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PhD thesis

The Prevalence and Risk Factors of Contact Allergy in the Adult General Population

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II Thyssen JP, Johansen JD, Menné T, Nielsen NH, Linneberg A. Contact allergy to allergens of the TRUE-test (panel 1 and 2) has decreased modestly in the general population. Br J Dermatol. 2009; 161: 1124–1129

III Thyssen JP, Nielsen NH, Linneberg A. The association between alcohol consumption and contact sensitization in Danish adults: the Glostrup Allergy Study. Br J Dermatol. 2008; 158:306-12

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Abbreviations

CE-DUR	Clinical epidemiology and drug utility research
CI	Confindence interval
DCDG	Danish Contact Dermatitis Group
ECDRG	European Contact Dermatitis Research Group
HICC	Hydroxyisohexyl-3-cyclohexene carboxaldehyde
HLA	Histocompatibility leukocyte antigen
IgE	Immunoglobulin E
IFN	Interferon
IL	Interleukin
IPPD	N-Isopropyl-N'-phenylparaphenylenediamine
LC	Langerhans cells
MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone
MDBGN	Methyldibromo glutaronitrile
OR	Odds ratio
PPD	p-Phenylenediamine
PTBP	p-Tert-butylphenol
ROAT	Repeated open application test
SPSS	Statistical Products and Service Solutions
TGF	Tumor transforming growth factor
Th	T-helper
TNF	Tumour necrosis factor
T-reg	T-regulatory
TRUE-test®	Thin-layer Rapid Use Epicutaneous-test®

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1.1 Summary (English)

Contact allergy is prevalent in the general population. A review, based on data from 9 patch test studies performed between 1966 and 2007 estimated that the median prevalence of contact allergy was 21.4% (range 15.2–26.3%) among 15-69 year old Scandinavians. The most prevalent contact allergens were nickel, fragrances, and thimerosal. Risk factors of contact allergy included female sex and ear-piercing. A possible effect of tobacco smoking on the prevalence of contact allergy has not been determined with certainty despite one Danish population based study found a significant dose-response relationship between contact allergy and smoking. Contact allergy is associated with hand eczema which may result in decreased quality of life and sick-leave.

This PhD had two aims: Firstly, to estimate the overall prevalence of contact allergy among adults from the general population in Denmark. Secondly, to investigate whether alcohol consumption and tobacco smoking affect the prevalence of (nickel) contact allergy.

Two different approaches were used to estimate the prevalence of contact allergy: A simple mathematical approach, the clinical epidemiology and drug utilization research (CE-DUR) method, used patch test data from dermatitis patients (n=14 284) tested within the Danish Contact Dermatitis Group during 2001-2005 in combination with patch test sales data from 1996-2005 to estimate the 10-year prevalence of contact allergy among adult Danes. The second estimate was based on results from a cross-sectional patch test study performed in adult volunteers from the general population in Copenhagen between 2006 and 2008. Participants (n=3 460) were patch tested with TRUE-tests® and readings were done on day 2. As a cross-sectional patch test study using similar methods was also performed in 1990 (n=543), the development in the prevalence of contact allergy could be assessed. A possible effect of alcohol consumption on the prevalence of (nickel) contact allergy was investigated by using questionnaire data from the 1990 (n=1 056) and 2006 (n=3 460) studies, and by using follow-up data from a similar patch test study performed in 1998 (n=734) were 69% participants from the 1990 were patch tested again. Finally, an association between tobacco smoking and (nickel) contact allergy was investigated by using questionnaire and patch test data from the 2006 study.

The CE-DUR method estimated that the 10-year prevalence of contact allergy ranged between 7.3% and 12.9% among adult Danes (>18 years). Based on German experience, the worst case scenario may reveal the most accurate estimate, i.e. 12.9%. Despite inherent inaccuracies of the CE-DUR method, it may work as a rapid and in-expensive way to monitor the prevalence of contact allergy in the general population. The 1990 and 2006 patch test studies found that the overall prevalence of contact allergy among 18-69 year olds decreased from 15.5% in 1990 to 10.0% in 2006 ($p<0.001$). This was mainly explained by a decrease in thimerosal-, cobalt-, Myroxylon Pereirae- and rubber allergy. The decrease of thimerosal allergy may be explained by the removal of this ingredient from vaccines in Denmark. Tobacco smoking was significantly associated with nickel allergy ($p_{trend}<0.05$). In contrast, there was no clear association between alcohol consumption and contact allergy (or nickel allergy), although the 8-year incidence of contact allergy tended to be inversely associated with alcohol consumption in women ($p_{trend}=0.045$).

1.2 Resumé (Danish)

Kontaktallergi er hyppigt forekommende. En videnskabelig oversigtsartikel baseret på 9 lappetest studier gennemført i de Skandinaviske lande i perioden 1966-2007 viste at den mediane prævalens af kontaktallergi var 21.4% (range 15.2-26.3%) blandt 15-69 årige. Nikkel, parfume og thimerosal udgjorde de hyppigste kontaktallergier. Kvindeligt køn og huller i ørerne udgjorde væsentlige risikofaktorer for udviklingen af kontaktallergi. Det er fortsat ikke med sikkerhed fastslået hvorvidt tobaksrygning påvirker forekomsten af kontaktallergi skønt et dansk studie påviste en signifikant dosis-respons sammenhæng mellem rygning og kontaktallergi. Personer med kontaktallergi har en øget forekomst af håndeksem. Denne sygdom kan medføre nedsat livskvalitet og sygefravær.

Dette ph.d.-studium havde to overordnede formål: 1) At bestemme prævalensen af kontaktallergi blandt voksne danskere. 2) At undersøge om alkoholindtag og tobaksrygning kan påvirke prævalensen af (nikkel) kontaktallergi.

Der blev anvendt 2 forskellige metoder til at estimerer prævalensen af kontaktallergi. Det ene estimat blev udregnet ved hjælp af en simpel matematisk metode der bestemte 10-års prævalensen af kontaktallergi blandt voksne danskere. Metoden kaldes på engelsk "the clinical epidemiology and drug utilization research (CE-DUR) method". Der blev anvendt lappetest data fra eksempatienter (n=14 284) testet i den danske kontakt dermatitis gruppes klinikker i perioden 2001-2005 samt lappetest salgsdata der viste hvor mange lappetests der blev solgt i Danmark mellem 1996 og 2005. Det andet prævalensestimat blev baseret på resultater fra et tværsnitsstudie udført i 2006. Her deltog tilfældige voksne danskere i alderen 18-69 år (n=3 460) i en helbredsundersøgelse der bl.a. omfattede lappetestning (TRUE-test®) med aflæsning på dag 2. Resultaterne af undersøgelsen blev sammenlignet med data fra et tværsnitsstudie fra 1990 hvor der blev anvendt næsten identiske metoder (n=543). Hvorvidt alkoholindtag kan påvirke prævalensen af (nikkel) kontaktallergi blev undersøgt ved hjælp af spørgeskemadata fra 1990- (n=1 056) og 2006 studiet (n=3 460) og ved hjælp af follow-up data fra et lignende lappetest studie udført i 1998 (n=734) hvor 69% af deltagerne fra 1990 studiet blev lappetestet igen. Endelig blev sammenhængen mellem tobaksrygning og nikkel kontaktallergi undersøgt ved hjælp af spørgeskema- og lappetest data fra tværsnitsstudiet i 2006.

CE-DUR studiet estimerede at 10-års prævalensen af kontaktallergi blandt danskere over 18 år var mellem 7.3% og 12.9%. På basis af erfaringer fra Tyskland anses det mest pessimistiske overslag for mest nøjagtigt, i dette tilfælde 12.9%. På trods af at CE-DUR metoden har mange indbyggede usikkerheder, synes metoden at være en hurtig og billig måde hvormed forekomsten af kontaktallergi i befolkningen kan monitoreres. En sammenligning af data fra tværsnitsstudier i 1990 og 2006 viste at prævalensen af kontaktallergi faldt fra 15.5% i 1990 til 10.0% i 2006 ($p<0.001$). Faldet i 2006 skyldtes primært lavere prævalenser af thimerosal-, kobolt-, Myroxylon Pereirae- og gummiallergi. Faldet i forekomsten af thimerosalallergi kan forklares ved at thimerosal generelt er blevet fjernet fra danske vacciner. Spørgeskema undersøgelsen viste at tobaksrygning var signifikant associeret med nikkelallergi ($p_{trend}<0.05$). En mulig sammenhæng mellem (nikkel) kontaktallergi og alkoholindtag blev påvist i et 8 års follow-up studie (1990 til 1998) men kunne ikke bekræftes i 2 tværsnitsstudier (henholdsvis 1990 og 2006). Ved opfølgning i 1998, var forekomsten af kontaktallergi invers associeret med alkoholindtag blandt kvinder ($p_{trend}=0.045$) mens forekomsten af (nikkel) kontaktallergi blandt henholdsvis 1 056 deltagere i 1990 og 3 460 deltagere i 2006 var uafhængig af alkoholindtag.

2 Background

Contact allergy is frequent in the general population [1]. Continuous surveillance is necessary to determine the prevalence of various contact allergies. Thus, reliable and inexpensive epidemiological tools are required. Risk factors of contact allergy include female sex and ear-piercing whereas little is known about the influence of life-style factors such as alcohol consumption and tobacco smoking [1]. This thesis aimed to further explore these areas.

2.1 Allergic contact dermatitis

Contact allergy may develop following repeated or prolonged skin contact with allergens such as nickel, fragrances and preservatives and is a delayed type hypersensitivity reaction (type IV). Genetic predisposition seems to play a minor role although null-mutations in the filaggrin gene complex may be associated with nickel allergy [2-5]. When a contact sensitized subject is re-exposed to an allergen in concentrations that exceed the individual threshold, allergic contact dermatitis is elicited. Thus, two distinct phases are recognized, an induction phase referred to as contact sensitization and an effector phase referred to as allergic contact dermatitis [6].

Allergic contact dermatitis may involve all body parts (figure 1) but is most frequently located on the hands, feet, and face. The clinical picture varies depending on its chronicity. Acute dermatitis is characterized by erythema, edema, papules, vesicles and weeping whereas chronic dermatitis is scaly, dry and fissured. Risk factors of allergic contact dermatitis include the inherent sensitizing potential of an allergen [7], elevated allergen concentration (dose per unit area) [8], high frequency of exposure [9], occlusion [10], long exposure time [11], the presence of penetration enhancing factors and an altered skin barrier function [12].

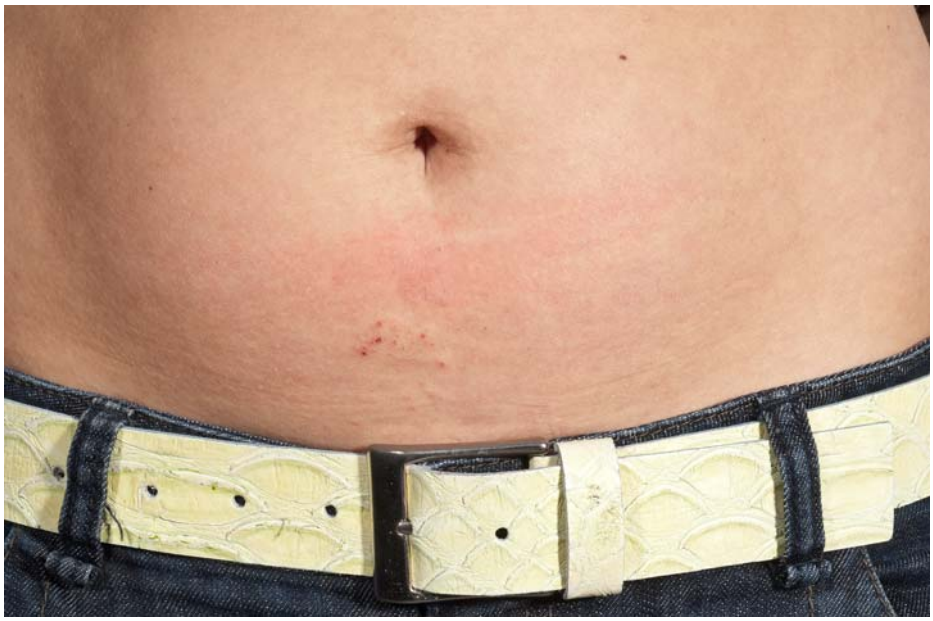


Figure 1. Mild allergic nickel dermatitis caused by nickel release from a belt buckle.

2.2 Allergen skin penetration & immunological mechanisms

Skin sensitization results in immunological responsiveness to a substance. Contact allergens are small molecules (haptens) with a molecular weight of less than 500 Dalton [13]. Before allergens can elicit a cutaneous immune response, they must gain access to the viable epidermis. Thus, contact allergens should have certain physicochemical characteristics which are necessary for passage across the stratum corneum as it normally works as an effective barrier to many chemicals. In their native state, chemical allergens are haptens and as such are of insufficient size to provoke an immune response. For this to be achieved, the chemical must form stable conjugates with proteins once inside the skin [14]. Consequently, contact allergens are either inherently protein-reactive or can be metabolized in the skin to protein-reactive species [15]. However, it was recently shown that some contact allergens, e.g. nickel, may interact directly with the human histocompatibility leukocyte antigen (HLA) in a peptide-independent manner [16].

The epidermis contains an interdigitating network of Langerhans cells (LC). These cells form part of a wider family of dendritic cells, the main function of which is to present antigen to the immune system. The hapten is internalized, processed, and transported by LC or macrophages to the regional skin draining lymph node where it is presented to specific naive T-cells that become activated and clonally expand to memory T-cells [17]. Re-exposure to the relevant allergen initiates the efferent phase and clinical expression of allergic contact dermatitis [18]. LC and macrophages produce large amounts of interleukin (IL)-12 which promotes T-helper (Th) 1 cell differentiation in the tissue through the suppression of IL-4 and concurrent promotion of interferon (IFN)-γ secretion [19]. A positive feedback is generated since INF-γ released by Th1-cells promotes further release of IL-12 from LC and macrophages. CD4+ T-cells activate keratinocytes through the secretion of IFN-γ, tumour necrosis factor (TNF)-α and IL-17 which in turn contributes to the amplification of the inflammatory response [20]. However, CD8+ cytotoxic T-cells is considered the major effector population in allergic contact dermatitis [21].

Recently, the key role of LC was debated by Kaplan et al. [22]. This research group has performed experimental studies using genetically modified mice to observe LC function. They identified a novel population of dermal dendritic cells that appeared to capture, process and present antigens rather than traditional LC [22]. Further research is necessary to establish the function of LC.

Th1 cells are crucial for humans to control the replication of intracellular pathogens whereas Th2 cells support the development of humoral immunity. The Th1/Th2 dichotomy has traditionally been the cornerstone of immunological thinking. The basic idea is that naïve precursor CD4+ T-cells differentiate to either Th1 or Th2-cells under the influence of cytokines (namely IL-12 and IL-4, respectively) secreted by various bystander cells [23]. Thus, the hypothesis postulates that Th1-cell predominance leads to delayed type hypersensitivity reactions (e.g. contact allergy and autoimmune diseases) whereas Th2-cell predominance leads to immunoglobulin (Ig)-E mediated allergic respiratory diseases (i.e. allergic rhinitis, asthma and urticaria). A Th2-mediated immune response relies on the secretion of IL-4, IL-5, IL-6, IL-10 and IL-13 [24]. The early source of IL-4 remains unclear [25]. These cytokines favour antibody production since IL-4 promotes B-cells to produce IgE [26]. Furthermore, a Th1-mediated immune response is inhibited by IL-4 and IL-10 [27;28]. Apart from Th2 cells and T-regulatory (T-reg) 1 cells, IL-10 is also being synthesised by monocytes, macrophages and dendritic cells [29]. The initial differentiation between the pathway for Th1 and Th2 cell, respectively, can be explained by multiple mechanisms [23].

It was recently demonstrated that a subgroup of T-cells, Tregs may suppress both Th1 and Th2 mediated immune responses. Furthermore, allergen specific Th1 and Th2-cells has been isolated from skin biopsies [30] and co-expression of Th1 and Th2-cytokines following sensitization have been observed in a murine model [31]. Thus, the dichotomy may only partially explain the development of various immune responses. To further complicate things, another subtype of T-cells (Th17 cells) have recently entered the field of immunology [32]. Th17 cells secrete IL-17 which

mediates inflammation through T-cell proliferation. Furthermore, IL-17 provides defence against extra-cellular bacteria and is involved in the inflammatory process of cancer and autoimmune diseases [33]. The differentiation of Th17-cells is inhibited by cytokines from both Th1 and Th2 cells (INF-γ and IL-4) and is stimulated by tumor transforming growth factor (TGF)-β1 and IL-23 [32]. Similar to the reciprocal interaction of Th1 and Th2 cells, Th17 cells are involved in reciprocal interaction with Th1 cells (i.e. Th1 cells inhibits the inflammatory damage caused by Th17 cells via the secretion of INF-γ) [34]. Finally, Th17 cells and Treg cells show reciprocal interactions through the action of IL-6 [34]. Recently, it was shown that inflamed skin of nickel-challenged allergic individuals contained infiltrating cells expressing IL-17 [35]. The Th1/Th2 hypothesis remains illuminating although it is acknowledged that no single cytokine can regulate a vital process like tissue damage and that a refinement of the model is necessary. However, it demonstrates that a reciprocal interaction between whole subsets of T-cells (i.e. Th1 and Th2) is a key point in inflammatory responses.

2.3 The influence of tobacco smoking & alcohol consumption on the immune system

Tobacco smoke contains more than 4 500 chemicals, many of which have toxic and/or carcinogenic activity. Chronic cigarette smoking alters a wide range of immunological functions, including innate and adaptive immune responses [36]. Furthermore, studies have shown that long-term smoking significantly reduces serum levels of Ig in humans [36]. Despite this reduction, smoking also increases autoantibody levels which may explain the association between smoking and autoimmune disease, e.g. lupus erythematosus, multiple sclerosus, Grave's hyperthyroidism, rheumatoid arthritis [37;38]. Immunological study results can be controversial and sometimes contradictory which may be explained by differences in smoking history, genetic susceptibility and socioeconomic status (as this may be influenced by exercise, nutrition, occupation and air quality) [39]. Thus, the patterns of smoke exposure are so varied individually and geographically that no single experimental smoke exposure system can replicate the diversity of human smoking patterns, and each experimental system probably reflects only facets of the overall picture [39]. Nevertheless, it seems plausible that tobacco smoking favours Th1 mediated immune responses and suppresses Th2 mediated immune responses [39].

