophony. It has been shown for lanoline that proper information is beneficial for the prognosis in contact allergy (165) and three subjects with a history of dermatitis of the hands and face in previous soldering jobs, where colophony-containing flux was used, were free of dermatitis at examination and had been so ever since they changed jobs. Christoffersen et al. found in an evaluative study of clinical patch test data a correlation between colophony-

sensitivity and leg ulcer/leg eczema in elderly women (50) while in a North American multicentre study (166) an overrepresentation of positive patch-test reactions to colophony (among other substances) was found in individuals with hand dermatitis and facial dermatitis (particularly eyelid dermatitis).

The only significant factor for *current* hand dermatitis found in the present study was location of dermatitis at onset, since 59% of those with hand involvement at onset had hand dermatitis on follow-up examination, compared to 16% among the others.

The exposure to colophony was in most cases difficult to estimate, and only few subjects could mention a particular product that induced or worsened the dermatitis. Among those with probable occupational exposure to colophony (*n=36*), 22 had hand dermatitis at the follow-up examination while 19 of 47 with no such exposure had hand dermatitis. The difference is not statistically significant. There is a risk that exposure was over-reported among those with dermatitis, since it is probable that they were more prone to look for colophony in the environment as an explanation for the dermatitis, than those with no skin disease.

Colophony and zinc oxide

The previously proposed inhibitory effect of zinc oxide (ZnO) on elicitation of contact allergy to colophony (45) was not confirmed in the present study. As shown in Table 6, there was no difference in reactivity to the mixtures of ZnO and unmodified colophony and the mixtures of ZnO and methyl-esterified colophony. In a mixture of zinc oxide and unmodified colophony, zinc salts (resinates) may theoretically form, while in methyl-esterified gum rosin and ZnO, zinc resinate formation is impossible.

One individual (no. 7, Table 6) showed overall weaker reactions than the others. This subject was also patch-test negative to all but one of the adhesive bandages both "as is" and as extract. In the others, five of the seven bandages tested caused positive patch-test reactions. In those bandages that gave *no* positive patch-test reactions no abietic acid was detected, with HPLC technique (15). One of these test-negative bandages was declared not to contain colophony and the other to contain hydrogenated derivatives of tall-oil rosin. Hydrogenation reduces allergenicity of colophony (25) and in this bandage the hydrogenated tall-oil rosin had been further modified, which may have reduced the amount of ordinary colophony allergens. IR analyses offered no proof of formation of zinc resinates in the mixtures of colophony and ZnO.

Results of the ROATs in guinea pigs and humans

There was a correlation between the threshold concentrations at patch testing and the outcome of ROATs in guinea pigs, both with cobalt chloride and colophony, and there was a dose-response relationship at the ROATs (Fig 2). In humans, too, a correlation was found between the threshold concentration at patch testing with colophony and the outcome of ROATs (Table 8). There was a dose-response relationship with 10/13 colophony-sensitive participants reacting to 20% colophony, 4/13 to 1% and only 1 showing a trancient reaction to 0.1%. Johansen et al also found a correlation between patch test results and outcome of ROATs performed in humans with cinnamic aldehyde and isoeugenol (133-134). With Kathon CG, a

correlation between the result of a serial dilution patch test and the outcome of ROATs has also been shown (167).

The ROAT reactions in guinea pigs were considered positive when there was a confluent erythema. In humans, however, the ROAT reactions differed from ordinary patch test reactions, in that they started as scattered red macules and papules, which eventually, with further applications of colophony, became more numerous and finally turned into clinical eczema, in some subjects; including also erythema, dryness and itching. A ROAT reaction was considered positive when five or more macules/papules were seen within the test area. A similar morphology was described in ROATs performed with cinnamic aldehyde, isoeugenol and formaldehyde (133-134, 168).

Guinea pigs have the advantage over humans that they can be treated under controlled conditions in experiments. In the human study efforts were made to perform the experiments as "controlled" as possible: all applications of test substances were performed by an assistant and not by the participants themselves; test substances were applied in random order, blind to the investigator; participants had to wait for approximately 10 min for the test solutions to dry; and the test sites were read daily, except for Saturday and Sunday. Hannuksela and Salo when presenting the repeated open application test (ROAT) (132) suggested that application of test substances should be performed twice daily for seven days. In the present studies, test substances were applied only once daily for practical reasons. In the guinea pigs the applications went on for seven days, while in humans, where no treatments were performed during the weekends, the treatments went on for two weeks. In ROATs performed with isoeugenol and cinnamic aldehyde (133-134) the test did not become positive until the second week or even later in some subjects. It cannot be known whether more guinea pigs or humans would have reacted if applications of cobalt and colophony had been applied over a longer period or twice a day. It is notable though that with colophony only one subject became "ROAT positive" during the second week of treatment. With further applications the risk of sensitizing control animals and humans would probably have been higher. The time difference in response between Johansen's studies (133-134) and the present ROAT study in humans might depend on differences in test materials, in test concentrations and in vehicles. The pilot study showed that colophony in petrolatum gave stronger patch test reactions than colophony in acetone/arachis oil of the same concentration, when patch tested simultaneously, and it can be assumed that the vehicle might influence also ROATs.