Alcohol consumption has an effect on the immune system. In mice models, alcohol leads to impaired Th1 lymphocyte regulated cell mediated immune responses favoring a Th2 lymphocyte deviation of the immune system [40]. In addition, chronic alcohol consumption increases the susceptibility to viral and bacterial infections of the lower respiratory tract (e.g. pneumococcal pneumonia and tuberculosis) due to an impaired Th1 lymphocyte regulated cell mediated immune response [41]. In alcoholic abstinence syndrome, an increased level of Th2 lymphocyte related cytokines has been demonstrated in comparison to healthy controls [42]. Thus, alcohol consumption is associated with changes in the cytokine profile consistent with a relative Th2 lymphocyte deviation of the immune system. However, the exact mechanism alcohol consumption plays in allergic skin diseases has only partly been elucidated whereas the effect on pulmonary host defence has been investigated to a higher extent [43]. Alcohol mainly displays its effect on antigen presenting cells such as monocytes and dendritic cells (both in vitro and in vivo) where it leads to a decreased T-cell activation [44]. It can inhibit the antigen-presenting capacity of these cells for nearly 7 days [44]. Gluthatione levels in antigen-presenting cells may influence whether a Th1 or Th2 response will develop [45]. Gluthatione inhibition prevents IL-12 synthesis in antigen-presenting cells and lead to an increased production of IL-4 and thus a Th2-mediated immune response [46]. Since alcohol is an inhibitor of gluthatione synthesis, consumption may lead to IgE-mediated allergic diseases and possibly prevent contact allergy. Furthermore, ethanol leads to increased gut permeability which in turn leads to increased absorption of endotoxins (lipopolysaccharides) [47]. Monocytes CD14 receptors may then interact with absorbed lipopolysaccharides and indirectly favour IgE synthesis [47]. In addition, it has been demonstrated that alcohol consumption inhibits Th1 mediated immune responses both in vitro and in vivo (and in acute and chronic alcohol intake) [48-50]. Apparently, alcohol interferes with early cell surface-associated signal transduction phosphorylation events leading to impaired IFN-γ and IL-12

secretion whereas IL-2 synthesis is almost unaffected [51]. Administration of IL-12 can restore IFN-γ levels and delayed type hypersensitivity reactions in mice [52]. The effect of alcohol consumption on Th2-mediated immune responses is due to ethanol itself and not to non-ethanol content of alcoholic drinks [53]. Finally, it has been suggested that genetic variations in alcohol metabolism may influence both alcohol drinking behaviour and susceptibility to the immunological effects of alcohol [54]. Such genetic influence would tend to bias associations between alcohol and immune effects.

2.4 Contact allergy & patch testing

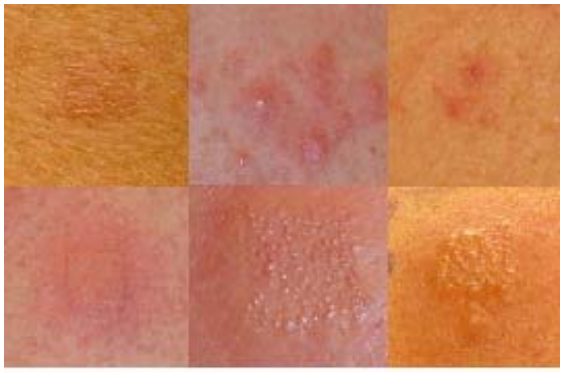
Contact allergy and contact sensitization are often used unanimously. Contact sensitization refers to immunological reactions towards contact allergens whereas contact allergy refers to positive patch test reactions [55]. Definitions of sensitization include "the process in which exposure to an antigen results in the development of hypersensitivity" or "the act or process of inducing an acquired sensitivity or allergy"[56] whereas definitions of allergy include "an altered body reaction, usually hypersensitivity, as a response to exposure to a specific substance" and "an altered reactivity following second or subsequent exposure to antigen (allergen)" [57].

Patch testing is widely used to establish a diagnosis of contact allergy although other in vitro methods exist [58]. The reproducibility of the patch test is generally high but is allergen dependent [59]; e.g. non-reproducibility is high for formaldehyde but low for nickel. In Europe, patch testing is mostly performed with the European baseline series. It should ideally contain contact allergens that will result in positive patch reactions in at least 1% of a local dermatitis patient population [60]. Currently, 28 allergens are included [61] but these only represents a subset of contact allergens as more than 3 700 have been described [60]. To evaluate specific occupational exposures, dermatologists may apply screening series such as dental and hairdresser series. Patch tests are applied to the upper back and usually occluded for 48 hr. Readings are ideally performed on day 3 or 4 and if possible on more than one occasion [60;62]. Patch test studies performed among dermatitis patients have suggested that 24-34.5% of positive patch test reactions potentially are missed when readings are not performed beyond day 2 [62-64]. Furthermore, a general population study showed that 27% of positive patch test reactions to nickel are missed when readings are only done on day 2 and not also on day 4 [65]. Early reactors include fragrance mix I and Myroxylon Pereirae whereas late reactors include nickel, neomycin, MCI/MI, corticosteroids, gold, and p-phenylenediamine (PPD) [60;66]. Ready-to-use test systems such as the Thin-layer Rapid Use Epicutaneous (TRUE) test® generally have a good concordance with conventional patch test systems using e.g. Finn Chambers® [67-69].

Patch testing intends to identify contact sensitized subjects by distinguishing between negative, irritant and allergic reactions. Thus, a valid positive patch test reaction (i.e. one that measures contact allergic reactions) requires a trained and experienced person that adheres to a set of valid criteria. Currently, the recommendations from the International Contact Dermatitis Research Group (ICDRG) dictate that homogeneous redness and infiltration in the entire test area is scored as a 1+ reaction, homogeneous redness, infiltration, and vesicles in the test area are scored as a 2+ reaction, and homogeneous redness, infiltration, and coalescing vesicles in the test area as a 3+ reaction [70]. 1+, 2+, or 3+ readings should be interpreted as positive responses whereas irritant responses, doubtful (+?) responses, or negative readings should be interpreted as negative responses (figure 2). However, the definition of a 1+ reaction is currently not uniform [60;71]. Thus, Menné and White recently suggested a new reading scale that would include both schools of patch test reading; 1+ reactions should display homogeneous redness in the test area with scattered papules; 2+ reactions should display homogeneous redness and homogeneous infiltration in the test area; 3+ reactions should be homogeneous redness and infiltration with vesicles and 4+ reactions should display homogeneous redness and infiltration with coalescing vesicles [71]. Digital images showing various patch test reactions were recently read by dermatologists at a dermatology conference in Berlin [72]. Subsequent analysis revealed a high validity but also that context information resulted in reclassification of patch test readings [73]. This

underscores that patch test readings should never be mixed with interpretation as this may weaken the objectivity.

Figure 2. Patch test reactions following 48 hours of nickel sulphate exposure interpreted in the 2006 Glostrup study. Upper line from left to right: irritative reaction, doubtful reaction (+?), and doubtful reaction (+?). Lower line from left to right: weak positve (1+), moderate positive (2+), and strong positive (3+) allergic reactions.



False positive and negative reactions are often encountered and they may have several explanations (table 1) [60]. Dermatologist tend to prefer false positive reactions rather than false negative reactions as one is alerted about the possibility of contact allergy [60]. In case readings are unclear, the dermatologist may perform additional readings, increase test concentrations, perform serial dilution testing or initiate repeated open application testing (ROAT) to better categorize the reaction and separate irritant from allergic ones [60]. However, determination of clinical relevance remains a challenge to dermatologists and patients [55]. Together, they should seek to establish whether the relevance of positive patch test reactions is current, past, unknown or non-existent [55]. Current or past relevance should be based on definite exposure to an allergen in combination with presence of dermatitis. Dermatologists may facilitate this assessment by performing product patch- and use testing [55].

Table 1. Selected causes of false positive and negative patch test reactions. (Modified from [60]).

False negative reactions	**False positive reactions**
Insufficient penetration of allergen: ▪ The duration of contact was too brief due to accidental removal ▪ Incorrect anatomical application ▪ Too low a test concentration ▪ Insufficient occlusion	Too high test concentrations: ▪ Uneven distribution of test substance in vehicle ▪ Excess test concentration applied ▪ Contaminated test preparation
Failure to perform late patch test readings	Misinterpretation of irritant reactions
Exposure to UV-light	Reactions to the patch itself
Meteorological conditions	Angry back syndrome
Immunosuppressive treatment	Artifacts
Testing in a population with a low prevalence of disease	Spillover reaction from a nearby positive reaction
Compound allergy (i.e. patch test positivity to formulated products but not to individual ingredients)	Testing of subjects with active dermatitis

2.5 Epidemiology of contact allergy

The prevalence of contact allergy has previously been studied in the general population [74-78] (table 2). In a literature review, Mortz et Andersen, showed that 13-23% children had positive patch test reactions to standard allergens [79]. Also, a recent review based on data from 17 patch test studies performed between 1966 and 2007 in mainly Europe and North America suggested that the median and weighted average prevalence of contact allergy was 21.2% (range 12.5-40.6%) and 19.5%, respectively [1]. Among Scandinavians aged 15-69 years, a median prevalence of 21.4% (range 15.2-26.3%) was identified [1]. The most prevalent contact allergens were nickel, thimerosal and fragrance mix I. Other frequent allergens were cobalt, chromium, PPD, and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI). The proportion of nickel allergy out of the overall prevalence of contact allergy in the general population increased significantly from approximately 5% in 1966 to 60% in 2007 ($p<0.003$) (figure 3) [1]. This finding underscores the importance of nickel allergy when addressing contact allergy in the general population. Various patch test populations were studied (e.g. infants, school children, adolescents, adults, twins, and cadets) over the years [1]. Patch test systems included ready-to-use test systems as well as conventional ones. Patch test readings were most frequently performed on day 2 or day 3 and rarely on more than one occasion. Readings were done by dermatologists, pediatricians and trained general physicians. The variations in study designs obviously troubled a direct comparison of prevalence estimates between studies. In addition, one should be aware that environmental allergen exposure may vary over time and result in different prevalences between age-groups [80;81]. Thus, when comparing contact allergy prevalences between different countries, one should preferably use data from same periods. Several studies did not present participation rates or characteristics of non-participants [1;82]. Low participation rates will generally make it difficult to assess the overall prevalence of contact allergy in the general population as it is obviously impossible to determine contact allergy in subjects that do not show up for testing [82]. Finally, a new epidemiological tool was recently used to estimate the number of subjects with contact allergy in Germany based on information on total annual patch test sales and clinical patch test data [83]. The study suggested that the 9-year prevalence of contact allergy in the general population was between 4.0% and 16.6% and that the worst case scenario (16.6%) was likely to be most accurate [83].

A review on nickel allergy in the general population showed that the median prevalence was 8.6% (range 0.7%-27.8%) and that it was higher among women than men (17.1%, range 3.9-38.8% versus 3%, range 0.7-6.8%) [1]. The single most important risk factor for nickel allergy was pierced ears [74;79;80;84-86]. Thus, the higher prevalence of nickel allergy in women was explained by a higher median prevalence of pierced ears in women when compared to men (81.5%, range 38.0-91.6% versus 12%, range 4.9-29.2%) [1]. In 1990, the Danish government began to regulate nickel release from consumer products including jewelry [87]. A Danish study showed that the prevalence of nickel allergy was higher among school girls that had their ears pierced before the Danish nickel regulation was introduced than among girls who had their ears pierced after its implementation [80].

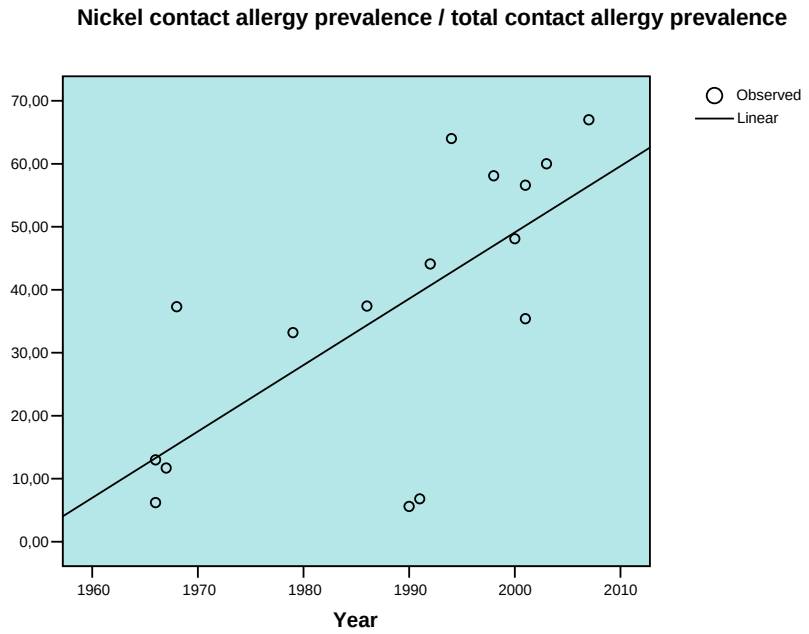


Figure 3. The proportion of nickel contact allergy prevalence out of the proportion of contact allergy prevalence to at least one allergen derived from published studies between 1966 and 2007.

Based on 13 studies performed in adults from the general population, the weighted average prevalence of fragrance mix I and Myroxylon Pereirae allergy was 3.7% and 1.6%, respectively [82]. Among 12-16 year olds, the prevalence of fragrance mix I and Myroxylon Pereirae allergy was 1.8% and 0.6%, respectively, in a large study from Odense, Denmark [75]. The different prevalences in different age-groups are in accordance with previous studies that have suggested a higher prevalence of fragrance allergy in older age-groups [78;88]. No studies have so far investigated the prevalence of fragrance mix II allergy. Thus, the overall prevalence of contact allergy to fragrances is therefore expected to be higher as allergy to fragrance mix II in the general population is likely to replicate the incline observed among dermatitis patients [89].

Few population based epidemiological studies have so far addressed the possible association between tobacco smoking and contact allergy. A Danish study with 1 056 participants found a strong association [90] whereas a Norwegian study with 1 236 adult participants only identified a weak association in women [74]. Also, among 520 young Swedish men doing compulsory military training, no association was found [91]. Of interest, prospective population based studies have suggested that tobacco smoking may decrease the risk of IgE-mediated allergic sensitization to aeroallergens [92;93]. Also, cross-sectional population based studies have demonstrated a lower prevalence of allergy to common aeroallergens among smokers and ex-smokers than among non-smokers [94;95]. Finally, no epidemiological studies have investigated an association between alcohol consumption and contact allergy although studies have demonstrated that alcohol consumption is associated with IgE mediated immune diseases, probably due to an imbalance in favor of Th2 cell predominance [96;97].

At present, little research has been conducted on the possible association between contact allergy and social status. However, older data from Malmö, Sweden, showed that the prevalence of nickel allergy was significantly higher among immigrants, unemployed, and patients on social security than among patients from higher socio-economic groups [98]. Furthermore, a German study showed that the prevalence of nickel allergy was higher among nurses (24.9%) and receptionist (29.3%) than among physicians (12.1%), indicating that nickel allergy may be more prevalent in low income groups [99].

Table 2. Studies on contact allergy in the general population conducted between 1966 and 2007.

Author	Population	*n* =	Allergens used for patch testing	Patch test reading done at day	Positive reaction to at least one allergen Total (%)	Three most common allergens
Röckl [100]	Children	357	*	3+4	40.6	Chromium, $HgCl_2$, formaldehyde
Forsbeck [101]	Relatives to patients with allergic contact dermatitis	93	Standard series†	-	24.7	Nickel, fragrance mix I, Myroxylon Pereirae /formaldehyde/ p-phenylendiamine procaine
Sipos [102]	Subjects with intact skin	659	**	3	13.7	$HgCl_2$, formaldehyde, nickel/PPD
Forsbeck [103]	Twins	202	Standard series†	3	15.8	Nickel, chromium, methyl thiuram disulfide
Magnusson [104]	Patients awaiting hip surgery	274	Standard series†	3	22.0	Nickel, Myroxylon Pereirae, Formaldehyde
Weston [105]	Children	314	Standard series†	3	20.3	Neomycin, nickel, chromium
Seidenari [106]	Cadets	593	Standard and textile series †	3	12.5	Thimerosal, nickel, $HgCl_2$
Barros [107]	School-children	562	Standard series†	2	13.3	Neomycin, thimerosal, p-tertiary-butylphenol-formaldehyde
Nielsen. [76]	Adult population	567	TRUE-tests	2	15.2	Nickel, thimerosal, cobalt/ Myroxylon Pereirae
Dotterud [108]	School children	424	Epiquick test	2	23.3	Nickel, cobalt, MCI/MI
Mangelsdorf [109]	Young adults	41	Standard series†	2,3	15.0	Myroxylon Pereirae, fragrance mix I, neomycin
Mangelsdorf [109]	Aged adults	82	Standard series†	2,3	37.0	-
Nielsen [77]	Adult population	469	TRUE-tests	2	18,6	Nickel, fragrance mix I, thimerosal
Bruckner [110]	Infants	85	TRUE-tests	4,5	24.5	Nickel, thimerosal, MCI/MI
Greig [111]	Adult volunteers	219	Standard series†	2, 4-7	35.0	Nickel, chromium, cobalt
Mortz [75]	School-children	1146	TRUE-tests	3***	15.2	Nickel, fragrance mix I, thimerosal/colophony/cobalt
Schäfer [78]	Adult population	1141	Standard Series†	3	28	Nickel, fragrance mix I, thimerosal
Bryld**** [112]	Twins	627	TRUE-tests	3	21.4	Thimerosal, nickel, colophony/fragrance mix I
White [113]	Adult population	1178-2545	*****	2	-	Nickel, PPD, chromium
Spiewak [114]	Students	135	Standard series†	2	28.1	Thimerosal, nickel, cobalt
Dotterud [74]	Adult population	1236	TRUE-tests	3	26.3	Nickel, cobalt, thimerosal
Svedman [115]	Stented population	715	Standard series† and other	3,7	48.4	Gold, Myroxylon Pereirae, nickel,

- Not given
* Chromium, nickel (2%), formalin, benzocaine, mercury chloride ($HgCl_2$), turpentine.
** $HgCl_2$, formaldehyde, nickel, chromium, novocaine, p-phenylenediamin (PPD), turpentine, lanoline.
*** 40 children were not read at day 3 but rather on day 2,4 or 7. Some were also read by parents who had been previously instructed.
**** Calculations made on subjects without hand eczema.
***** Nickel, Fragrance, formaldehyde, PPD, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), colophonium, chromate.
† Conventional patch testing using e.g. Finn Chambers®.