For the two ROAT studies using colophony, a vehicle of acetone and arachis oil was chosen. This was done since petrolatum had a tendency to spread outside the test site. Mixtures of acetone and arachis oil were tested in different proportions prior to the studies, and in guinea pigs acetone/arachis oil 3:1 was found to be the best, while in humans it was easier to apply a mixture of 1:1. The latter mixture was, however, not absolutely ideal, since it also spread outside the test sites in some subjects.

It would have been interesting if guinea pigs and humans both could have been tested with additional concentrations of cobalt and colophony, respectively. In animals as well as in humans the number of possible test sites was, however, limited, since it was desirable to have the different test sites well separated from each other to avoid interference of the test solutions between sites and also to include control sites. To be able to use three test sites on each

forearm the sites were smaller than what Hannuksela and Salo originally suggested (132). Later studies show, however, that the size of application areas in ROATs is of minor importance (169).

There were great variations in the reactivity to colophony in the human ROATs, from one subject developing eczema after only one application of a small amount of colophony (5µl of a 20% colophony solution) to others experiencing only small macules with no discomfort despite nine applications. There is no obvious explanation of this. Immunological factors (and the role of the skin barrier may be involved. It is however an interesting finding, and shows that dermatitis may develop from a single open exposure of colophony in some subjects, while others may not react at all to open applications - even if repeated.

The use of bioengineering techniques in evaluating test reactions

Non-invasive bioengineering techniques have been used for experimental evaluation of patch-test reactions (140). Laser Doppler flowmetry has been found useful in evaluating both allergic and irritant patch-test reactions (139, 170). Studies have shown a three-fold increase in laser Doppler blood flow values before a test reaction has been visible to the naked eye (144). In the present study, where ROAT reactions and not closed patch-test reactions were assesed, laser Doppler flowmetry added nothing to what was seen with the naked eye. In most test sites with a visible ROAT reaction there was also a rise in blood flow value, but the rise was recorded at the same time as or later than the visual reaction. In some cases the blood flow value sank again despite a continuous visible reaction. As shown by the spotty erythema, there was probably no even increase in blood flow at the site of an early ROAT reaction. Although the probe holder, thanks to skin marking, was attached in the same site every day, even a slight variation of the probe position within the holder might have made the laser beam hit a spot with no blood flow increase and thus explaining the variations.

Impairment of barrier function in allergic reactions is likely to be secondary to inflammation (171), while SLS has a direct cytotoxic effect on the skin (151-152). A poor correlation between the visual test reaction and an increase in TEWL was seen in this study when the allergen was applied as a ROAT. Few of the positive test sites showed an increase in TEWL, which appeared one to several days after the reaction had become visible. Most test sites treated with SLS 25% showed an increase in TEWL of 50% or more. An increase was seen also in some subjects with no visible reaction, sometimes prior to the visual reaction. In most previous studies SLS has been applied under occlusion. However, Lammintausta et al applied 2%, 5% and 7.5% SLS as repeated open applications. They also found that some individuals reacted with an increase in TEWL while others did not, and that SLS sometimes caused an increase in TEWL without erythema (172). SLS 25% was less useful as a "positive control" for irritancy than anticipated, since not all participants reacted when treated with open applications.

The use of TEWL measurment and LDF did not add further information compared to visual assessment when evaluating ROAT reactions to colophony.

CONCLUSIONS

The studies have shown that contact allergy to colophony is of clinical relevance for development of dermatitis when the skin is exposed under occlusion, to e.g adhesive bandages, and that addition of zinc oxide to colophony does not inhibit this reaction.

Colophony in "sticky" preparations to which the skin was exposed for some time caused dermatitis in sensitive subjects. Exposure to colophony in soldering flux caused occupational dermatitis, which healed with changes of jobs.

Accidental and temporary occupational exposure to colophony seemed to be of minor importance for dermatitis and contact allergy to colophony was not a significant problem in the workplace studies.

At least 1/3 of the colophony-sensitive patients examined had hand dermatitis, when followed-up after 10 years. Exposure to colophony could not be shown to be of significance for *ongoing* hand dermatitis in colophony-sensitive subjects. The most important factor correlated to ongoing hand dermatitis was previous hand eczema.

The repeated open application test (ROAT) is a useful method for evaluating contact allergy to colophony and cobalt in guinea pigs; a correlation between threshold concentrations at patch testing and the outcome of ROAT was found, and also a dose-response relationship.

Also in humans there was a correlation between the outcome of ROATs and threshold concentrations at patch testing with colophony. Objective readings with two bioengineering methods were of no additional value when assessing ROAT reactions to colophony.