Study aims

This thesis aimed to estimate the prevalence of contact allergy in the adult general population in Denmark and furthermore, to investigate whether contact allergy was associated with life-style factors such as tobacco smoking and alcohol consumption.

Aims:

Study I To estimate the 10-year prevalence of contact allergy in Denmark by using a novel epidemiological tool, the CE-DUR.

Study IIa To estimate the current prevalence of contact allergy among adults in Denmark by patch testing a sample of the general population.

Study IIb To compare the prevalence of contact allergy among patch tested adults in Denmark with the CE-DUR prevalence estimate and with the prevalence of contact allergy among patch tested adults in 1990.

Study III To investigate whether alcohol consumption was associated with contact allergy in the general population by using data from a cross-sectional and a prospective patch test study.

Study IV To investigate whether alcohol consumption and tobacco smoking was associated with contact allergy in a cross-sectional patch test study.

3 Materials and Methods

3.1 Part 1: Clinical epidemiology & drug utilization research (CE-DUR) method

To estimate the 10-year prevalence of contact allergy in the general population in Denmark via the CE-DUR method, information was collected from different sources and assumptions made on available evidence.

3.1.1 Patch test sales data

Data on total patch test sales regarding the European baseline series (the overall number of sold syringes), the TRUE-test® (the total number of sold tests containing panel 1 and 2) and 0.5% methyldibromo glutaronitrile (MDBGN) (the number of sold single syringes containing MDBGN) were supplied by the three main manufacturers on the Danish market (Mekos Laboratories, Hillerød, Denmark; Hermal, Reinbeck, Germany; and Chemotechnique, Malmö, Sweden). Sales data were collected for a total period of 4-10 years depending on available company sales data. This was done to adjust for possible changing trends in the use of patch testing in Denmark over the last decade. However, no consistent trends were found, except for an increased sale of MDBGN after 1998 that had stabilized after 2001. The number of applications per sold syringe was estimated to 150; i.e., one syringe resulted in patch testing of 150 patients. This number was an average conservative estimate derived from retrospective registrations at the patch test clinic at Gentofte Hospital. However, scientific staff at Trolab and Chemotechnique estimated that 100 and 120 chambers could be filled per syringe, respectively. If the hypothetical maximum number of applications per syringe (petrolatum) was calculated (5000 µL/20µL), an estimated 250 applications was possible. Although the estimates differed markedly, we believe the most accurate estimate was 150 according to local registration. Thus, patch test material sufficient to test approximately 25 000 patients per year was sold in Denmark between 1996 and 2005 (table 3).

Table 3: A stepwise estimation of the number of subjects eligible for patch testing per year in Denmark based on the number of patch tests sold annually and published evidence regarding the proposed selection process. With model I (worst case scenario), the number of subjects eligible for patch testing would be 118 750 per year. With model III (medium case scenario), the number would be 87 750 subjects/year (Study I).

	Model I (Worst case scenario)	Model II (Best case scenario)	Model III (Medium case scenario)
The number of patch tests sold per year	25 000	25 000	25 000
Correction factor 1: The proportion of discarded patch tests (0-5%)	0%	- 5%	-2.5%
The number of actually applied patch tests	25 000	23 750	24 375
Correction factor 2: The proportion of previously tested persons (5-15%)	- 5%	- 15%	- 10%
First time patch tested subjects	23 750	20 188	21 938
Correction factor 3: The proportion of diseased persons that seek medical consultation (20-30%)	/ 20%	/ 30%	/ 25%
Persons eligible for patch testing per year	118 750	67 290	87 750

3.1.2 Patch test reading data

Patch test results were collected from the Danish Contact Dermatitis Group (DCDG) database for the period 1.1.2001-31.12.2005 (n=14 284). Thimerosal was omitted from our analyses since patch test results for thimerosal was not included in the baseline series and as thimerosal allergy rarely has any clinical relevance [116]. The DCDG network consists of 3 university clinics and 7 private dermatology clinics and represents an average clinical (patch test) population in Denmark. It is believed that the network is fairly representative of the entire population eligible for patch testing in Denmark. Patch test occlusion time was 48 hours and readings were performed at least on day 3 according to the criteria defined by the ICDRG [70]. Patients with positive patch test reactions were only included once in the database.

3.1.3 The proportion of discarded patch test

The proportion of purchased patch test that were discarded rather than used for testing was estimated. In the German CE-DUR investigation, it was assumed that 10-20% of purchased patch tests were discarded [83]. The experience from the patch test clinic at Gentofte Hospital reveals that the expiry dates of syringes are never or very rarely lapsed. However, in smaller departments or offices also contributing to the database, this may be the case to some extent. Thus, it is

estimated that 2.5% of all purchased patch tests are discarded each year in Denmark. As a consequence, the estimated number of patients patch tested will be lower (table 3).

3.1.4 The proportion of previously tested subjects

Experience from the Department of Dermatology in Göttingen determined that 38% of all patients have been tested on a previous occasion [83]. However, according to the DCDG database, only 15% have been tested previously. This figure is comparable to data (7.9%) published from St. John's Institute in London [117]. Hence, in order to establish the number of subjects eligible for patch testing, patch test sales figures had to be corrected downwards accordingly (table 3).

3.1.5 The proportion of diseased persons that seek medical consultation

In the German CE-DUR investigation, it was estimated that only 15-38% of patients with allergic contact dermatitis consult a physician, based on Swedish and German observations [118-120]. Furthermore, two consecutive Danish surveys from 1987 and 1994 estimated that approximately 25% of patients with allergic contact dermatitis are patch tested [121]. Thus, the sales figures had to be corrected upwards (table 3).

3.1.6 Subjects eligible for patch testing per year

The absolute number of diseased subjects eligible for patch testing per year was estimated through the use of the above presented information (table 3), summarised as three correction factors, ranging from very liberal assumptions ("worst case") to a combination of the most conservative, in terms of a low number of persons tested, assumptions ("best case"). Applying these correction factors, three different scenarios were defined, namely model I (worst case), model II (best case) and model III (medium case).

3.1.7 Population estimate

The Danish population, according to Statistics Denmark was 5 400 000 persons in 2006 [122]. Out of these, 1 200 000 were children and adolescents (<18-years). Hence, the adult Danish population was 4 200 000 persons.

3.1.8 Prevalence estimate

The 10-year prevalence of contact allergy to at least one allergen of the respective baseline series (and MDBGN) was calculated on the basis of patch test reactions:

$$PP_{I,II,III} = \frac{CP \times 10\,yrs \times n(elig)_{I,II,III}}{n(popul)}$$

PP denoting the derived prevalence on the population level in the three (country specific) scenarios I (worst), II (medium), and III (best), CP the clinical prevalence (in patients patch tested), 10 yrs the number of years considered as sampling frame for the clinical data, n(elig) the number of persons eligible for patch testing in the three scenarios and n(popul) the size of the total population.

3.2 Part 2: Cross-sectional & prospective patch test studies

This thesis used patch test and questionnaire data from two cross-sectional studies performed in the general population in 1990 and 2006. Furthermore, participants from the 1990 study were

re-invited in 1998 for a second health examination including patch testing. These prospective data were used to investigate a possible association between alcohol consumption and contact allergy.

In all studies, Danish adults (with Danish citizenship and born in Denmark) living in one of the same 11 municipalities (Albertslund, Ballerup, Brøndby, Glostrup, Herlev, Høje Taastrup, Hvidovre, Ishøj, Ledøje-Smørum, Rødovre, and Vallensbæk) of the Copenhagen County were randomly drawn from the Danish Civil Registration System and invited to participate in a general health examination including patch testing. Participants completed a questionnaire using the same questions. The Ethical Committee of Copenhagen County approved all studies (KA-20060011). Torkil Menné and Niels Henrik Nielsen participated in study preparations for the 1990, 1998 and 2006 studies whereas Allan Linneberg participated in the preparation of the 1998 and 2006 studies and finally, Jacob Thyssen and Jeanne Duus Johansen participated in the preparation of the 2006 study.

3.2.1 Baseline study 1990

The 1990 study was conducted according to a two-stage protocol. In the first stage a screening questionnaire on respiratory symptoms was mailed to a random sample of 15-69 year-olds (n=8 000) [123]. A total of 6 998 (87.5%) responded to the screening questionnaire. In the second stage, a random group and a symptom group selected among the respondents were invited to a health examination. The symptom group comprised all respondents (n=788) who reported upper ("itchy or stuffy nose or sneezing") or lower ("shortness of breath or trouble breathing") respiratory symptoms on exposure to either pollen or furry animals in the screening questionnaire [123]. The random group was comprised of 793 subjects who were randomly selected from all respondents (n=6 998). By chance, 146 subjects were comprised in both the random and the symptom group. Subsequently, 635 (80.5%) and 599 (75.5%) subjects from the symptom and random group, respectively, were examined. As 122 subjects were included in both groups, only 1 112 subjects (overall participation rate 77.5%) underwent general health examination. Furthermore, some participants were not patch tested and therefore data from only 1 056 participants were achieved (figure 4). On the test day, all participants filled out another questionnaire with a variety of health questions including questions on life-style factors, social-economic factors, ear-piercing and dermatitis. Examinations took place between February 1990 and January 1991. Patch test data from all subjects (n=1 056) examined in 1990 were used to investigate a possible association between alcohol consumption and contact allergy (study III) whereas cross-sectional patch test data from the random- and overlap groups were used for comparison between patch test data from the 1990 and 2006 study (study II) (figure 4). However, 15-17 year olds were excluded in this study in order to allow for comparison with data from the 2006 study. Thus, analyses were based on 543 participants aged 18-69 years.

3.2.2 Follow-up study 1998

Between October 1997 and November 1998, a prospective study was performed. 15-69 year old subjects from the 1990 study were re-invited for patch testing. At the time of follow-up, 28 subjects had died, 8 had emigrated and 12 could not be located. Thus, a total of 1 064 participants were invited to the follow-up study and 734 (69.5%) were examined. Participants were patch tested in the same months as they had been examined in the 1990 baseline study to avoid potential seasonal differences. Hence, a total of 63.9% (469/734) of the participants in the follow up-study were examined on a date within 2 months (62 days) of the date of examination in the baseline study. The median follow-up time was 7 years and 10 months (range: 6 years and 10 months to 8 years and 8 months). A comparison of characteristics among participants and non-participants revealed that male sex and high educational level at baseline were significantly associated with participation in the follow-up study whereas no differences were found regarding the prevalence of contact allergy, smoking status or alcohol consumption in participants and non-participants [93]. Follow-up data were only used for the incidence study investigating a possible association between contact allergy and alcohol consumption (study III).

3.2.3 Cross-sectional study 1998

Between January and November 1998, 1 138 15-41 year-old subjects responded to a screening questionnaire on respiratory symptoms (response rate 81.5%) [124]. A random sample of 902 subjects were invited to a health examination including patch testing, and 482 (53.5%) participated. None of these data were used in this thesis.

3.2.4 Cross-sectional study 2006

Between June 2006 and May 2008, 3 471 (43.7%) of 7931 invited subjects aged 18-69 years showed up for health examination and 3 460 participants were patch tested. For practical reasons, persons below the age of 18 years were not invited in 2006, since an informed, written consent from the parents is now mandatory in Denmark. Data from this study was used to estimate the prevalence of contact allergy in 2006 (study II) and furthermore, to investigate the association between contact allergy and alcohol consumption and tobacco smoking, respectively (study IV).

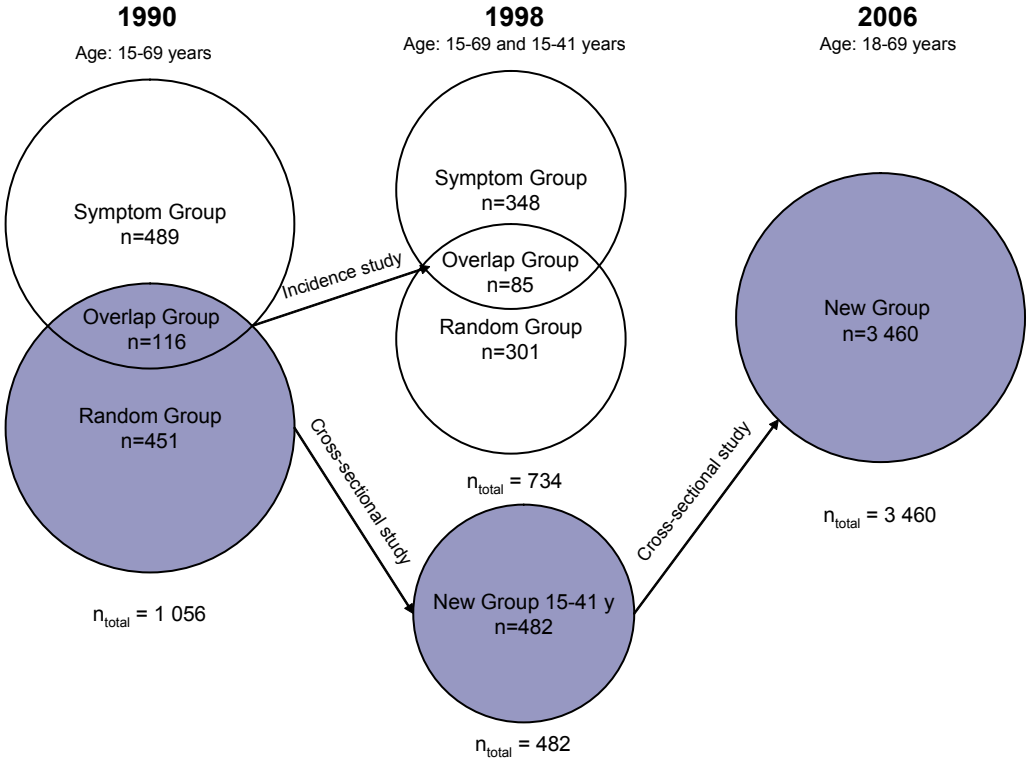


Figure 4. Overview of the Glostrup allergy studies performed in 1990, 1998 and 2006, respectively. n and n_{total} indicate the number of subjects that underwent patch testing. Symptom group indicates subjects that reported respiratory symptoms whereas random group indicates subjects that were randomly invited from all the respondents. Data from the 1998 cross-sectional study were not used for this thesis. When a comparison of patch test results was made between the 1990 and 2006 study, only 18-69 year olds (n=543) from the random- and overlap group in the 1990 study were included (study II).

3.2.5 Patch testing

Patch testing in all studies was performed by using the panel 1 and 2 of the TRUE-test® (suppliers were ALK-Abelló A/S, Hørsholm, Denmark in 1990 and 1998 and Mekos Laboratories, Hillerød, Denmark in 2006). The haptens in the TRUE-test® differ from the European baseline series by including thimerosal and excluding primine, sesquiterpene lactone mix, tixocortol pivalate, budesonide, hydroxyisohexyl-3-cyclohexene carboxaldehyde (HICC), MDBGN, and

fragrance mix II. Furthermore, the TRUE-test® contain black-rubber mix instead of N-Isopropyl-N'-phenylparaphenylenediamine (IPPD) and caine mix (tetracaine, benzocaine and dibucaine) instead of benzocaine. Finally, formaldehyde was not included in the TRUE-test® in 1990.

Directions to apply the patch tests panels to the upper back 2 days before examination were mailed together with the patch tests. They were read and photographed 1-1½ hour after removal by Nielsen in 1990, by Linneberg in 1998 and by trained health care personnel in 2006 (supervised by Thyssen and Linneberg) at the Research Centre for Prevention and Health, Glostrup Hospital. Subsequently, photos were reviewed in a blinded manner by Nielsen and Menné in 1990, by Nielsen, Menné and Linneberg in 1998, and by Nielsen, Menné, Linneberg and Thyssen in 2006. The group had no information about sex, age or previous medical history of participants when reviewing the photos. The set-up should ensure that the ICRDG criteria were used consistently over time [70]. Prior to study start, the health care staff (n=6) that performed patch test readings in the 2006 study underwent one week of training at the patch test clinic at Gentofte Hospital. A combination of theoretical education provided by Jacob Thyssen and hands-on training provided by experienced nurses at Gentofte Hospital was given to ensure a high validity of patch testing. A laboratory technician with 10 years of patch test reading experience from the Department of Dermatology at Bispebjerg Hospital had the daily overall patch test reading responsibility when Jacob Thyssen was not present at Glostrup Hospital. Hand-out patch test and contact allergen information for participants and standard operational procedures for the staff was developed by Jacob Thyssen for the 2006 study.

Contact allergy was defined as a positive (at least grade 1+ according to ICRDG) patch test to at least one allergen or mixes of haptens. Rubber allergy was defined as a positive patch test reaction to at least one of the following allergens: thiuram mix, mercapto mix, mercaptobenzothiazole, and carba mix. In case the patch had no skin contact upon patch test reading, or if the subject had removed it prior to testing as a result of known contact allergy, it was regarded as missing data.

3.2.6 Measurement of immunoglobulin E antibodies

Venous blood was taken on the day of examination and was left to coagulate for 2 h. The serum was then separated by centrifugation at 3000 r.p.m. for 10 min and frozen immediately afterward. The serum samples were analyzed for IgE specific to birch, grass (timothy), cat, and mite (Dermatophagoides pteronyssinus) with the ADVIA Centaur IgE antibody assay system (Bayer Corporation) [125]. The analysis for IgE antibodies was judged to be positive if the measurement was in excess of 0.35 kU/l. Specific IgE positivity was defined as a positive test to at least one of the four allergens tested.

3.2.7 Calculation of alcohol & tobacco consumption

One standard drink was assumed equivalent with one normal beer, one glass of wine, or one serving of spirits as they usually contain 12 gram/1.5 cL ethanol. As one strong beer contained 18 gram/1.5 cL ethanol, it was equivalent with 1½ standard drink. Total weekly consumption was calculated by adding the intake of beer, wine, and spirits.

The amount of tobacco in grams among current smokers were calculated for cigarettes, cheroots, cigars and pipe tobacco by equating one cigarette or one gram of pipe tobacco with 1 g tobacco, one cheroot with 3 g tobacco and one cigar with 5 g tobacco.

3.2.8 Questionnaire

Participating researchers prepared a questionnaire prior to the 1990, 1998 and 2006 studies. Overall, questions were identical although new ones were introduced and some were abandoned. Questionnaires were mailed to participants prior to health examination in 1998 and 2006 but not in 1990. At the day of health examination, the personnel or the secretary made sure that all

questions had been answered to ensure high response rates. Selected questions used in the questionnaire are displayed in table 4. Personal data and questions were used to define the following independent variables that were used in logistic regression analyses:

- Sex: “men”, “women” (study II, III, IV)
- Age-group: “18-35”, 36-55”, “56-69” (Study II and IV).
- Age-group: “15-34”, “35-49”, “50-69” (Study III).
- Study year: “1990”, “2006” (Study II).

- Ear-piercing status: “yes”, “no” (Study II, III, IV).

- Smoking status: “never”, “previously”, “≤15 g daily”, “>15 g daily” (Study III and IV).
- Lifetime smoking: “0 pack-years”, “≤15 pack-years”, “>15 pack-years” (Study III).
- Total alcohol consumption per week: “0”, “1-7”, “8-14”, “≥15” (Study III and IV).

- Vocational training: “yes”, “no” (Study III and IV).
- Years of educational: “≤9”, “10-11”, “12-13”, “≥14” (Study III).
- Educational level: “skilled or unskilled blue-collar workers”, “short-cycle higher education”, “medium higher education”, “long-cycle higher education”, “other education” (Study IV).

- Type of residence: “house”, “apartment”, “other” (Study III and IV).
- Ownership of residence: “yes”, “no” (Study III).

- Self-estimated social status: “very high”, “high”, “middle”, “below middle”, “low”,
- Social group: “self-employed”, “white-collar workers”, ”skilled blue-collar workers”, “unskilled blue-collar workers”, “other”. (Study III).

3.2.9 Statistical methods

Comparisons were generally made by using the chi-square (x^2) test. Logistic regression models were performed to adjust for known confounders when investigating associations between contact allergy and selected variables. All logistic regression models were performed with contact allergy as the dependent variable and with various independent variables as indicated above (3.2.8 Questionnaire). Associations were expressed as odds ratios (ORs) with confidence intervals (CIs) of 95%. Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, USA) for windows (release 15.0).

Table 4. Selected questions from the postal questionnaire.

Category	Group questioned	Question	Possible answers
Tobacco smoking	All participants	Do you smoke?	Yes, daily
			Yes, occasionally (less than 1 cigarette, or 1 cheerot, or 1 pipe of tobacco per day)
			No, but previously
			No, never
	Daily smokers only	Please indicate how much tobacco you smoke on average per day?	Number of cigarettes
			Number of cheroots
			Number of cigars
			Grams of pipe tobacco
Alcohol consumption	All participants	Have you consumed any alcoholic drinks during the past 12 months?	Yes
			No
	Drinkers within the past 12 months	How many of the following have you been drinking on average per week during the past 12 months?	Number of normal beer
			Number of strong beer
			Number of glasses of wine (1 bottle of wine equals 6 glasses)
			Number of glasses/units of spirits (standard drinks)
Ear piercing	All participants	Have you ever had your ears pierced?	Yes
			No
Vocational training	All participants	Have you ever had vocational training?	Yes
			No
	All participants	What is your educational level?	Skilled or unskilled blue-collar workers
			Short-cycle higher education (<3 years, e.g. dental technician and nursing assistants)
			Medium higher education (3-4 years, e.g. nurse, school teacher, and physiotherapist)
			Long-cycle higher education (>4 years, e.g. medical physician, psychologist, and engineer
			Other education
Social status	All participants	What is your self-estimated social status based on education, job, income, etc.	High
			Middle
			Below middle
			Low
			Very high
Type of residence	All participants	What kind of residence do you live in?	House
			Apartment
			Other

4 Results

4.1 Part 1: Clinical epidemiology & drug utilization research (CE-DUR) method

4.1.1 10-year contact allergy prevalence based on the period 2001-2005

Among 14 284 dermatitis patients tested between 2001 and 2005, 6 299 (44.1%) had at least one positive patch test reaction. The estimated 10-year prevalence of contact allergy is presented in table 5. The 10-year prevalence of contact allergy among adult Danes above 18-years ranged between 7.3 and 12.9% whereas the prevalence estimate for Danes of all ages ranged between 5.5% and 9.7%.

Table 5. 10-year prevalence estimates of contact allergy in the general population in Denmark. Worst, medium and best case scenarios for all age groups and adults > 18 years are presented. (Study I)

	10-year prevalence (%) Age > 18 years	**10-year prevalence (%) All ages**
Worst case	12.9	9.7
Medium case	9.5	7.2
Best case	7.3	5.5

The 10-year prevalences of contact allergy to individual allergens were calculated for the worst and medium case scenarios (table 6). The worst case scenario suggested that nickel was the most prevalent allergen in Denmark since 3.9% (all ages) and 3.7% (adults above 18-years) positive reactions were estimated.

Table 6. Selected 10-year prevalences of contact allergy based on patch test data from a 5-year period (2001-2005) in the Danish Contact Dermatitis Group (n=14 284) for both sexes. Prevalences in the general population were estimated on the basis of model I (worst case) and model III (medium case) and related to the total Danish population (5 400 000). (Study I)

	Population (%)		
Allergen	**Clinical patients**	**Model I (worst)**	**Model III (medium)**
Nickel sulphate	17.50	3.85	2.84
Fragrance mix I	7.70	1.69	1.25
MDBGN*	4.90	1.08	0.80
Cobalt chloride	4.50	0.99	0.73
Colophony	3.80	0.84	0.62
Myroxylon Pereira	3.50	0.77	0.57
Potassium dichromate	3.30	0.73	0.54
Carba mix	3.30	0.73	0.54
Formaldehyde	3.10	0.68	0.50
p-Phenylendiamine	2.40	0.53	0.39
Thiuram mix	2.30	0.51	0.37
MCI/MI **	1.90	0.42	0.31
Epoxy resin	1.70	0.37	0.28
Quaternium-15	1.60	0.35	0.26
Neomycin	1.60	0.35	0.26
Sesquiterpene lactone mix	1.50	0.33	0.24
p-tert-Butylphenol-formaldeyde resin	1.50	0.33	0.24
Quinolin mix	1.10	0.24	0.18
Ethylendiamine	1.00	0.22	0.16
Black rubber mix	1.00	0.22	0.16
Wool alcohol	1.00	0.22	0.16
Cliquinol	0.80	0.18	0.13
N-isopropyl-n-phenyl-p-phenylendiamine	0.60	0.13	0.10
Primin	0.60	0.13	0.10
Mercaptomix	0.60	0.13	0.10
Mercaptobenzothiazole	0.60	0.13	0.10
Paraben mix	0.50	0.11	0.08
Benzocaine	0.50	0.11	0.08

* MDBGN (methyldibromo glutaronitrile)
** MCI/MI = Methylchloroisothiazolinone (+)/methylisothiazolinone

4.2 Part 2: Cross-sectional & prospective patch test studies

4.2.1 Participation rate

Sex and age specific participation rates in the 1990 and 2006 studies are presented in table 7. Decreasing rates were observed in both sexes and all age-groups when the 1990 study was compared with the 2006 study. Especially young men had a low participation rate in the 2006 study (25.0%).

Table 7. The rate of participation among 753 and 7 931 subjects invited in 1990 and 2006, respectively to a cross-sectional study in Copenhagen, the Capital of Denmark. (Study II & IV)

	Participation rate % (attendants/invited)					
	Men			Women		
Age (years)	**1990**	**2006**	**Crude OR with 95% CI†**	**1990**	**2006**	**Crude OR with 95% CI†**
18-35	70.1 (94/134)	25.0 (265/1058)	0.14 (0.10-0.21)	78.7 (111/141)	38.4 (382/996)	0.17 (0.11-0.26)
36-55	79.5 (128/161)	43.0 (742/1724)	0.19 (0.13-0.29)	78.2 (129/165)	53.8 (938/1742)	0.33 (0.22-0.48)
56-69	81.3 (65/80)	45.9 (546/1189)	0.20 (0.11-0.35)	63.9 (46/72)	48.9 (598/1222)	0.54 (0.33-0.89)
Total	76.5 (287/375)	39.1 (1553/3971)	0.20 (0.15-0.25)	75.6 (286/378)	48.4 (1918/3960)	0.30 (0.24-0.39)

† Odds ratio (OR) with 95% confidence intervals (CI) comparing the age and sex specific rate of participation in 2006 to that in 1990.

4.2.2 Contact allergy prevalence based on a cross-sectional patch test study

Patch test results from 3 460 18-69 year olds tested in 2006 are presented in table 8. The overall prevalence of contact allergy was 10.0% (CI 95%=9.0-11.0). The most common contact allergens were nickel (5.9%), fragrance mix I (1.6%), colophony (0.6%) and thimerosal (0.5%). A positive patch test reaction to more than one allergen was observed among 21 (0.6%) subjects. All were nickel allergic and the most common combination was allergy to fragrance mix I and nickel (42.9%) whereas concomitant nickel and cobalt allergy was less common (14.0%).

4.2.3 Changes in the prevalence of contact allergy between 1990 and 2006

Patch test results revealed that the overall prevalence of contact allergy decreased from 15.5% (CI 95% = 12.4-18.5) in 1990 to 10.0% in 2006 ($p<0.001$). Significant decreases were observed in both men (1990=11.9% and 2006=4.7%, $p<0.001$) and women (1990=19.0% and 2006=14.2%, $p<0.05$). Furthermore, decreasing prevalences were observed in all male age-groups and in women aged 18-55 years whereas an increase was observed among older women aged 56-69 years (table 9). The increase in older women may be explained by a cohort effect following the Danish nickel regulation [81]. The observed decreases were mainly explained by a decrease in the prevalence of thimerosal allergy as 3.5% were allergic in 1990 in comparison to 0.5% in 2006. The decrease of thimerosal allergy was observed in all age-groups except among 56-69 year old women where a slight increase was registered from 0% to 0.2% (table 9). If thimerosal allergy was left out of the analyses, an overall decrease of contact allergy to at least one allergen was observed from 12.7% in 1990 to 9.6% in 2006 ($p<0.03$). Other prevalent allergens that also showed decreasing prevalences between 1990 and 2006 (table 8) included cobalt (1.1% to 0.2%), p-tert-butylphenol (PTBP) formaldehyde resin (1.1% to 0.1%), MCI/MI (0.7% to 0.2%), and rubber related allergens (1.5% to 0.2%). No significant increases were observed between 1990 and 2006.

A logistic regression analysis with "contact allergy to at least one allergen" as the dependent variable and with sex, age-group, study year and ear-piercing status as the independent variables

was performed (table 10). It showed that “contact allergy to at least one allergen” was associated with “female sex” (OR=2.11; CI=1.58-2.82) and that a positive trend test was identified for age (OR=1.18; CI=1.01-1.37). Finally, it showed that study year “2006” was inversely associated with contact allergy (OR=0.55; CI=0.42-0.78). A similar logistic regression analysis with “contact allergy to at least one allergen but not nickel” as the dependent variable and with sex, age, and study year as the explanatory variables showed that “contact allergy to at least one allergen but not nickel” was also associated with female sex (OR=1.33; CI=1.00-1.76) and inversely associated with study year “2006” (OR=0.43; CI=0.31-0.60). No association with age-group was identified. The outcome of the analysis did not change if the dependent variable was replaced with “contact allergy to at least one allergen but not nickel, fragrance mix I, and Myroxylon Pereirae” or with “contact allergy to at least one allergen but not thimerosal”. Thus, a decrease in the prevalence of contact allergy was observed between 1990 and 2006 for the categories “thimerosal” as well as “contact allergens from the TRUE-test panel and 2 but not nickel, fragrance mix I, and Myroxylon Pereirae”.

Table 8. The prevalence of contact allergy in 543 (1990) and 3 460 (2006) adults aged 18-69 years from the general population in Copenhagen, Denmark. (Study II)

	Positive reactions % (n)					
	Men		**Women**		**Total**	
Allergens and mixtures of allergens	**1990** (n=269)	**2006** (n=1 547)	**1990** (n=274)	**2006** (n=1 913)	**1990** (n=543)	**2006** (n=3 460)
Nickel sulphate	2.2 (6)	1.0 (15)	10.9 (30)	9.9 (189)	6.6 (36)	5.9 (204)
Wool Alcohols*	0.4 (1)	0	0	0	0.2 (1)	0
Neomycin Sulfate	0	0	0	0.1 (2)	0	0.1 (2)
Potassium Dichromate	0.7 (2)	0	0.4 (1)	0.3 (5)	0.6 (3)	0.1 (5)
Caine Mix	0	0	0	0.1 (2)	0	0.1 (2)
Fragrance Mix I	1.1 (3)	1.4 (21)	1.1 (3)	1.8 (34)	1.1 (6)	1.6 (55)
Colophony	0.4 (1)	0.7 (11)	1.1 (3)	0.5 (10)	0.7 (4)	0.6 (21)
Epoxy Resin	0.4 (1)	0.8 (12)	0.7 (2)	0.3 (6)	0.6 (3)	0.5 (18)
Quinoline mix*	0.4 (1)	0.1 (2)	0.4 (1)	0	0.4 (2)	0.1 (2)
Myroxylon pereirae*	0.7 (2)	0.1 (1)	1.5 (4)	0.1 (2)	1.1 (6)	0.1 (3)
Ethylenediamine Dihydrochloride	0.4 (1)	0.1 (2)	0	0.3 (6)	0.2 (1)	0.2 (8)
Cobalt Dichloride*	0.7 (2)	0.1 (1)	1.5 (4)	0.4 (7)	1.1 (6)	0.2 (8)
p-tert-Butylphenol Formaldehyde Resin*	1.1 (3)	0	1.1 (3)	0.1 (1)	1.1 (6)	0.1 (1)
Paraben Mix*	0.4 (1)	0	0.4 (1)	0	0.4 (2)	0
Carba Mix	0.7 (2)	0.1 (2)	0	0.1 (2)	0.2 (4)	0.1 (4)
Black Rubber Mix	0.4 (1)	0	0	0.2 (4)	0.2 (1)	0.1 (4)
MCI/MI *	0.4 (1)	0.3 (4)	1.1 (3)	0.1 (2)	0.7 (4)	0.2 (6)
Quaternium-15	0.4 (1)	0.2 (3)	0	0.2 (3)	0.2 (1)	0.2 (6)
Mercaptobenzothiazole* †	0.4 (1)	0	0	0	0.2 (1)	0
p-Phenylenediamine	0	0	0	0.2 (4)	0	0.1 (4)
Formaldehyde	-	0.1 (2)	-	0.3 (5)	-	0.2 (7)
Mercapto Mix*	0.7 (2)	0	0	0	0.4 (2)	0
Thimerosal*	3.7 (10)	0.4 (6)	3.3 (9)	0.6 (12)	3.5 (19)	0.5 (18)
Thiuram Mix*	0.7 (2)	0	0.4 (1)	0.2 (3)	0.6 (3)	0.1 (3)
Total	11.9 (32)	4.7 (73)	19.0 (52)	14.2 (272)	15.5 (84)	10.0 (345)

* = Allergens that decreased significantly between 1990 and 2006
† = MCI/MI = Methylchloroisothiazolinone (+)/methylisothiazolinone
\- = Formaldehyde not tested in 1990

Table 9. The prevalence of contact allergy* among adults from the general population in Copenhagen, Denmark (1990 and 2006). (Study II)

		Study year					
		Men			Women		
Age group (years)		**1990** n=269	**2006** n=1 547	**Crude OR with 95% CI†**	**1990** n=274	**2006** n=1 913	**Crude OR with 95% CI†**
		% (n)	% (n)		% (n)	% (n)	
18-35 n=837	Contact allergy to at least one allergen*	13.1 (11)	4.9 (13)	0.34 (0.15-0.80)	24.5 (26)	15.2 (58)	0.55 (0.33-0.93)
	Contact allergy to at least one allergen but not nickel*	8.5 (8)	4.2 (11)	0.47 (0.18-1.06)	9.9 (11)	5.2 (20)	0.50 (0.23-1.08)
	Contact allergy to thimerosal	4.8 (4)	0.4 (1)	0.08 (0.01-0.72)	3.8 (4)	0	-
36-55 n=1 917	Contact allergy to at least one allergen*	9.9 (12)	4.1 (30)	0.39 (0.19-0.78)	18.5 (23)	17.2 (161)	0.91 (0.56-1.48)
	Contact allergy to at least one allergen but not nickel*	8.6 (11)	2.8 (21)	0.31 (0.15-0.66)	13.2 (17)	6.1 (57)	0.42 (0.24-0.76)
	Contact allergy to thimerosal	3.3 (4)	0.3 (2)	0.08 (0.02-0.45)	4.0 (5)	1.2 (11)	0.29 (0.01-0.84)
56-69 n=1 249	Contact allergy to at least one allergen*	14.1 (9)	5.5 (30)	0.35 (0.16-0.59)	6.8 (3)	8.9 (53)	1.33 (0.40-4.45)
	Contact allergy to at least one allergen but not nickel*	12.3 (8)	5.1 (28)	0.38 (0.17-0.89)	4.3 (2)	4.3 (26)	1.00 (0.23-4.35)
	Contact allergy thimerosal	3.1 (2)	0.6 (3)	0.18 (0.03-1.07)	0	0.2 (1)	-

* = In 1990, formaldehyde was not included in the TRUE-test®.
OR = Odds ratio
CI = Confidence interval
† = Odds ratio (95% confidence interval) comparing the prevalence of contact allergy in 2006 to that in 1990

Table 10. Logistic regression analysis with the outcome "contact allergy"* and with different explanatory variables performed in 4 003 subjects patch tested in 1990 (n=543) and 2006 (n=3 460). (Study II)

Explanatory variables	**Adjusted OR † with 95% CI**
Study year:	
1990	1 (reference)
2006	0.55 (0.42-0.78)
Sex:	
Male	1 (reference)
Female	2.11 (1.58-2.82)
Age:	
18-35	1 (reference), * p<0.04
36-55	0.97 (0.76-1.26)
56-69	1.42 (1.04-1.94)
Ear piercing:	
No	1 (reference)
Yes	1.62 (1.22-2.16)

† = Mutually adjusted for variables shown in table.
* = p-value of trend test
OR = Odds ratio
CI = Confidence intervals

4.2.4 Association between alcohol consumption & contact allergy (Study III)

An association between alcohol consumption and contact allergy was investigated in the 1990 baseline study as well as in the 1998 follow-up study (figure 4). Table 11 and 12 show the baseline characteristics among 1 111 participants in the 1990 study. The prevalence of contact allergy and nickel allergy was substantially higher among women than among men (table 11). Alcohol abstinence as compared to drinking ≥ 1 drink/week was associated with female sex ($p<0.001$), a history of ear piercing ($p<0.001$) and never-smoking status ($p<0.02$) whereas no significant age differences were detected (table 11). Similarly, consumption of 1-7 alcoholic drinks/week was associated with female sex ($p<0.001$), young age (15-34 years) ($p<0.001$), a history of ear piercing ($p<0.01$) and never-smoking status ($p<0.01$). Finally, heavy drinking (≥15 drinks/week) as compared to drinking ≤15 drinks/week was associated with male sex ($p<0.001$), high age (50-69 years) ($p<0.001$), non-history of ear-piercing ($p<0.001$) and heavy smoking (≥15 /day) ($p<0.001$).