The variation in reactivity to repeated open exposure to colophony is great. These findings imply that, although *open* exposure to colophony in many individuals may be of little importance for developing dermatitis, in some even temporary open exposure could cause eczema.

An important field for further investigation is to study what factors cause the difference in reactivity. Till then ROATs and/or serial dilution patch tests seem to be of value for establishing the clinical relevance of contact allergy to colophony, diagnosed by a standard patch test.

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REFERENCES

1. Foussereau J. History of epicutaneous testing: the blotting-paper and other methods. Contact Dermatitis 1984; 11: 219-223.
2. Scheper RJ, von Blomberg BME. Cellular mechanisms in allergic contact dermatitis. In: Rycroft RJG, Menné T, Frosh PJ (eds) Textbook of Contact Dermatitis, 2nd edition. Heidelberg: Springer-Verlag, 1995: 11-27.
3. Lachapelle JM. First Jadassohn lecture: A century of patch testing. Jadassohn Centenary Congress. London, UK, 9-12 October, 1996.
4. Stauffer DF. Production, Markets and Economics. In: Zinkel DF, Russel J (eds). Naval Stores. Production-Chemistry-Utilization, 1st edition. New York: Pulp Chemicals Association, 1989: 39-80.
5. Karlberg A-T. Contact allergy to colophony. Chemical identifications of allergens, sensitization experiments and clinical experiences. Acta Derm Venereol. (Stockh) 1988; suppl 139: 1-43.
6. Gäfvert E. Allergenic components in modified and unmodified rosin. Chemical characterization and studies of allergenic activity. Acta Derm Venereol (Stockh) 1994; Suppl. 184: 1-36.
7. Hausen BM, Kuhlwein A, Schulz KH. Kolophonium-Allergie. Ein Beitrag zur Herkunft, Chemie und Verwendung von Kolophonium und Kolophonium-modifizierten Produkten. (1. Mitteilung). Dermatosen 1982; 30: 107-115.
8. Stoltes EJ, Zinkel DF. Chemistry of Rosin. In: Zinkel DF, Russel J (eds) Naval Stores. Production-Chemistry-Utilization, 1st edition. New York: Pulp Chemicals Association, 1989: 261-345.
9. Hausen BM, Kuhlwein A, Schulz KH. Kolophonium-Allergie. Ein Beitrag zur Herkunft, Chemie und Verwendung von Kolophonium und Kolophonium-modifizierten Produkten. (2.Mitteilung). Dermatosen 1982; 30: 145-152.
10. Taylor JS. Adhesives, gums and resins. In: Fisher AA (ed): Contact Dermatitis 3rd edition. Philadelphia: Lea & Febiger,1986: 665-674.
11. Flyvholm M-A. Contact allergens in registered chemical products. Contact Dermatitis 1991; 25: 49-56.
12 Sadhra S, Foulds IS, Gray CN, Koh D, Gardiner K. Colophony - uses, health effects, airborne measurement and analysis. Ann. Occup. Hyg. 1994; 38: 385-396.
13. National chemicals inspectorate, Solna, Sweden.
14. Karlberg A-T, Lidén C. Colophony (rosin) in newspapers may contribute to hand eczema. Br J Dermatol 1992; 126: 161-165.
15. Ehrin E, Karlberg A-T. Detection of rosin (colophony) components in technical products using an HPLC technique. Contact Dermatitis 1990; 23: 359-366.
16. The Council Directive 88/379/EEC.
17 Anderson KE, Burrows D, White IR. Allergens from the standard series. In: Rycroft RJG, Menné T, Frosch PF (eds): Textbook of Contact Dermatitis 2nd edition. Berlin: Springer-Verlag, 1995: 429-430.
18. Lachapelle J-M. Historical aspects. In: Rycroft RJG, Menné T, Frosch PF (eds): Textbook of Contact Dermatitis 2nd edition. Berlin: Springer-Verlag, 1995: 3-8.
19. Karlberg A-T, Bergstedt E, Boman A, Bohlinder K, Lidén C, Nilsson JLG, Wahlberg JE. Is abietic acid the allergenic component of colophony? Contact Dermatitis 1985; 13: 209-215

20. Karlberg A-T. Air oxidation increases the allergenic potential of tall-oil rosin. Colophony contact allergens also identified in tall-oil rosin. Am J Contact Dermatitis 1991; 2: 43-49.
21. Hausen BM, Krohn K, Budianto E. Contact allergy due to colophony (VII). Sensitizing studies with oxidation products of abietic and related acids. Contact Dermatitis 1990; 23: 352-358.
22. Hausen BM, Börries M, Budianto E, Krohn K. Contact allergy due to colophony (IX). Sensitizing studies with further products isolated after oxidative degradation of resin acids and colophony. Contact Dermatitis 1993; 29: 234-240.
23. Sahdra S, Foulds IS, Gray CN. Identification of contact allergens in unmodified rosin using a combination of patch testing and analytical chemistry techniques. Br J Dermatol 1996; 134: 662-668.
24. Karlberg A-T, Lidén C. Clinical experience and patch testing using colophony (rosin) from different sources. Br Journal Dermatol 1985; 113: 475-481.
25. Hausen BM, Mohnert J. Contact allergy due to colophony (V). Patch test results with different types of colophony and modified-colophony products. Contact Dermatitis 1989; 20: 295-301.
26. Karlberg A-T, Boman A, Nilsson JLG. Hydrogenation reduces the allergenicity of colophony (rosin). Contact Dermatitis 1988; 19: 22-29.
27. Hausen BM, Jensen S, Mohnert J. Contact allergy to colophony (IV). The sensitizing potency of commercial products. An investigation of French and American modified colophony derivatives. Contact Dermatitis 1989; 20: 133-143.
28. Karlberg A-T, Gäfvert E, Hagelthorn G, Nilsson JLG. Maleopimaric acid - a potent sensitizer in modified rosin. Contact Dermatitis 1990; 22: 193-201.
29. Shao LP, Gäfvert E, Karlberg A-T, Nilsson U, Nilsson JLG. The allergenicity of glycerol esters and other esters of rosin (colophony). Contact Dermatitis 1993; 28: 229-234.
30. Gäfvert E, Shao LP, Karlberg A-T, Nilsson U, Nilsson JLG. Allergenicity of rosin (colophony) esters (II). Glyceryl monoabietate identified as contact allergen. Contact Dermatitis 1994; 31. 11-17.
31. Gäfvert E, Shao LP, Karlberg A-T, Nilsson JLG. Maleopimaric acid - a contact allergen in fumaric acid-modified rosin used for paper size. Nordic Pulp & Paper Research Journal 1995; 10: 139-144.
32. Gäfvert E, Bordalo O, Karlberg A-T. Patch testing with allergens from modified rosin (colophony) discloses additional cases of contact allergy. Contact Dermatitis 1996; 34: 290-298.
33. Karlberg A-T, Boman A, Holmbom B, Lidén C. Contact allergy to acid and neutral fractions of rosins. Sensitization experiments in guinea pigs and patch testing in patients. Dermatosen 1986; 34: 31-36.
34. Karlberg A-T, Lidén C. Comparison of colophony patch test preparations. Contact Dermatitis 1988; 18: 158-165.
35. Karlberg A-T, Bohlinder K, Boman A, Hacksell U, Hermansson J, Jacobsson S, Nilsson JLG. Identification of 15-hydroperoxyabietic acid as a contact allergen in Portuguese colophony. J. Pharm. Pharmacol. 1988; 40: 42-47.
36. Karlberg A-T, Boman A, Hacksell U, Jacobsson S, Nilsson JLG. Contact allergy to dehydroabietic acid deriatives isolated from Portuguese colophony. Contact Dermatitis 1988; 19: 166-174.

37. Gäfvert E, Nilsson U, Karlberg A-T, Magnusson K, Nilsson JLG. Rosin allergy: identification of dehydroabietic acid peroxide with allergenic properties. Archives of Dermatological Research 1992; 284: 409-413.
38. Gäfvert E, Shao LP, Karlberg A-T, Nilsson U, Nilsson JLG. Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. Chemical Research in Toxicology 1994; 7: 260-266.
39. Karlberg A-T, Gäfvert E. Isolated colophony allergens as screening substances for contact allergy. Contact Dermatitis 1996; 35 201-207.
40. Shao LP, Gäfvert E, Nilsson U, Karlberg A-T, Nilsson JLG. 15-hydroperoxy dehydroabietic acid - a contact allergen in colophony from pinus species. Phytochemistry 1995; 38: 853-857.
41. Hausen BM, Krueger A, Mohnert J, Hahn H, König WA. Contact allergy due to colophony (III). Sensitizing potency of resin acids and some related products. Contact Dermatitis 1989; 20: 41-50.
42. Hausen BM, Hessling C. Contact allergy due to colophony (VI). The sensitizing capacity of minor resin acids and 7 commercial modified-colophony products. Contact Dermatitis 1990; 23: 90-95.
43. Hausen BM, Loll M. Contact allergy due to colophony (VIII). The sensitizing potency of commercial products: an investigation of French and German modified-colophony derivatives. Contact Dermatitis 1993; 29: 189-191.
44. Khan L, Saeed MA. 13β, 14β-dihydroxy-13α-isopropylabietic acid, an elicitor of contact allergy. J Pharm Sci 1994; 83. 909-910.
45. Söderberg TA, Elmros T, Gref R, Hallman G. Inhibitory effect of zinc oxide on contact allergy due to colophony. Contact Dermatitis 1990; 23: 346-351.
46. Han JS, Zinkel DF. Gas chromatography of resin acids with a methyl silicone fused-silica capillary column. Naval Stores Review January/February 1990: 11-15.
47. Lee BL, Ong HY, Koh D, Ong CN. High-performance liquid chromatographic method for determination of dehydroabietic and abietic acids, the skin sensitizers in bindi adhesive. Journal of Chromatography A 1994; 685: 263-269.
48. Seidenari S, Manzini BM, Danese P, Motolese A. Patch and prick test study of 593 healthy subjects. Contact Dermatitis 1990; 23: 162-167.
49. Nielsen NH, Menné T. Allergic contact sensitization in an unselected Danish population. The Glostrup allergy study, Denmark. Acta Derm Venereol (Stockh) 1992; 72: 456-460.
50. Christophersen J, Menné T, Tanghøj P, Andersen KE, Brandrup F, Kaaber K, Osmundsen PE, Thestrup-Pedersen K, Veien NK. Clinical patch test data evaluated by multivariate analysis. Contact Dermatitis 1989; 21: 291-299.
51. Freeman S. Fragrance and nickel: old allergens in new guises. Am J Contact Dermatitis 1990; 1: 47-52.
52. Veien NK, Hattel T, Laurberg G. Patch test results from a private dermatologic practice for two periods of 5 years with a 10-year interval. Am J Contact Dermatitis 1992; 3: 189-192.
53. Marks Jr JG, Belsito DV, DeLeo VA, Fowler Jr JF, Fransway AF, Maibach HI, Mathias CGT, Nethercott JR, Rietschel RL, Rosenthal LE, Sherertz EF, Storrs FJ, Taylor JS. North American Contact Dermatitis group standard tray patch test results (1992 to 1994). Am J Contact Dermatitis 1995; 6: 160-165.
54. Brasch J, Geier J, Gefeller O. Dynamic patterns of allergic patch test reactions to 10 European standard allergens. *An analysis of data recorded by the Information*