Table 13 shows the association between alcohol consumption and the prevalence of contact allergy to at least one of 23 allergens in 1990. Among men, no statistical significant association was found and the adjusted ORs were almost similar for drinkers and non-drinkers. Among women, the prevalence of contact allergy was significantly lower among non-drinkers (adjusted OR= 0.53; CI 95%=0.31-0.94) in comparison to women that consumed 1-7 drinks/week (reference group). However, the prevalence of contact allergy was not increased among women drinking 8-14 or ≥15 drinks/week. No relation between the type of alcoholic drink and contact allergy was found.

Table 14 shows the association of alcohol consumption with the incidence of contact allergy between 1990 and 1998. A total of 573 (292 men and 281 women) persons with negative patch test results in 1990 were patch tested again in 1998. Out of these, 69 (12%) developed a positive patch test reaction. No men were tested positive among non-drinkers and it was therefore impossible to calculate ORs for this category. Among women, individuals that reported no consumption of alcohol were more likely to develop contact allergy (adjusted OR=2.12; CI=0.98-4.61) during the 8 year follow-up period. A positive trend test among women was detected ($p=0.045$). No relation between the type of alcoholic drink and contact allergy was found.

Table 11. The prevalence of contact allergy (to at least one of 23 allergens), nickel contact allergy, allergic nickel contact dermatitis, a history of ear piercing, skin prick test reactivity, and smoking among women and men from the 1990 study. (Study III)

	Women	**Men**	**P-value †**
	% (n/total)	% (n/total)	
Contact allergy	23.2 (133/574)	10.4 (50/482)	< 0.001
Nickel allergy	15.2 (87/574)	1.5 (7/482)	< 0.001
Allergic nickel contact dermatitis *	13.8 (79/573)	0.8 (4/482)	< 0.001
Ear piercing	70.4 (419/595)	14.1 (73/517)	< 0.001
Skin prick test reactivity	38.1 (226/593)	48.0 (248/517)	< 0.001
Smoking status			
Never	35.3 (210/595)	30.6 (158/517)	< 0.001 (3 df)
Previously	16.6 (99/595)	23.6 (122/517)	
Current: <=15 g/day	33.4 (199/595)	20.5 (106/517)	
Current: > 15 g/day	14.6 (87/595)	25.3 (131/517)	
Lifetime smoking			
0 pack-years	38.5 (227/589)	33.6 (171/515)	< 0.001 (2 df)
<= 15 pack-years	40.4 (238/589)	30.7 (158/515)	
> 15 pack-years	21.1 (124/589)	35.7 (184/515)	

* Persons with a history of eczema from wearing earpins or earrings, under the tightener of one's watchstrap or under the button of one's jeans and a positive patch test to nickel were defined as cases of allergic nickel contact dermatitis.
† P-value of Chi-square test for the comparison of women and men.
df degrees of freedom.

Table 12. Baseline characteristics among 1 111 participants from the 1990 study.
Weekly alcohol consumption (drinks/week) stratified by sex, age, ear piercing and smoking. (Study III)

	Sex		**Age (year)**			**Ear piercing**		**Smoking**			
Alcohol consumption (drinks/week)	Men % (n)	Women % (n)	15-34 % (n)	35-49 % (n)	50-69 % (n)	No % (n)	Yes % (n)	Never smoker % (n)	Previous smoker % (n)	<15 g/day % (n)	>15 g/day % (n)
0	6.4 (33)	21.2 (126)	16.9 (73)	8.9 (34)	17.4 (52)	10.3 (64)	19.3 (95)	17.9 (66)	8.6 (19)	14.5 (44)	13.8(30)
1-7	47.6 (246)	60.4 (359)	63.7 (276)	50.5 (192)	46.0 (137)	50.2 (311)	59.9 (294)	61.4 (226)	54.3 (120)	57.9 (176)	38.1 (83)
8-14	20.9 (108)	14.3 (85)	12.7 (55)	24.2 (92)	15.4 (46)	19.5 (121)	14.7 (72)	13.0 (48)	18.6 (41)	20.4 (62)	19.3 (42)
>=15	25.1 (130)	4.0 (24)	6.7 (29)	16.3 (62)	21.1(63)	20.0 (124)	6.1 (30)	7.6 (28)	18.6 (41)	7.2 (22)	28.9 (63)
Total	100 (517)	100 (594)	100 (433)	100 (380)	100 (298)	100 (620)	100(491)	100 (368)	100 (221)	100 (304)	100 (218)
Chi-square	146,5			68.9		62.2		88.4			
p-value	<0.001			<0.001		<0.001		<0.001			

g/day = grams per day

Table 13. The association between alcohol consumption and the prevalence of contact allergy in the 1990 study. (Study III)

	Men			**Women**		
Alcohol consumption (drinks/week)	Contact allergy (%)	Odds ratio (95% CI)	Adjusted odds ratio† (95% CI)	Contact allergy (%)	Odds ratio (95% CI)	Adjusted odds ratio† (95% CI)
0	6.7 (2)	0.52 (0.12-2.23)	0.62 (0.13-2.83)	15.3 (19)	0.54 (0.31-0.95)	0.53 (0.31-0.94)
1-7	12.2 (28)	1.00	1.00	24.9 (85)	1.00	1.00
8-14	9.1 (9)	0.72 (0.33-1.59)	0.67 (0.30-1.51)	27.7 (23)	1.16 (0.67-1.99)	1.20 (0.68-2.20)
>= 15	8.9 (11)	0.71 (0.34-1.47)	0.67 (0.30-1.44)	25.0 (6)	1.00 (0.38-2.62)	0.90 (0.33-2.48)
	10.4 (50/482)			23.2 (133/573)		

† Adjusted for age, ear piercing and smoking.
CI = Confidence interval

Table 14. The effect of alcohol consumption on the incidence of contact allergy between 1990 and 1998. (Study III)

	Men			**Women**		
Alcohol consumption (drinks/week)	Contact allergy % (n)	Odds ratio (95% CI)	Adjusted odds ratio † (95% CI)	Contact allergy % (n)	Odds ratio (95% CI)	Adjusted odds ratio † (95%)
0	0 (0)	-	-	23.0 (14)	1.69 (0.82-3.48)	2.12 (0.98-4.61)
1-7	9.4 (13)	1.00	1.00	15.0 (27)	1.00 P=0.071 ‡	1.00 P=0.045 ‡
>=8	8.1 (11)	0.94 (0.38-2.23)	0.84 (0.37-1.96)	12.5 (4)	0.72 (0.23-2.26)	0.91 (0.28-2.89)
	8.2 (24/292)			16.0 (45/281)		

† Adjusted for age, ear piercing and smoking.
‡ P-value for test for trend
CI = Confidence interval

4.2.5 Alcohol consumption, tobacco smoking & nickel allergy (Study IV)

Characteristics of the 2006 study population according to sex are presented in table 15. The prevalence of ear piercing was markedly higher among women than among men whereas men consumed significantly more alcohol than women. The prevalence of never smokers and previous smokers was nearly identical among women and men whereas the prevalence of current light smokers (i.e. current smokers: ≤15 grams/day) was higher among women than men (9.5% versus 16.3%) and the prevalence of current heavy smokers (current smokers: >15 grams/day) was higher among men than women (12.6% versus 7.9%).

Table 16 shows the 2006 baseline characteristics of participants stratified by smoking status: The proportion of current light smokers was higher among subjects who were ear-pierced or were nickel allergic in comparison to subject who were not ear-pierced and who were not nickel allergic. Alcohol consumption tended to increase with smoking status and the proportion of current heavy smokers was higher among subjects with a short education.

Crude data analyses without adjustment for potential confounders showed that nickel allergy was significantly associated with female sex, ear-piercing, alcohol consumption (≥15 drinks per week), and tobacco smoking (table 17). The relationship between nickel allergy and educational level revealed no clear pattern except a higher prevalence of nickel allergy among subjects with a short-cycle higher education. It was evaluated whether it could be assumed that the effects of smoking were independent of sex. Thus, a logistic regression model was performed with nickel allergy as the dependent variable, and with sex, age-group ("18-35 years","36-55 years", "56-69 years"), smoking status ("never smokers", "previous smokers", "current smokers ≤15 g/day", "current smokers >15 g/day"), and an interaction term between sex and smoking status as the independent variables. It did not reveal any significant interaction between sex and smoking status (p=0.97) which means that the possible effect of smoking status on the prevalence of nickel allergy did not differ between men and women.

In order to examine possible confounding, several logistic regression models were performed in which one variable where added at a time while observing changes in the risk estimates for the exposure variables (smoking and alcohol consumption) (table 17). The regression analyses revealed that ear-piercing was an important risk factor for nickel allergy which indicates that nickel allergy to a high degree is an environmental disorder. Thus, when adjusting for ear-piercing status, the association between female sex and nickel allergy was dramatically lowered. Furthermore, the analyses showed that alcohol consumption was not associated with nickel allergy whereas a significant trend ($p<0.05$) was identified between smoking status and nickel allergy in the fully adjusted model (i.e. nickel allergy was higher among both previous smokers (OR=1.19; CI=0.81-1.76), current light smokers (OR=1.50; CI=0.94-2.37) and current heavy smokers (OR=1.56; CI=0.87-2.80) as compared to never smokers).

Finally, similar logistic regression analyses were performed with "contact allergy to at least one allergen" and "contact allergy to at least one allergen but not nickel", respectively, as the independent variable and with the explanatory variables listed in table 17. These analyses did not show any significant associations between contact allergy on one hand and alcohol consumption or smoking status on the other hand. Thus, the fully adjusted regression analysis with contact allergy to at least one allergen as the dependent variable revealed a non-significant trend test for smoking status ($p<0.6$) (data not shown). However, a positive association with smoking status was still identified (i.e. contact allergy was higher among both previous smokers (OR=1.24; CI=0.92-1.66), current light smokers (OR=1.14; CI=0.78-1.67) and current heavy smokers (OR=1.04; CI=0.65-1.69) as compared to never-smokers).

Table 15. Sex specific characteristics regarding contact allergy (to at least one of 24 allergens), nickel contact allergy, a history of ear piercing, specific immunoglobulin (Ig)E status, alcohol consumption, smoking status, and educational level. Data was based on a general health examination including patch testing performed among 3 471 18-69 year old participants from a cross-sectional study performed in Copenhagen, Denmark between 2006 and 2008. (Study IV)

	Men % (n/total)	**Women** % (n/total)	**P-value †**
Contact allergy to at least one allergen:	4.7 (73/1547)	14.2 (272/1913)	0.001
Nickel allergy:	1.0 (15/1495)	10.3 (189/1913)	0.001
Ear piercing:	17.0 (261/1538)	82.2 (1564/1902)	0.001
Specific immunoglobulin E:‡	27.3 (418/1531)	20.0 (378/1889)	0.001
Alcohol consumption (drinks per week within past 12 months) :			
0	9.0 (138/1532)	19.2 (367/1912)	< 0.001
1-7	33.7 (516/1532)	53.0 (1013/1912)	
8-14	24.3 (372/1532)	17.7 (338/1912)	
≥ 15	33.0 (506/1532)	10.1 (194/1912)	
Smoking status:			
Never smokers	43.3 (640/1478)	43.1 (795/1846)	< 0.001
Previous smokers	34.6 (512/1478)	32.7 (604/1846)	
Current smokers <=15 g/day	9.5 (140/1478)	16.3 (301/1846)	
Current smokers > 15 g/day	12.6 (186/1478)	7.9 (146/1846)	
Educational level:			
Skilled or unskilled blue-collar workers	44.8 (602/1345)	37.3 (609/1633)	< 0.001
Short-cycle higher education	14.1 (189/1345)	20.1 (328/1633)	
Medium higher education	17.4 (234/1345)	26.1 (426/1633)	
Long-cycle higher education	13.4 (180/1345)	7.5 (122/1633)	
Other	10.4 (140/1345)	9.1 (148/1633)	

† = P-value of Chi-square test for the comparison of women and men.
‡ = Analysis for IgE specific to birch, grass (timothy), cat, and mite (Dermatophagoides pteronyssinus). The analysis was judged to be positive if the measurement was in excess of 0.35 kU/l.

Table 16. Characteristics of 3 471 participants from a cross-sectional study performed in Copenhagen in 2006 grouped by smoking status. (Study IV)

	Smoking status				**P-value†**
	Never smokers % (n/total)	**Previous smokers** % (n/total)	**Current smokers ≤15 g/day** % (n/total)	**Current smokers >15 g/day** % (n/total)	
Age (years):					
18-35 (n=593)	57.8 (343)	20.4 (121)	14.2 (84)	7.6 (45)	0.001
36-55 (n=1613)	39.7 (641)	35.0 (565)	13.9 (224)	11.3 (183)	
56-69 (n=1118)	40.3 (451)	38.5 (430)	11.9 (133)	9.3 (104)	
Ear-piercing:					
Yes (n=1752)	38.7 (678)	34.1 (598)	17.1 (300)	10.0 (176)	0.001
No (n=1563)	48.0 (751)	33.0 (516)	9.0 (140)	10.0 (156)	
Nickel allergy:					
Yes (n=1752)	31.4 (678)	36.6 (598)	21.1 (300)	10.8 (176)	0.001
No (n=1563)	44.4 (751)	33.2 (516)	12.6 (140)	9.7 (156)	
Alcohol consumption (drinks/week within past 12 months):					
0 (n=472)	43.2 (204)	31.8 (150)	12.5 (59)	12.5 (59)	0.001
1-7 (n=1484)	50.1 (743)	30.1 (447)	12.7 (188)	7.1 (106)	
8-14 (n=673)	39.5 (266)	36.4 (245)	15.5 (104)	8.6 (58)	
≥15 (n=668)	31.0 (207)	40.0 (267)	13.0 (87)	16.0 (107)	
Educational level:					
Skilled or unskilled blue collar worker (n=1174)	38.4 (451)	35.2 (413)	14.1 (166)	12.3 (144)	0.001
short cycle higher education (n=499)	37.7 (188)	36.5 (182)	14.0 (70)	11.8 (59)	
Medium cycle higher education (n=631)	46.0 (290)	37.1 (234)	11.6 (73)	5.4 (34)	
Long cycle higher education (n=289)	64.7 (187)	24.9 (72)	6.2 (18)	4.2 (12)	
Other education (n=275)	44.4 (122)	35.3 (97)	12.7 (35)	7.6 (21)	

† = P-value of Chi-square test for the comparison of different categories of smoking status.