Network of Departments of Dermatology (IVDK). Contact Dermatitis 1996; 35: 12-22.
55. Schehade SA, Beck MH, Hillier VF. Epidemiological survey of standard series patch test results and observations on day 2 and day 4 readings. Contact Dermatitis 1991; 24: 119-122.
56. Bruze M. Simultaneous reactions to phenol-formaldehyde resins colophony/hydroabietyl alcohol and balsam of Peru/perfume mixture. Contact Dermatitis 1986; 14: 119.
57. Holness DL, Nethercott JR, Adams RM, Belsito D, Deleo V, Emmett EA, Fowler J, Fisher AA, Larsen WG, Maibach HI, Marks J, Reitschel RL, Rosenthal LE, Schorr WF, Storrs FJ, Taylor JS. Concomitant positive patch test results with standard screening tray in North America 1985-1989. Contact Dermatitis 1995; 32: 289-292.
58. Shenefelt PD. Two-way tables listing irritant or allergen versus occupation. Am J Contact Dermatitis 1995; 6: 105-109.
59. Widström L. Contact allergy to colophony in soldering flux. Contact Dermatitis 1983; 9: 205-207.
60. Lidén C. Patch testing with soldering flux. Contact Dermatitis 1984; 10: 119.
61. Goh CL, Ng SK. Airborne contact dermatitis to colophony in soldering flux. Contact Dermatitis 1987; 17: 89-91.
62. Koh D, Foulds IS, Aw TC. Dermatological hazards in the electronics industry. Contact Dermatitis 1990; 22: 1-7.
63. Moura C, Dias M, Vale T. Contact dermatitis in painters, polishers and varnishers. Contact Dermatitis 1994: 31: 51.
64. Fregert S. Colophony in cutting oil and in soap water used as cutting fluid. Contact Dermatitis 1979, 5: 52.
65. Matos J, Mariano A, Gonçalo S, Freitas JD, Oliviera J. Occupational dermatitis from colophony. Contact Dermatitis 1988; 18: 53.
66. Grattan CEH, English JSC, Foulds IS, Rycroft RJG. Cutting fluid dermatitis. Contact Dermatitis 1989; 20: 372-376.
67. Calnan CD. Colophony dermatitis from insulated tools. Contact Dermatitis Newsletter 1972; 11: 281.
68. Castelain P-Y, Pirious A, Raulot-Lapointe H, Robaglia J-L. Sensitization to abieto-formo-phenolic resin in printing ink. Contact Dermatitis 1980; 6: 145.
69. Bergmark G, Meding B. Allergic contact dermatitis from newsprint paper. Contact Dermatitis 1983: 9: 330.
70. Lidén C, Karlberg A-T. Colophony in paper as a cause of hand eczema. Contact Dermatitis 1992; 26: 272.
71. Karlberg A-T, Gäfvert E, Lidén C. Environmentally friendly paper may increase the risk of hand eczema in rosin-sensitive persons. J Am Acad Dermatol 1995; 33: 427-432.
72. Meding B, Torén K, Karlberg A-T, Hagberg S, Wass K. Evaluation of skin symptoms among workers at a Swedish paper mill. Am J Industrial Medicine 1993; 23; 721-728.
73. Meding B, Åhman M, Karlberg A-T. Skin symptoms and contact allergy in woodwork teachers. Contact Dermatitis 1996; 34: 185-190.
74. Burry JN. Contact dermatitis from radiata pine. Contact Dermatitis 1976; 2: 262-263.
75. Daly BM, Stevenson CJ. Contact dermatitis to wood wool. Contact Dermatitis 1984; 11: 123.

76. Watsky KL. Airborne allergic contact dermatitis from pine dust. Am J Contact Dermatitis 1997; 8: 118-120.
77. Angelini G, Vena GA. Allergic contact dermatitis to colphony in a violoncellist. Contact Dermatitis 1986, 15: 108.
78. Helm TN, Taylor JS, Adams RM, Fisher AA, Nethercott JR. Skin problems in performing artists. Am J Contact Dermatitis 1993; 4: 27-32.
79. Aberer W. Allergy to colophony acquired backstage. Contact Dermatitis 1987; 16: 34-36.
80. James WD. Allergic contact dermatitis to a colophony derivative. Contact Dermatitis 1984; 10: 6-10.
81. Sjöborg S, Fregert S. Allergic contact dermatitis from a colophony derivative in a tape skin closure. Contact dermatitis 1984; 10: 114.
82. Dooms-Goossens A, Boden G, Aupaix F, Bruze M. Allergic contact dermatitis from adhesive plaster due to colophony and epoxy resin. Contact Dermatitis 1993; 28; 120.
83. Mallon E, Powell SM. Allergic contact dermatitis from Granuflex hydrocolloid dressing. Contact Dermatitis 1994; 30: 110.
84. Lachapelle J-M, Leroy B. Allergic contact dermatitis to colophony included in the formulation of flexible collodion BP, the vehicle of a salicylic and lactic acid wart paint. Dermatologic Clinics 1990; 8: 143-146.
85. Veraldi S, Schianchi-Veraldi R. Allergic contact dermatitis from colophony in a wart gel. Contact Dermatitis 1990; 22: 184.
86. Cameli N, Vassilopoulou A, Vincenzi C. Contact allergy to colophony in a wart remover. Contact Dermatitis 1991; 24: 315.
87. Lodi A, Leuchi S, Mancini L, Chiarelli G, Crosti C. Allergy to castor oil and colophony in a wart remover. Contact Dermatitis 1992; 26: 266.
88. Agathos M. Bavarian tiger balm. Contact Dermatitis 1982: 8: 215.
89. Barbaud A, Mougeolle JM, Tang JQ, Protois JC. Contact allergy to colophony in Chinese Musk and Tiger-Bone Plaster. Contact Dermatitis 1991; 25: 324
90. Li L-F. A clinical and patch test study of contact dermatitis from traditional Chinese medicinal materials. Contact Dermatitis 1995; 33: 392-395.
91. de Argila D, Ortic-Frutos J, Iglesias L. Occupational allergic dermatitis from colophony in depilatory waxes. Contact Dermatitis 1996; 34: 369.
92. Calnan CD. Colophony in eye-shadow. Contact Dermatitis Newsletter 1971: 10: 235.
93. Foussereau J. A case of allergy to colophony in a facial cosmetic. Contact Dermatitis 1975; 1: 259.
94. Rademaker M, Kirby JD, White IR. Contact cheilitis to shellac, Lanopol 5 and colophony. Contact Dermatitis 1986; 15: 307.
95. Batta K, Bourke JF, Foulds IS. Allergic contact dermatitis from colophony in lipsticks. Contact Dermatitis 1997; 36: 171.
96. Dooms-Goossens A, Degreef H, Luytens E. Dihydroabietyl alcohol (Abitol®) A sensitizer in mascara. Contact Dermatitis 1979; 5: 350-353.
97. Karlberg A-T, Lidén C, Ehrin E. Colophony in mascara as a cause of eyelid dermatitis. Chemical analyses and patch testing. Acta Derm Venereol (Stockh) 1991; 71: 445-447.
98. Sainio EL, Henriks-Eckerman M-L, Kanerva L. Colophony, formaldehyde and mercury in mascaras. Contact Dermatitis 1996; 34: 364.
99. Koh D, Lee BL, Ong HY, Ong CN, Wong WK, Ng SK, Goh CL. Colophony in bindi adhesive. Contact Dermatitis 1995; 32. 186.