Table 17. The relationship of different potential risk factors to the prevalence of nickel allergy. (Study IV)

	Nickel allergy % (n/total)	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted OR†† (95%)	Adjusted OR††† (95%)	Adjusted OR†††† (95%)
Smoking status						
Never smokers	4.4 (61/1397)	1.00	1.00, * p<0.001	1.00, * p<0.005	1.00, * p<0.009	1.00, * p<0.05
Previous smokers	6.6 (71/1071)	1.56 (1.09-2.21)	1.60 (1.11-2.91)	1.45 (1.00-2.09)	1.41 (0.98-2.05)	1.19 (0.81-1.76)
Current smokers <=15 g/day	9.7 (41/421)	2.36 (1.57-3.57)	1.91 (1.25-2.31)	1.72 (1.13-2.63)	1.65 (1.08- 2.53)	1.50 (0.94-2.37)
Current smokers > 15 g/day	6.7 (21/313)	1.58 (0.94-2.63)	1.97 (1.15-3.35)	1.78 (1.04-3.05)	1.73 (1.01-2.98)	1.56 (0.87-2.80)
Sex						
Men	1.0 (15/1495)	1.00	1.00	1.00	1.00	1.00
Women	10.3 (189/1843)	11.27 (6.63-19.16)	11.03 (6.36-19.16)	5.50 (2.95-10.2)	5.83 (3.10-10.97)	5.55 (2.85-10.81)
Age (years)						
18-35	7.2 (45/622)	1.00	1.00	1.00	1.00	1.00
36-55	7.9 (128/1625)	1.10 (0.77-1.56)	1.04 (0.71-1.52)	1.09 (0.75-1.60)	1.03 (0.70-1.52)	0.99 (0.65-1.51)
56-69	2.8 (31/1091)	0.38 (0.24-0.60)	0.41 (0.25-0.64)	0.48 (0.29-0.79)	0.45 (0.27-0.74)	0.41 (0.24-0.73)
Ear piercing						
No	1.2 (19/1567)	1	-	1.00	1.00	1.00
Yes	10.6 (184/1741)	9.63 (5.97-15.52)	-	3.35 (1.89-5.96)	3.44 (1.93-6.13)	3.01 (1.66-5.46)
Alcohol consumption (drinks/week within past 12 months)						
0	7.6 (36/475)	1.00	-	-	1.00	1.00
1-7	6.7 (98/1472)	0.87 (0.59-1.29)	-	-	1.02 (0.67-1.56)	0.96 (0.61-1.52)
8-14	6.4 (44/683)	0.84 (0.53-1.32)	-	-	1.42 (0.86-2.34)	1.33 (0.77-2.29)
>= 15	3.8 (26/682)	0.48 (0.29-0.81)	-	-	1.34 (0.77-2.37)	1.05 (0.56-1.97)
Educational level						
Skilled or unskilled blue collar worker	5.8 (67/1151)	1.00	-	-	-	1.00
Short cycle higher education	9.3 (46/495)	1.66 (1.12-2.45)	-	-	-	1.16 (0.76-1.76)
Medium cycle higher education	6.4 (41/638)	1.11 (0.74-1.66)	-	-	-	0.87 (0.57-1.33)
Long cycle higher education	4.0 (12/297)	0.68 (0.36-1.28)	-	-	-	0.71 (0.33-1.49)
Other education	5.3 (15/281)	0.91 (0.51-1.62)	-	-	-	0.99 (0.54-1.84)

† = Logistic regression analysis adjusted for sex, age and smoking.
†† = Logistic regression analysis adjusted for sex, age, smoking, and ear- piercing.
††† = Logistic regression analysis adjusted for sex, age, smoking, ear-piercing, and alcohol consumption.
†††† = Logistic regression analysis adjusted for sex, age, smoking, alcohol consumption, ear- piercing and educational level.
* = Trend test
OR = Odds ratio
CI = Confidence intervals

5 Discussion

5.1 Contact allergy prevalence

5.1.1 The overall prevalence of contact allergy among adult Danes.

When applying the CE-DUR method in Denmark, the 10-year prevalence of contact allergy was estimated to 7.3-12.9% (adults >18 years) based on data from the period 2001-2005. According to the previous German CE-DUR study, the worst case estimate may be the most accurate [83]. Thus, the CE-DUR analysis suggested that 12.9% of adult Danes had contact allergy over a 10-year period. In comparison, the cross-sectional patch test studies in Glostrup showed that among 18-69 year olds, the prevalence of contact allergy was 15.5% in 1990 and 10.0% in 2006. At first sight, the CE-DUR estimate therefore seems to be a rapid and in-expensive way to monitor the overall prevalence of contact allergy. That said, there are many factors that should be taken into consideration when these prevalence estimates are interpreted and compared.

5.1.2 Methodological considerations when comparing prevalence estimates

The 10-year prevalence estimate generated via the CE-DUR method relies on the quality of available information regarding patch test sales- and reading data as well as the accuracy of assumptions. It is estimated that the validity of this information in the Danish CE-DUR study was generally good. However, only patch test data from a 5-year period was included and it is possible that a longer sampling period would reveal a more accurate prevalence estimate (as more dermatitis patients would be included). Also, the representativity of the DCDG database can be questioned as it (at the time) only covered approximately 12% of patch tested subjects in Denmark. Furthermore, the DCDG- and the Glostrup study patch test data hold some inherent inaccuracies and differences when they are used for comparison: Firstly, the use of different patch test systems may have influenced the estimated prevalence of contact allergy as the TRUE-test®, depending on the allergen tested, may have a lower as well as a higher sensitivity in comparison to conventional test systems (table 1) [67;68]. Secondly, patch test readings were performed on either day 3 or 4 in the DCDG clinics whereas readings were only done on day 2 in the Glostrup studies. This could tend to lower the prevalence estimates generated in the Glostrup studies in comparison to the CE-DUR estimate. Thirdly, allergens used for patch testing within the DCDG and in the Glostrup studies differed as e.g. MDBGN but not thimerosal were included in the DCDG patch test series and vice versa in the Glostrup studies. Fourthly, the DCDG patch test database had a higher proportion of female patients in comparison to the samples in the Glostrup studies, which would tend to increase the overall prevalence of contact allergy in the CE-DUR estimate (as contact allergy is more common in women than in men). Fifthly, subjects in the Glostrup studies were 18-69 years old whereas the CE-DUR estimate was based on persons above 18 years. Finally, several assumptions were made in the CE-DUR study based on available evidence. It is evident that they hold inaccuracies but it is also conceivable that they may have a fair validity. Finally, it should be noted that after the Danish CE-DUR study was made, a Danish questionnaire study showed that 67% and 44% of subjects with hand eczema consulted their general practitioner and dermatologists, respectively [126]. The number of persons that were also patch tested was not given.

One should be aware that the CE-DUR method estimated the 10-year prevalence of contact allergy and not the prevalence of contact allergy in a traditional manner (as in e.g. the Glostrup allergy studies). The 10-year prevalence estimate is less straightforward conceptually: Although patients are commonly patch tested for incident allergic contact dermatitis (but sometimes for chronic suspected allergic contact dermatitis), the spectrum of contact allergy diagnosed upon patch testing does not necessarily relate exclusively to the current episode of allergic contact dermatitis. Moreover, allergic contact dermatitis is far from always confirmed after patch testing, with positive test results relating to past episodes of allergic contact dermatitis, or having

uncertain clinical relevance in these cases. Hence, the contact allergy frequencies derived from patch test databases cannot be interpreted as contact allergy incidence rates (although an incidence rate may be an appropriate measure for the continual surveillance of contact allergy). Rather, the CE patch test data and the DUR estimation of the annual number of patients eligible for patch testing can be regarded to represent prevalence, estimated during a sampling period of several years. In this period, which was set to a standard of 10 years in our analysis, "nearly all" patients from the general population, potentially affected by contact allergy, are assumed to consult a dermatologist and be patch tested. Basically, it is assumed that 10 years is a reasonable sampling period during which incident cases accumulate in the clinical networks. This is of course an arbitrary number and if e.g. 8 or 12 years was used instead the prevalence estimate would have changed accordingly. However, as long as the same sampling period is used when the CE-DUR method is used, e.g. in Denmark and Germany, the estimates may be used for comparison.

Table 18. The type, source, year span and validity of data, and its resulting correction factor, collected for the estimation of the 10-year prevalence of contact allergy in Denmark using the CE-DUR method. (Study I)

Data type	Data source	Period of data collection	Resulting correction factor (%)	Validity of data
National patch test sales data	Chemotechnique, Hermal & MEKOS Laboratories	1997-2006	-	Good
Patch test reading data	Danish Contact Dermatitis Group database	2001-2005	-	Good
Proportion of discarded test	Laboratory staff at Gentofte Hospital	2005-2006	2.5 (0-5)	Good
Proportion of diseased persons that seek medical consultation	National Institute of Public Health in Denmark [127]	1987-1994	25 (20-30)	Fair
Persons previously tested	Danish Contact Dermatitis Group database	2001-2005	10 (5-15)	Fair
Magnitude of the Danish Population	Statistics Denmark	2006	-	Good

- = Not applicable.

The CE-DUR study also estimated the prevalence of various contact allergies (table 6). Although, these estimates may be interesting to compare between countries, e.g. Denmark and Germany, they may not be very useful for comparison with patch test data from the Glostrup studies due to different distributions of age and sex. Also, the CE-DUR prevalence estimates were not calculated in men and women separately which would have been necessary to make proper comparisons. Two studies have investigated the correlation between patch test results from the general population and from patients suspected with allergic contact dermatitis. The first study delineated the relationship through an extensive literature review in hope of opening the door to more specific investigations of contact allergy in general populations and, ultimately, to use this information for refining the clinical relevance of predictive toxicologic assays [128]. The second study compared the prevalence of contact allergy to specific allergens among unselected individuals as well as patients in Augsburg, Germany and made further comparisons with patient patch test

data from the entire IVDK network [129]. Both studies identified a differential effect of selection until presentation in dermatological departments and found that certain exposures (e.g. Neomycin) were greater in patients than in the general population. Finally, materials of ubiquitous exposure, e.g. fragrances and nickel had more similar prevalences among dermatitis patients and subjects from the general population. The above perspectives are interesting when one interprets and compares the patch test results from the CE-DUR- and the 2006 Glostrup study.

5.1.3 Future use of the CE-DUR method

The CE-DUR methods may be a useful future tool in countries that have an organized system of patch test clinics but where large, expensive cross-sectional patch test studies are not performed. It could possibly be repeated every 10 years to estimate the population at risk of allergic contact dermatitis. Furthermore, the CE-DUR method was recently used in a reverse manner to make delineations between the 10-year prevalence of contact allergy in the general population and the corresponding theoretical prevalences of contact allergy observed among patients with dermatitis in Denmark and Germany [130]. The backward CE-DUR tool is different from the normal CE-DUR method as it does not apply CE data. However, as for the traditional CE-DUR, the validity of the outcome largely depends on the accuracy of the correction factors. Results indicated that if 1/100 subjects in the general population in Denmark and Germany had contact allergy; dermatologists would observe a prevalence of contact allergy among dermatitis patients was between 4.5-8.0% and 2.5-10.4%, respectively. The reverse CE-DUR approach may therefore be used to alert public health authorities if the estimated number of sensitized persons in the general population is above a certain threshold. In further discussion about acceptable risk of contact allergy in the general population, a categorization of contact allergy epidemics was suggested (table 20)[130]. Thus, nickel may be regarded as an allergen that has caused an outbreak whereas fragrance mix allergy is characterized as a generalized epidemic.

5.1.4 Perspectives

Although the CE-DUR method holds much potential, the method still needs further validation in other countries than Denmark and Germany. The accuracy of assumptions should also be further investigated. The CE-DUR method can not completely replace cross-sectional patch test studies as one can not test for associations. The CE-DUR- as well as the Glostrup studies estimated the overall (10-year) prevalence of contact allergy but their accuracy was limited by the methods used. Thus, a more accurate contact allergy prevalence estimate would demand a cross-sectional study where a large representative sample with a high participation rate was patch tested with the European baseline series and with readings performed on at least 2 occasions. If such an approach had been used among adult Danes, it is likely that a higher overall prevalence of contact allergy had been demonstrated.

Table 19. Categorization of contact allergy epidemics [130].

Number of contact sensitized subjects in the general population:	**Epidemic category**
> 1/20	Outbreak
> 1/100	Generalized
> 1/1.000	Concentrated
> 1/10.000	Low level
> 1/100.000	-
> 1/1.000.0000	-

5.1.5 Changes in the prevalence of contact allergy between 1990 and 2006

The 1990 and 2006 studies showed that the overall prevalence of contact allergy to allergens of the TRUE-test (panel 1 and 2) decreased in Denmark from 15.5% in 1990 to 10.0% in 2006. When the recent estimate was compared to prevalence estimates from past studies performed in other general populations, it was evident that the 2006 prevalence was much lower. However, one should be aware that the Glostrup study estimates traditionally have been lower than those from other countries (table 2). This finding may owe to the use of day 2 patch test readings only; to conservative patch test readings in the Glostrup study; to an overall lower prevalence of contact allergy in Denmark in comparison to other countries; or to the use of different patch test systems. The strength of the two studies was that identical patch test methods were used. Also, seasonal influence was expected to be limited as sampling was performed throughout the year. So far, only one other repeated patch test study has investigated the development of the prevalence of contact allergy in the same general population. A comparison between two samples of 15-41 year olds in Glostrup (figure 4), showed that the prevalence increased from 15.9% in 1990 to 18.6% in 1998 [77].

The overall low participation rate in the 2006 study was problematic as it is possible that selection bias influenced the results. This could tend to overestimate the burden of contact allergy in the general population. However, patch testing was only a little part of the approximately 2 hour long general health examination that participants underwent in the 2006 study. Therefore, selection could rather concern e.g. cardiovascular diseases or other large disease entities. To challenge these speculations, one could perform e.g. phone interviews in non-participants. Furthermore, the representativity of the study populations was unknown. Thus, the prevalence estimate can not be directly transferred to the entire Danish population. However, it is conceivable that the overall homogenous Danish population is fairly represented in this sample.

Stratification by sex and age-group revealed decreasing prevalences of contact allergy between 1990 and 2006 in all male age-groups and in the young and middle-aged female age-groups (18-55 years) whereas increasing prevalences were observed among older women (56-69 years) (table 9). The diverging trend observed in young and middle-aged women versus older women was probably explained by a cohort effect due to a change in the prevalence of nickel allergy as it was decreasing in young women ear-pierced later than 1990 (i.e. after the Danish nickel regulation was passed) but increasing in women ear-pierced before 1990 [81]. However, when nickel allergy (and also fragrance mix I and Myroxylon Pereirae) was omitted from the analysis, a significant decrease was still observed in most age-groups between the two study years which owed to decreasing prevalences of other allergens (table 8 and 9). Of note, female sex remained associated with contact allergy even when nickel (and also fragrance mix I and Myroxylon Pereirae) was omitted from the logistic regression analysis. This finding suggests that women may have a heavier exposure to chemicals than men and therefore a higher prevalence of contact allergy [131].

A weakness of the study was the significantly decreasing rate of participation in all age-groups from 1990 to 2006. To limit the influence of bias, analyses were stratified by age-group and sex, and therefore the observed trends were unlikely to be explained by differences in these variables between the studies. However, it can not be ruled out that other characteristics differed between participants in the two studies (leading to an over- or underrepresentation of sensitized subjects). Although we were unable to stratify for these unknown factors, we believe that data on the prevalence of contact allergy in repeated cross-sectional general population studies is likely to more reliably show the development of time trends of contact allergy than data obtained in series of patients admitted to specialized clinics. However, it should be acknowledged that increasing rates of non-participation in general population studies represents an important limitation.

The overall decrease of contact allergy to the allergens in the TRUE-test (panel 1 and 2) was mainly explained by a significant decrease in the prevalence of thimerosal allergy (table 8). The decrease of thimerosal allergy between 1990 and 2006 was observed in all age-groups among men whereas a slight increase was observed among older women (56-69 years), perhaps owing to a

cohort-effect. Thimerosal allergy is known to be prevalent in the general population due to its use as a preservative in vaccines and ophthalmic solutions [106]. Furthermore, thimerosal has traditionally been widely used in biologics and vaccines in the United States which has resulted in a significant increasing prevalence of thimerosal allergy among North American dermatitis patients between 1984 and 1994 [132]. However, positive patch test reactions to thimerosal are generally very poor predictors of dermatitis reactions to thimerosal-containing vaccines [116]. In Denmark, thimerosal allergy mainly derives from childhood vaccination as it has been an ingredient in vaccines since the 1950's and until March 1992. Since the Danish childhood vaccination program is voluntary and free of charge, the vast majority of Danish children have been exposed to thimerosal through 4 decades. Furthermore, thimerosal may be an ingredient in vaccines against e.g. hepatitis and influenza virus. However, steps have been taken in Denmark and in the rest of the world to strongly reduce or totally remove thimerosal from vaccines as thimerosal is a mercuric compounds that may be nephrotoxic and neurotoxic at high doses. Furthermore, it has been suspected of causing neurodevelopmental disorders such as autism although this association has been rejected [133]. The observed decrease of thimerosal allergy in Denmark is interesting as it shows that when exposure to a contact allergen (whether nickel, thimerosal or other) is strongly reduced by an administrative initiative, a clear decrease of contact allergy can be registered in the general population.

Besides the decrease of thimerosal allergy, the overall decrease of contact allergy between 1990 and 2006 was explained by a decrease in the prevalence of allergy to nickel, cobalt, MCI/MI, PTBP formaldehyde resin, and rubber related allergens. The observed decreases were all significant (except for nickel) but it may off course be a result of random error. Furthermore, one should remember that the sample size was small when compared to clinical databases. It is possible that the Danish general population is less exposed to contact allergens today than almost 20 years ago as a result of personal precautions and protection in an increasingly educated population. Cobalt is a hard metal that is common in combination with other metals such as nickel, chromium and tungsten to increase hardness and durability. Because it is often mixed with, or is an impurity in other metals, cobalt allergy may go along with nickel allergy in women or chromate allergy in men [134]. It has been suggested that nickel sensitivity and irritant hand eczema precede cobalt allergy in metal workers whereas cross-sensitization is rare [135]. Ear-piercing has been associated with cobalt allergy in the general population [91]. Some 11 (78.6%) of 14 positive cobalt reactions were observed in women in 1990 and 2006. However, 7 women and 2 men were sensitized to cobalt only. The findings therefore suggest that combined cobalt and nickel allergy is not that prevalent in the general population. It has been speculated whether cobalt has replaced nickel in jewelry after the introduction of the nickel regulation [136]. In comparison, the prevalence of concomitant patch test reactivity to cobalt and nickel is much higher among patients with dermatitis [137]. This area needs further attention to clarify the significance of these finding.