100. Saha M, Srinivas CR, Shenoy SD, Balachandran C, Acharya S. Footwear dermatitis. Contact Dermatitis 993; 28: 260-264.
101. Freeman S. Shoe dermatitis. Contact Dermatitis 1997; 36: 247-251.
102. Hindson C, Lawlor F, Downey A. Cross sensitivity between zinc oxide plaster and Cupressus Leylandii shrubs. Contact Dermatitis 1982; 8: 335.
103. Dooms-Goossens A, Maertens M, van Lint L, Ruys-Catlender CM, Scheffer JJC. Colophony-induced sensitivity to Juniperus chinensis L "Hetzii"? Contact Dermatitis 1984; 10 185.
104. Castiglioni G, Carosso A, Nebiolo F. Contact dermatitis from colophony in a horticulturalist. Contact Dermatitis 1992; 26: 271.
105. Satyawan I, Oranje AP, van Joost Th. Perioral dermatitis in a child due to rosin in chewing gum. Contact Dermatitis 1990; 22: 182.
106. Koch Patrick. Occupational contact dermatitis from colophony and formaldehyde in banknote paper. Contact Dermatitis 1995; 32: 371-372.
107. Bergh M, Menné T, Karlberg A-T. Colophony in paper-based surgical clothing. Contact Dermatitis 1994; 31: 332
108. van Ketel WG, Bruynzeel DP. Occupational dermatitis in an accordian repairer. Contact Dermatitis 1992; 27: 186.
109. Thörneby-Andersson K, Hansson C. Allergic contact dermatitis from colophony in waxes for polishing spectacle frames. Contact Dermatitis 1994; 31: 126.
110. Karlberg A-T, Gäfvert E, Meding B, Stenberg B. Airborne contact dermatitis from unexpected exposure to rosin (colophony). Rosin sources revealed with chemical analyses. Contact Dermatitis 1996; 35: 272-278.
111. Fisher AA. Contact stomatitis and cheilitis. In: Fisher AA (ed): Contact Dermatitis 3rd edition. Philadelphia: Lea & Febiger,1986: 784-785.
112. Gar ia-Bravo B, Pons A, Rodriguez-Pichardo A. Oral lichen planus from colophony. Contact Dermatitis 1992; 26: 279.
113. Thune P. Contact allergy due to lichens in patients with a history of photosensitivity. Contact Dermatitis 1977; 3: 267-272.
114. Menagé H duP, Ross JS, Norris PG, Hawk JLM, White IR. Contact and photocontact sensitization in chronic actinic dermatitis: sesquiterpene lactone mix is an important allergen. Br J Dermatol 1995; 132: 543-547.
115. Burge PS. Occupational asthma, rhinitis and alveolitis due to colophony. In: Pepys J (ed): Clinics in immunology and allergy. Philadelphia: W.B Saunders Co, 1984: 55-81.
116. Cullen RT, Cherrie B, Soutar CA. Immune responses to colophony, an agent causing occupational asthma. Thorax 1992; 47: 1050-1055.
117. Rivers JK, Rycroft RJG. Occupational allergic contact urticaria from colophony. Contact Dermatitis 1987; 17: 181.
118. Wahlberg JE. Patch testing. In: Rycroft RJG, Menné T, Frosh PJ (eds) Textbook of Contact Dermatitis, 2nd edition. Heidelberg: Springer-Verlag, 1995: 241-261.
119. Paramsothy Y, Collins M, Smith AG. Contact dermatitis in patients with leg ulcers. The prevalence of late positive reactions and evidence against systemic ampliative allergy. Contact Dermatitis 1988; 18: 30-36.
120. Edman B, Björkner B, Bruze M, Möller H, Svensson Å, Malmquist-Padoan S. Utvärdering av två avläsningar med standardserien. Svenska Läkaresällskapets Riksstämma 1994: 142.

121. Macfarlane AW, Curley RK, Graham RM, Lewis-Jones S, King CM. Delayed patch test reactions at days 7 and 9. Contact Dermatitis 1989; 20: 127-132.
122. Fregert S, Gruvberger B. Patch testing with colophony. Contact Dermatitis 1984; 11: 141-143.
123. Bruze M, Dahlquist I, Fregert S. Patch testing with colophony at 60% concentration. Contact Dermatitis 1986; 15: 193.
124. Wilkinson JD, Rycroft RJG. Contact dermatitis. In: Champion RH, Burton JL, Ebling FJG (eds): Rook/Wilkinson/Ebling: Textbook of Dermatology, 5th edition. Oxford: Blackwell Scientific publications, 1992: 611-128.
125. TeLintum JCA, Nater JP. On the persistence of positive patch test reactions to balsam of Peru, turpentine and nickel. Br J Dermatol 1973; 89: 629-634.
126. Thormann J, Jespersen NB, Joensen HD. Persistence of contact allergy to chromium. Contact Dermatitis 1979; 5: 261-264.
127. Keczkes K, Basheer AM, Wyatt EH. The persistence of allergic contact sensitivity: a 10-year follow-up in 100 patients. Br J Dermatol 1982; 194: 461-465.
128. Schubert H, Kohanka V, Korossy S, Nebenführer L, Prater E, Rothe A, Szarmach H, Temesvári E, Ziegler V. Epidemiology of nickel allergy: results of a follow-up analysis of patients with positive patch tests to nickel. Contact Dermatitis 1988; 18: 237-239.
129. Valsecchi R, Rossi A, Bigardi A, Pigatto PD. The loss of contact sensitization in man. Contact Dermatitis 1991; 24; 183-186.
130. Ayala F, Balato N, Lembo G, Patruno C, Fabbrocini G, Nofron I, Magliocchetti N, Schena D, Rafanelli A, Seidenari S, Motolese A, Angelini G, Tosti A, Saccabusi S, Pigato P, Lisi P. Statistical evaluation of the persistence of acquired hypersensitivity by standardized patch tests. Contact Dermatitis 1996; 34: 354-358.
131. Katsarou A, Baxevanis C, Armenaka M, Volonakis M, Balamotis A, Papamihail M. Study of persistence and loss of patch test reactions to dichromate and cobalt. Contact Dermatitis 1997; 36: 87-90.
132. Hannuksela M. Salo H. The repeated open application test (ROAT). Contact Dermatitis 1986; 14: 221-227.
133. Johansen JD, Andersen KE, Rastogi SC, Menné T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. Contact Dermatitis 1996; 34: 165-171.
134. Johansen JD, Andersen KE, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. Contact Dermatitis 1996; 34: 414-418.
135. Agner T. Noninvasive measuring methods for the investigation of irritant patch test reactions. Acta Derm Venereol (Stockh) 1992; Suppl 173: 10-13.
136. Belcaro G, Nicolaides AN. Laser-Doppler flowmetry: Principles of technology and clinical applications. In: Serup J, Jemec GBE (eds). Handbook of non-invasive methods of the skin. Boca Raton: CRC Press, 1995: 405-410.
137. Ollmar S, Nyrén M, Nicander I, Emtestam L. Electrical impedance compared with other non-invasive bioengineering techniques and visual scoring for detection of irritation in human skin. Br J Dermatol 1994; 130: 29-36.
138. Fischer T, Bjarnason B. Sensitizing and irritant properties of 3 environmental classes of diesel oil and their indicator dyes. Contact Dermatitis 1996; 34: 309-315.
139. Staberg B, Klemp P, Serup J. Patch test responses evaluated by cutaneous blood flow measurements. Arch Dermatol 1984; 120: 741-743.

140. Berardesca E, Maibach HI. Review article. Bioengineering and the patch test. Contact Dermatitis 1988; 18: 3-9.
141. Agner T, Serup J. Skin reactions to irritants assessed by non-invasive bioengineering methods. Contact Dermatitis 1989; 20: 352-359.
142. Seo KI, Eun HC. Loss of contact sensitization evaluated by laser Doppler blood flowmetry and transepidermal water loss measurement. Contact Dermatitis 1996; 34: 233-236.
143. Shepherd AP. History of laser Doppler blood flowmetry. In: Shepherd AP, Öberg PÅ (eds). Laser-Doppler blood flowmetry. Norwell, Massachusetts: Kluwer Academic Publishers, 1990: 1-16.
144. Wahlberg JE. Nickel: the search for alternative, optimal and non-irritant patch test preparations. Assessment based on laser Doppler flowmetry. Skin Research and Technology 1996; 2: 136-141.
145. Bircher A, de Boer EM, Agner T, Wahlberg JE, Serup J. Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1994; 30: 65-72.
146. Pinnagoda J. Hardware and measuring principles: evaporimeter. In: Elsner P, Berardesca E, Maibach HI (eds). Bioengineering of the skin: water and stratum corneum. Boca Raton: CRC Press, 1994: 51-58.
147. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1990; 22: 164-178.
148. Wahlberg JE, Boman A. Guinea Pig Maximization Test. In: Andersen KE, Maibach HI (eds). Contact Allergy Predictive Tests in Guinea Pigs. Basel: Karger, 1985: 59-114.
149. Klecak G. The Freund's Complete Adjuvant Test and the Open Epicutaneous Test. In: Andersen KE, Maibach HI (eds). Contact Allergy Predictive Tests in Guinea Pigs. Basel: Karger 1985: 152-171.
150. Wahlberg JE, Lidén C. Attempts to mimic the repeated open application test in the guinea pig. Contact Dermatitis 1994; 30: 295-298.
151. Lee CH, Maibach HI. The sodium lauryl sulfate model: an overview. Contact Dermatitis 1995; 33: 1-7.
152. Tupker RA, Willis C, Berardesca E, Lee CH, Fartasch M, Agner T, Serup J. Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis 1997; 27: 53-69.
153. Altman DG. Statistics with confidence. - Confidence intervals and statistical guidelines. London: BMJ, 1989.
154. Objectives of epidemiologic study design. In: Rothman KJ. Modern epidemiology. Boston/Toronto: Little, Brown and Company, 1986: 83-84.
155. Uter WJC, Geier J, Schnuch A. Good clinical practice in patch testing: readings beyond day 2 are necessary: A confirmatory analysis. Am J Contact Dermatitis 1996; 7: 231-237.
156. Belsito DV, Storrs FJ, Taylor JS, Marks JG Jr, Adams RM, Rietschel RL, Jordan WP, Emmett EA. Reproducibility of patch tests: A United States multicentre study. Am J Contact Dermatitis 1992; 3: 193-200.