The decrease of allergy to rubber related allergens may possibly be a result of the focus on rubber gloves during the 1990's [138]. Rubber manufacturers reduced the content and use of accelerators as they are considered to be the most frequent contact sensitizer in rubber gloves. Hence, the use of thiurams were strongly reduced in single use natural rubber latex gloves [138]. The decrease observed in the general population in Denmark parallels the decrease observed among dermatitis patients from the Gentofte University Hospital between 1995 and 2004 [138]. Also, the prevalence of thiuram mix, mercapto mix, mercaptobenzothiazole, and carba mix allergy has decreased recently among dermatitis patients in the US [132;139]. The prevalence of chromate allergy in men decreased from 0.7% in 1990 to 0% in 2006 whereas it remained stable in women with 0.4% and 0.3% positive patch test reactions in 1990 and 2006, respectively. The decrease in the prevalence of chromate allergy in men could possibly be explained by an effect of the cement chromate regulation in Denmark [140] whereas the persistence of chromate allergy in women may be explained by continuous exposure to chromate in leather goods [141]. Finally, since patch test readings were only performed on day 2, the prevalence of allergy to late reacting allergens such as p-phenylenediamine (PPD) and neomycin may be underestimated. To better study such allergens properly, later readings should be carried out.

5.2 Part 2: Association between contact allergy & life-style factors

5.2.1 Contact allergy & tobacco smoking

The 2006 association study showed that nickel allergy was significantly associated with tobacco smoking. This association was dose-dependent and independent of sex. The results were in line with those from another cross-sectional population-based study performed in 1 056 Danish adults [90] and were also supported by a Norwegian patch test study in which a significant association with contact allergy was identified in adult women [74].

It is important to evaluate to which extent confounding by other factors could explain the positive association observed between smoking and nickel allergy (table 17). The association remained relatively unchanged after adjustment for confounders by multivariable regression analyses although it can not be ruled out that residual confounding (insufficient adjustment) or confounding by factors not included in this study could play a role. When the logistic regression analysis was adjusted for educational level, the association between smoking and nickel allergy was weakened. Thus, it is possible that we were not able to sufficiently adjust for social status in our analyses as an association between nickel allergy and socio-economic status has been suggested previously [98]. A further limitation of the study was that questions on smoking status and educational level have not been validated previously. Thus, it is possible that the study outcome may have been biased. Finally, the results may be a result of random error although the consistency of the association in three studies supports a true association.

The 2006 study did not identify any significant associations between smoking status and ”contact allergy to at least one allergen but not nickel” and “contact allergy to at least one allergen”, respectively. It should be emphasized that the prevalence of contact allergy to other contact allergens than nickel was low in this general population (table 8). This will necessarily lead to reduced statistical power in the regression analyses. However, a previous Danish study also showed that nickel allergy had a slightly stronger association with smoking than contact allergy to at least one allergen [90]. The stronger association observed for nickel allergy may be explained by the fact that nickel is found in tobacco plants as a result of absorption from soil, fertilizing products or pesticides. Furthermore, the nickel content in cigarettes and tobacco is high regardless of its kind and origin [142]. One study examined the nickel concentration in 123 blood samples and 147 urine samples from smokers and non-smokers. It revealed a significantly higher concentration of nickel in the urine but not in the blood of smokers in comparison to non-smokers [142]. It is therefore possible that T-cells in smokers are exposed to nickel in concentrations that may lead to nickel allergy. However, nickel exposure from cigarettes is probably of minor importance in terms of inducing nickel contact allergy as the prevalence of nickel allergy in men was approximately 1% whereas nearly 50% of men reported current or previous smoking.

5.2.2 Contact allergy & alcohol consumption

The 1990 and 1998 studies revealed an inverse dose-response relationship between alcohol consumption and incident contact allergy among women (i.e. women that consume alcohol were less likely to develop contact allergy than non-drinkers) (table 14). A possible association could not be evaluated among men since the number of incident positive patch test reactions was too low. Furthermore, it appeared that alcohol abstinence was associated with a lower prevalence of contact allergy among women in the 1990 baseline study (table 13). The findings of the 1990 cross-sectional and the 1990-1998 prospective analyses were contradictory. The reason for this discrepancy was not clear. Furthermore, the 2006 study did not identify any association between alcohol consumption and the prevalence of nickel allergy (or contact allergy). No other epidemiological studies on the possible association between alcohol consumption and contact allergy have been performed.

In general, the results of prospective analyses are considered as more reliable when determining associations and cause-effect relationships, as they are less prone to bias and confounding. Confounding was expected to be limited since independent well-known determinants of contact allergy were included in the analyses (i.e. female sex, history of ear-piercing and cigarette smoking). Also, adjustment for socio-economic factors was performed although its influence on contact allergy is generally considered to be limited. A possible source of bias in the studies could be that persons with excess alcohol consumption were less likely to participate. Also, participants were only asked about alcohol consumption within the past 12 months. If alcohol consumption has an effect on the prevalence of contact allergy, life-time alcohol consumption may be of relevance. However, a strength was that the questions used for assessment of alcohol consumption were previously validated against increased levels (≥80 IU/L) of serum γ-glutamyl transferase (GGT), a marker of alcohol exposure [143]. The results revealed that self-reported total alcohol intake (total number of drinks/week) was significantly and positively associated with increased levels of GGT [144]. It could be of interest to take into account genetic variations in alcohol metabolism as certain genetic variations may influence both alcohol drinking behaviour and susceptibility to the immunological effects of alcohol [54]. Husemoen et al., showed that an aldehyde dehydrogenase variant (ALDH1b ala69val) was associated with nondrinking as well as total alcohol intake. Furthermore, an aldehyde dehydrogenase promoter variant (ALDH2) was associated with binge-drinking. Such genetic influence could tend to bias associations between alcohol and immune effects. Finally, random error can not be out ruled as the results were diverging in the two study designs. In conclusion, an effect of alcohol consumption on the prevalence of contact allergy could not be determined based on the present studies. To investigate a possible association, a large prospective study using supplementary questions on alcohol consumption should be performed. Whether this should cover more than 8 years is likely as incident contact allergy is relatively low.

6 Conclusion

This thesis investigated the prevalence of contact allergy using two different epidemiological tools. The CE-DUR method used national patch test sales information as well as clinical data and assumptions to estimate the 10-year prevalence of contact allergy (12.9%) among adult Danes for the period 2001-2005 whereas a cross-sectional patch test study from 2006-2008 estimated the prevalence of contact allergy to panel 1 and 2 from the TRUE-test® (10.0%) among 18-69 year olds. A comparison with patch test data from a similar study performed in 1990 among 543 18-69 year olds showed that the prevalence decreased from 15.5%. Although it requires further validation, the CE-DUR methods may be useful for rapid and inexpensive surveillance of contact allergy in the general population. Investigations were also made on the possible association between (nickel) contact allergy and alcohol consumption and tobacco smoking, respectively. They suggested that tobacco smoking was associated with nickel allergy in a dose-response manner whereas no definite conclusions could be made regarding an association between (nickel) contact allergy and alcohol consumption.

7 Perspectives & future studies

Cross-sectional patch test studies investigating the prevalence of contact allergy should be repeated in the future. For the purpose of monitoring the development in the prevalence of contact allergy, Glostrup studies should continue to be performed using day 2 patch test readings. In addition, prospective studies using e.g. day 3 or 4 readings should be performed to test for possible associations with e.g. gene mutations, life-style factors, and systemic disorders [145]. Finally, the CE-DUR method should be performed again in Germany and Denmark and in other countries with well-organized patch test databases. Data from such studies may be helpful for the future prevention of contact allergy and associated disorders.

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CONTACT DERMATITIS

10-year prevalence of contact allergy in the general population in Denmark estimated through the CE-DUR method

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The prevalence of contact allergy in the general population has traditionally been investigated through population-based epidemiological studies. A different approach is the combination of clinical epidemiological (CE) data and the World Health Organization-defined drug utilization research (DUR) method. The CE-DUR method was applied in Denmark to estimate the prevalence of contact allergy in the general population and compare it with the prevalence estimates from the Glostrup allergy studies. Contact allergy prevalence estimates ranging from very liberal ('worst case') to conservative ('best case') assumptions were based on patch test reading data in combination with an estimate of the number of persons eligible for patch testing each year based on sales data of the 'standard series'. The estimated 10-year prevalence of contact allergy ranged between 7.3% and 12.9% for adult Danes older than 18 years. The 10-year prevalence of contact allergy measured by CE-DUR was slightly lower than previous prevalence estimates from the Glostrup allergy studies. This could probably be explained by a decrease in nickel allergy. The CE-DUR approach holds the potential of being an efficient and easy monitoring method of contact allergy prevalence.

Key words: CE-DUR; contact allergy; contact dermatitis; DUR; fragrance mix; nickel; prevalence clinical epidemiology. © Blackwell Munksgaard, 2007.

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The prevalence of contact allergy in the general population has previously been estimated by population-based epidemiological studies (1–6). Such large studies demand planning as well as resources and are therefore not applicable in all countries. Furthermore, they cannot be repeated frequently to monitor trends in contact allergy.

The drug utilization research (DUR) was defined by the World Health Organization in 1977 as research addressing 'the marketing, distribution, prescription, and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences' (7). Briefly, this method estimates the number of diseased persons in a population based on information about specific drug prescription and consumption (8, 9). In 2002, the DUR approach was used in combination with clinical epidemiological (CE) data to estimate the prevalence of contact allergy in Germany (10). The CE-DUR method is mainly based on the total annual patch test sales, which is justified as patch testing is uniquely used for the diagnosis of contact allergy as a cause of allergic contact dermatitis (ACD). The results of the study were largely in accordance with previous epidemiological studies but not directly comparable with prevalence estimates from Denmark because of different medical systems as well as the methods itself.

The Glostrup allergy studies from 1990 to 1998 estimated the prevalence of contact allergy in a general population in Denmark (3, 5). The main objective of the present investigation was to apply the CE-DUR approach in Denmark to estimate the 10-year prevalence of contact allergy and compare it with prevalence outcomes from the Glostrup allergy studies. It is our hope that this will help determine the quality of the CE-DUR

method as it is foreseen to be an inexpensive and rapid future tool for epidemiological monitoring in many countries.

Materials and Methods

The CE-DUR method requires a variety of information and data that should be collected before an adequate prevalence estimate is calculated and presented (Table 1).

Patch test sales data

Information on the total patch test sales data regarding the standard series (the overall number of sold syringes for the Danish standard test series), the TRUE-test (the total number of sold tests containing panel 1 and 2), and methyldibromo glutaronitrile (MDBGN) (the number of sold single syringes containing MDBGN) was supplied by the 3 main manufacturers on the Danish market (Mekos Laboratories, Hillerød, Denmark; Hermal, Reinbeck, Germany; and Chemotechnique, Malmö, Sweden). MDBGN is not included in the standard series in Denmark. Sales data were collected for a total period of 4–10 years depending on available company sales data. This was performed to adjust for possible changing trends in the use of patch testing in Denmark over the past decade. However, no consistent trends were found, except for an increased sale of MDBGN since 1998 that has stabilized since 2001.

The number of applications per sold syringe was estimated to be 150, that is 150 patients on average were patch tested until the material in 1 syringe was used up. This number was an average conservative estimate taken from retrospective registrations at the laboratory at Gentofte University Hospital. The estimate offered by scientific staff at Trolab and Chemotechnique was 100 and 120 applications per syringe, respectively. If the hypothetical maximum number of applications per syringe (petrolatum) is calculated (5000 μl/20 μl), an estimated 250 applications is possible. Thus, the numbers vary significantly, but we believe that the most accurate estimate is 150 according to local registration. In total, patch test material sufficient to test approximately 25 000 patients/year was sold in Denmark between 1996 and 2005 (Table 2).

Patch test reading data

Patch test outcomes regarding the patch test standard series, MDBGN, and the TRUE-test (except thimerosal) were collected from the Danish Contact Dermatitis Group (DCDG) database. Thimerosal was omitted from our analysis because patch test results for thimerosal were only available for a minority of patients (i.e. patients tested with TRUE-tests). The DCDG network is based on 3 different university clinics as well as 7 local dermatology clinics. This network represents an average clinical (patch test) population in Denmark, and we venture to assume that this is fairly representative of the entire population eligible for patch testing in Denmark. Patch test readings were performed at least on D3 according to the criteria defined by the International Contact Dermatitis Research Group (11). Patients were included in the study based on positive readings on at least 1 day. Patients were included only once in the database. A 5-year period was selected as the database does not go further back (1 January 2001 to 31 December 2005). The patch test outcomes were assessed for Danes of all ages and for

Table 1. The type, source, year span and validity of data, and its resulting correction factor collected for the estimation of the 10-year prevalence of contact allergy in Denmark using the CE-DUR method (10)

Data type	Data source	Period of data collection	Resulting correction factor (%)	Validity of data
National patch test sales data	Chemotechnique, Hermal, and Mekos Laboratories	1997–2006	NA	Good
Patch test reading data	Danish Contact Dermatitis Group database	2001–2005	NA	Good
Proportion of discarded test	Laboratory staff at Gentofte Hospital	2005–2006	2.5 (0–5)	Good
Proportion of diseased persons who seek medical consultation	National Institute of Public Health in Denmark (16)	1987–1994	25 (20–30)	Fair
Persons previously tested	Danish Contact Dermatitis Group database	2001–2005	10 (5–15)	Fair
Magnitude of the Danish population	Statistics Denmark (17)	2006	NA	Good

CE, clinical epidemiological; DUR, drug utilization research; NA, not applicable.

Table 2. Stepwise estimation of number of patients eligible for patch testing, based on the number of patch tests sold annually and published evidence concerning the selection process[a]

	Model I – worst-case scenario	Model II – best-case scenario	Model III – medium-case scenario
The number of sold patch tests per year	25 000	25 000	25 000
Correction factor 1: the proportion of discarded patch tests (0–5%)	0%	(- 5%)	(- 2.5%)
The number of actually applied patch tests	25 000	23 750	24 375
Correction factor 2: the proportion of previously tested persons (5–15%)	(- 5%)	(- 15%)	(- 10%)
First time patch tests	23 750	20 188	21 938
Correction factor 3: the proportion of diseased persons who seek medical consultation (20–30%)	/20%	/30%	/25%
Persons eligible for patch testing per year	118 750	67 290	87 750

[a]With model III, a more conservative estimation process, the number of patients eligible for patch testing would be 87 750/year. With model I, a worst-case scenario, the number would be 118 750/year.

adult Danes older than 18 years and were used for the prevalence estimates. This was performed to allow a comparison with results from the German CE-DUR (based on all ages) as well as the Glostrup allergy studies (based on adult Danes). However, as the contact sensitization prevalence estimates to individual allergens hardly differed between the 2 groups of calculations (i.e. 'all ages' and 'adults older than 18 years'), the estimate for adult Danes has only been stated when it was specifically needed for comparison with the Glostrup allergy studies.

The proportion of discarded patch test

The proportion of purchased patch test that was discarded rather than used for testing was estimated. In the German CE-DUR investigation, it was assumed that 10–20% of purchased patch test was discarded (10). The experience from the laboratory at Gentofte University Hospital shows that the expiry dates of syringes are never or very rarely lapsed. However, in smaller departments or offices also contributing to the database, this may be the case to some extent. We thus estimated that 2.5% of all purchased patch tests are discarded each year in Denmark. As a consequence, the estimated number of patients patch tested will be lower (Table 2).

The proportion of previously tested persons

Experience from the Department of Dermatology in Göttingen determined that 38% of all patients have been tested on a previous occasion (10). However, according to the DCDG database, only some 15% have been tested previously. This figure is comparable with data (7.9%) published from St John's Institute in London (12). Hence, to establish the number of persons eligible for patch testing, the patch test sales figures have to be corrected downwards accordingly (Table 2).

The proportion of diseased persons who seek medical consultation

In the German CE-DUR investigation, it was estimated that only 15–38% of patients with ACD consult a physician, based on Swedish and German observations (13–15). 2 consecutive Danish surveys from 1987 to 1994 estimated that approximately 25% of patients with ACD are patch tested (16). Thus, the sales figures have to be corrected upwards (Table 2).

Persons eligible for patch testing per year

The absolute number of diseased persons eligible for patch testing per year was estimated through the use of the above presented information (Table 2), summarized as 3 correction factors, ranging from very liberal assumptions ('worst case') to a combination of the most conservative, in terms of a low number of persons tested, assumptions ('best case'). Applying these correction factors, 3 different scenarios were defined, namely model I (worst case), model II (best case), and model III (medium case).

Population

The Danish population, according to Statistics Denmark, is currently 5 400 000 persons (17). Of these, 1 200 000 are children and adolescents younger than 18 years. Hence, the Danish adult population older than 18 years was 4 200 000 persons.

Prevalence estimation

Population-based, cross-sectional studies such as the Glostrup allergy studies estimate the prevalence

of contact allergy in samples of the population. The morbidity estimate derived from CE-DUR is less straightforward conceptually: although patients are commonly patch tested for *incident* ACD (but sometimes for chronic suspected ACD), the spectrum of contact allergy diagnosed on patch testing does not necessarily relate exclusively to the *current* episode of ACD. Moreover, ACD is far from always confirmed after patch testing, with positive test results relating to past episodes of ACD or having uncertain clinical relevance in these cases. Hence, the contact allergy frequencies derived from patch test databases cannot be interpreted as contact allergy incidence rates. Rather, the CE patch test data and the DUR estimation of the annual number of patients eligible for patch testing can be regarded to represent prevalence, estimated during a sampling period of several years. In this period, which was set to a standard of 10 years in our analysis, 'nearly all' patients from the general population, potentially affected by contact allergy, are assumed to consult and be patch tested. The 10-year prevalence of contact allergy to at least 1 allergen of the respective standard series (and MDBGN) was calculated on the basis of patch test reactions (the percentage of patients with at least 1 positive reaction × 10 years × the number of patients eligible for patch testing per year for the different scenarios/the Danish population). For better comparison, the 9-year prevalence from the German study was recalculated to a 10-year prevalence.

Results

Between 2001 and 2005, a total of 14 284 patients were patch tested with the patch test standard series, MDBGN, or the TRUE-test (except thimerosal). Among these, 6299 (44.1%) patients had at least 1 positive reaction (+/++/+++) and 3720 (26%) had at least 1 strong positive reaction (++/+++). The patch test sales figure during the 10-year period was estimated to be 250 000 (25 000/year × 10 years). After the correction factors were applied to the number of sold patch test, 3 different scenarios of persons eligible for patch testing per year were defined, namely model I (worst case), 118 750; model II (best case), 67 290; and model III (medium case), 87 750. The estimated 10-year prevalence of contact allergy to at least 1 allergen of the respective standard series is presented together with the German prevalence estimates in Table 3 (10). The prevalence of contact allergy among adult Danes older than 18 years ranged between 7.3% and 12.9%, whereas the prevalence estimate for Danes of all ages ranged between 5.5% and 9.7%. The German CE-DUR investigation showed prevalence estimates between 4.4% and 18.4% based on Germans of all ages. Furthermore, when the Danish estimates were compared with the German CE-DUR estimates, it was observed that best-case estimates were higher in Denmark than in Germany, whereas worst-case estimates were considerably lower in Denmark. The Danish study applied correction factors ranging from 2.69 to 4.75 [i.e. 118 750 and 67 290 persons eligible for patch testing per year divided by 25 000 sold patch test/year (Table 2)], whereas the German model incorporated factors ranging from 1.31 to 5.40, that is allowed for much more diversity of scenarios. Table 3 also presents prevalence estimates of strong patch test reactions (++/+++) in Denmark and Germany and shows that a higher percentage of worst-case strong reactions were observed in the German CE-DUR (9.0% versus 5.7%).

The 10-year prevalences of contact allergy to individual allergens in Denmark and Germany (in all ages) were calculated based on models I (worst case) and III (medium case), including + to +++ and ++/+++ test reactions, respectively, and are presented in Tables 4 and 5. The worst-case scenario showed that nickel was the most prevalent allergen in Denmark as 3.9% (all ages) and 3.7% (adults older than 18 years) positive reactions were estimated. In comparison, the prevalence of nickel allergy in Germany was 6.1% for all ages. Furthermore, the prevalence of contact allergy to cosmetic-related allergens [fragrance mix, colophonium, balsam of Peru, quarternium-15, parabens, wool alcohol, and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI)] was estimated to be 4.5% among adult Danes as opposed to 3.6% among Danes of all ages. In the German CE-DUR, this estimate (without quarternium-15) for all ages showed that 12.7% were contact sensitized. The main contribution to the high German estimate was fragrance mix (4.6%) and balsam of Peru (3.3%). The prevalence of strong patch test reactions to individual contact allergens in Germany and Denmark was most pronounced for nickel (3.3% versus 2%), fragrance mix (1.6% versus 0.7%), balsam of Peru (1% versus 0.2%) and purified protein derivative (PPD) (0.8% versus 0.2%).

Discussion

10-year prevalence estimate

This study estimates that the 10-year prevalence of contact allergy in Denmark is between 5.5% and 9.7% for Danes of all ages and between 7.3% and 12.9% for adult Danes older than 18 years

Table 3. Calculated 10-year prevalence estimates of contact allergy to worst-, medium-, and best-case scenarios for all age groups and for adults >18 years of age in Denmark and for all age groups in Germany

Scenario	10-year prevalence, + to +++ reactions (%) (for adults aged >18 years in Denmark)	10-year prevalence, + to +++ reactions (%) (for all age groups in Denmark)	10-year prevalence, + to +++ reactions (%) (for all age groups in Germany)	10-year prevalence, ++/+++ reactions (%) (for all age groups in Denmark)	10-year prevalence, ++/+++ reactions (%) (for all age groups in Germany)
Worst case	12.9	9.7	18.4	5.7	9.0
Medium case	9.5	7.2	7.7	4.2	3.7
Best case	7.3	5.5	4.4	3.2	2.2

(Table 3). In comparison, the German CE-DUR estimate from 2002, based on all ages, was considerably higher (4.4–18.4%). It has earlier been assessed that the worst-case scenario is most suitable when applying the CE-DUR method, which makes 9.7% and 12.9%, respectively, the most reasonable estimates of the contact allergy prevalences in Denmark (10). The prevalence estimate for adult Danes (12.9%) should be used for comparison with the results from the Glostrup allergy studies because they were also based on adults Danes. The Glostrup studies showed that the contact allergy prevalences in Denmark were 15.2% in 1990 (Danes of age 15–69 years) and 18.6% in 1998 (Danes of age 15–41 years) (3, 5). When a comparison is made between our results and the results from the Glostrup allergy studies, it should be emphasized that the CE-DUR data are based on data from the DCDG database [standard test series, TRUE-test (except thimerosal), and MDBGN], whereas the Glostrup data are based on older versions of the TRUE-test (including thimerosal but not primin, MDBGN, and formaldehyde). In addition, the age difference

Table 4. Selected '10-year prevalences' of contact allergy to allergens in the standard patch test series, MDBGN, and TRUE-test (except thimerosal), tested over a 5-year period (2001–2005) in the Danish Contact Dermatitis Group (n = 14 284)[a]

			Population (%)			
	Clinical patients		Model I (worst)		Model III (medium)	
Allergen/reactions	+/+++	++/+++	+/+++	++/+++	+/+++	++/+++
Nickel sulfate	17.50	9.10	3.85	2.00	2.84	1.48
Fragrance mix	7.70	3.00	1.69	0.66	1.25	0.49
MDBGN	4.90	1.80	1.08	0.40	0.80	0.29
Cobalt chloride	4.50	1.70	0.99	0.37	0.73	0.28
Colophonium	3.80	1.60	0.84	0.35	0.62	0.26
Balsam of Peru	3.50	0.90	0.77	0.20	0.57	0.15
Potassium dichromate	3.30	1.20	0.73	0.26	0.54	0.20
Carba mix	3.30	0.90	0.73	0.20	0.54	0.15
Formaldehyde	3.10	1.10	0.68	0.24	0.50	0.18
p-Phenylendiamine	2.40	0.90	0.53	0.20	0.39	0.15
Thiuram mix	2.30	1.00	0.51	0.22	0.37	0.16
Methylchloroisothiazolinone/methylisothiazolinone	1.90	0.80	0.42	0.18	0.31	0.13
Epoxy resin	1.70	0.80	0.37	0.18	0.28	0.13
Quaternium-15	1.60	0.60	0.35	0.13	0.26	0.10
Neomycin	1.60	0.60	0.35	0.13	0.26	0.10
Sesquiterpene lactone mix	1.50	0.90	0.33	0.20	0.24	0.15
p-tert-Butylphenol-formaldeyde resin	1.50	0.60	0.33	0.13	0.24	0.10
Quinolin mix	1.10	0.40	0.24	0.09	0.18	0.07
Ethylendiamine	1.00	0.60	0.22	0.13	0.16	0.10
Black rubber mix	1.00	0.50	0.22	0.11	0.16	0.08
Wool alcohol	1.00	0.30	0.22	0.07	0.16	0.05
Cliquinol	0.80	0.40	0.18	0.09	0.13	0.07
N-isopropyl-*n*-phenyl-*p*-phenylendiamine	0.60	0.30	0.13	0.07	0.10	0.05
Primin	0.60	0.30	0.13	0.07	0.10	0.05
Mercaptomix	0.60	0.20	0.13	0.04	0.10	0.03
Mercaptobenzothiazole	0.60	0.30	0.13	0.07	0.10	0.05
Paraben mix	0.50	0.10	0.11	0.02	0.08	0.02
Benzocaine	0.50	0.20	0.11	0.04	0.08	0.03

MDBGN, methyldibromo glutaronitrile.

[a]Prevalences of contact allergy in the general population were estimated on the basis of models I (worst case) and III (medium case) and related to the total Danish population (5 400 000). To address potential diagnostic uncertainties of the positive patch test reactions, the frequencies of unequivocal allergic reactions (++/+++) are also presented.

Table 5. Selected '10-year prevalences' of contact allergy to allergens in the standard patch test series in the IVDK ($n = 78\,067$), Germany, patch tested over a 9-year period (1992–2002)[a]

			Population (%)			
	Clinical patients		Model I (worst)		Model III (medium)	
Allergen/reactions	+/+++	++/+++	+/+++	++/+++	+/+++	++/+++
Nickel sulfate	15.5	8.3	6.1	3.3	2.6	1.3
Fragrance mix	11.7	4.0	4.6	1.6	2	0.7
Balsam of Peru	8.5	2.5	3.3	1	1.4	0.4
p-Phenylendiamine	4.6	2.0	1.8	0.8	0.8	0.3
Potassium dichromate	4.2	1.6	1.7	0.7	0.7	0.2
Colophonium	3.9	1.8	1.6	0.7	0.7	0.3
Wool alcohol	3.8	0.9	1.6	0.4	0.7	0.1
Methyldibromo glutaronitrile/phenoxyethanol[b]	2.0	0.8	0.4	0.2	0.2	0.06
	4.2	1.2	0.8	0.2	0.3	0.08
Thiuram mix	2.7	1.1	1.1	0.4	0.4	0.2
Neomycin	2.7	0.8	1.1	0.3	0.4	0.1
Methylchloroisothiazolinone/methylisothiazolinone	2.5	0.8	1	0.3	0.4	0.1
Formaldehyde	2.0	0.5	0.7	0.2	0.3	0.1
Oil of turpentine	2.0	0.5	0.8	0.2	0.3	0.1
Benzocaine	1.6	0.65	0.7	0.2	0.2	0.1
Paraben mix	1.5	0.3	0.6	0.1	0.2	0.05
Epoxy resin	1.2	0.6	0.4	0.2	0.2	0.1
Cetearyl alcohol	1.2	0.3	0.4	0.1	0.2	0.05
p-tert-Butylphenol-formaldehyde resin	1.0	0.4	0.4	0.1	0.15	0.06

IVDK, Information Network of Departments of Dermatology.
[a]Prevalences of contact allergy in the general population were estimated on the basis of models I (worst case) and III (medium case) and related to the total German population (82 000 000). To address potential diagnostic uncertainties of the positive patch test reactions, the frequencies of unequivocal allergic reactions (++/+++) are also presented (10).
[b]Methyldibromo glutaronitrile/phenoxyethanol was tested in 2 different concentrations (0.5% and 1%) in 1992–1996 and 1997–2000, respectively.

between the studies should be taken into account. Despite these inconsistencies, the present CE-DUR generates a plausible prevalence estimate. The German CE-DUR assessed that the prevalence of contact allergy was 18.4% in 2002. This is based on all ages and would therefore be even higher if it was based on adult Germans only. Thus, the CE-DUR prevalence estimate is higher in Germany than in Denmark. This corresponds well with an estimate from a German population-based epidemiological study on 1141 adults aged 28–78 years (4). An overall frequency estimate assessed that 28% were contact sensitized in 2001 in Germany. It appears that the CE-DUR method might be slightly inaccurate but produces prevalence estimates that are adequately realistic.

Nickel has traditionally been responsible for a high proportion of contact allergy prevalence in the general population (1–5). Consequently, a decrease in the prevalence of nickel allergy will automatically decrease the total prevalence markedly. The present study estimated that 3.7% (adult Danes older than 18 years) had a positive reaction to nickel. In comparison, the German CE-DUR estimated that 6.1% were nickel sensitized. According to the Glostrup studies, the prevalence of nickel sensitization was 6.7% in 1990 and remained almost unchanged in 1998 (3, 18). The Danish CE-DUR result may therefore suggest that the prevalence of nickel allergy is decreasing in Denmark and with it the overall prevalence of contact allergy. However, it should be emphasized that the method itself may explain the different estimates. A possible decrease in the nickel allergy prevalence is supported by other studies that have showed a similar decrease, probably as a consequence of the Danish nickel regulation from 1990 (19, 20). However, it is unknown whether the CE-DUR prevalence estimate is biased downwards. Individuals sensitized to nickel are most often aware of its connection to allergen exposure, for example imitation jewellery, and therefore do not seek medical consultation leading to patch testing.

Besides a decrease in nickel allergy, a minor contribution to the lower prevalence estimate in the general population could be explained by a possible decrease of allergy against cosmetic-related allergens (fragrance mix, colophonium, balsam of Peru, quarternium-15, parabens, wool alcohol, and MCI/MI). The Glostrup allergy study assessed that the prevalence of cosmetic-related allergens was 3.7% in 1990 and 5.8% in 1998 as opposed to 4.5% in the present study (3, 5). An explanation for this decrease could be that the decline of fragrance mix allergy observed among German patients is also occurring in Denmark and is reflected in the general population (21). The prevalence of allergy to cosmetic-related

allergens (except quaternium-15) in the German CE-DUR investigation was surprisingly higher (12.7%), which can be explained not only by the different study periods but also by possible different personal habits and exposure in Germany and Denmark.

Finally, a reason for the lower prevalence of contact allergy in the general population could be the exclusion of thimerosal from the calculations. Thimerosal was included in the Glostrup allergy studies and contributed with 3.4% positive reactions in 1990 and 2.1% in 1998 (5). Thimerosal is also known from other studies to make a significant contribution to the contact allergy prevalence in unselected populations (22). However, MDBGN was included in the CE-DUR estimate but not in the Glostrup allergy studies. This could more or less make up for the exclusion of thimerosal in the calculation.

Besides the discussed findings, the estimated contact allergy prevalences to individual allergens in Denmark and Germany show some interesting differences (Tables 4 and 5). The comparisons are based on 10-year prevalence estimates for all ages in the 2 populations. It is notable that the proportions of strong (++/+++) patch test reactions are more similar than the accumulated positive reactions (+/++/+++) (Tables 3 and 4). One explanation may be different criteria in the definition of a weak positive reaction (+) in Germany and Denmark. The marked similarity of the frequency of strong patch test reactions suggests that allergens such as thiuram mix, (chloro-) methylisothiazolinone, and epoxy resin are responsible for a comparable extent of ACD in the 2 countries. However, the clinical prevalence of contact allergy to fragrance mix, balsam of Peru, and PPD was markedly higher in Germany than in Denmark, and this was reflected in the general population estimates. Even though the DCDG database represents an average clinical patch test population in Denmark, not only the prevalence of contact allergy but also the relative frequency of certain sensitizations may differ between patients and the general population (23). In patient data, there may be a higher proportion of positive reactions to occupationally related allergens and also an elevated number of persons with more that 1 positive allergic reaction, as the patients are selected for morbidity (suspected ACD). Hence, the prevalence of contact sensitization to individual allergens seems mostly reliable when focusing on high-prevalence allergens, such as nickel and fragrance mix, that are widespread and non-occupational.

An observation that deserves attention is the number of sold patch tests in Germany and Denmark. In Germany, an estimated 600 000 patch tests are sold each year as opposed to 25 000 in Denmark. When related to the size of the populations, patch testing is performed approximately 60% more often per inhabitant in Germany than in Denmark. This is partly explained by the different medical systems in the 2 countries. In Denmark, nearly all patients consult their primary physician if they develop a skin rash. Usually, the physician will prescribe corticosteroids, and consequently many patients are never tested. In Germany, patients may consult a dermatologist without prior visitation and this probably leads to a higher degree of patch testing. Furthermore, the reimbursement for patch testing was high in the early 1990s in Germany, which may have increased its use at the time (10). At least to some extent, this difference has been taken into account by setting the CE-DUR model parameter 'dermatological consultation' differently: the medium setting for Germany is 30%, whereas it is 25% for Denmark (Table 2).

General remarks

So far, the Glostrup allergy study has been carried out twice, and a new study has recently been initiated. The studies require significant funding as they are conducted over several years and also require an organization that can organize the invitation, the scheduling, and the patch test reading of numerous individuals. A subject of concern is that patch test reading is performed after only 2 days, which is known to introduce a significant number of false-negative readings (24). Furthermore, it has been discussed whether patch testing with certain allergens, especially PPD, may cause active sensitization (25). Recent data suggest that active sensitization from PPD is not a problem in population-based epidemiological studies (26). In favour of such studies is their principal ability to assess morbidity and the possible role of risk factors. This has led to previously un-described associations, such as smoking and contact allergy (27). Evidently, the CE-DUR approach does not offer such advantages.

To the best of our knowledge, the CE-DUR approach has so far only been applied in Germany and Denmark. Because it is inexpensive and very easily carried out, we believe that it holds the potential of estimating contact allergy prevalences in other countries as well. Before doing so, it is of uttermost importance that the necessary assumptions and model factors are defined as exactly as possible and that they are based on local observations and experience (Table 1). We believe that the CE-DUR approach holds the potential of being an economical and easy monitoring method of

contact allergy prevalence in the general population. Its prevalence estimates were in range with those from the Glostrup allergy studies. Future results from the recently initiated Glostrup allergy study will offer an additional opportunity to validate the present CE-DUR findings.

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Contact allergy to allergens of the TRUE-test (panels 1 and 2) has decreased modestly in the general population

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Summary

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Background The prevalence of contact allergy in the general population is nearly 20%.

Objectives This study aimed to monitor the development of contact allergy to allergens from the TRUE-test (panels 1 and 2) between 1990 and 2006.

Methods Two random samples of adults from the general population in Copenhagen, Denmark, were invited to participate in a general health examination including patch testing. In 1990 and 2006, we patch tested and questioned 543 and 3460 adult Danes. Patch test readings were performed on day 2 only.

Results The overall prevalence decreased significantly from 15·5% in 1990 to 10·0% in 2006, mainly as a result of a decrease in thimerosal allergy from 3·4% to 0·8%. Furthermore, the prevalence of cobalt allergy and rubber-related allergens decreased from 1·1% to 0·2% and from 1·5% to 0·2%, respectively. Stratification by sex and age group revealed decreasing prevalences of contact allergy in all male age groups and in young and middle-aged female age groups (18–55 years) whereas increasing prevalences were observed among older women (56–69 years). The diverging trend observed in women was probably explained by a cohort effect due to a change in the prevalence of nickel allergy following the Danish regulation on nickel exposure.

Conclusions Although the overall prevalence of contact allergy decreased in the general population, frequent contact allergens such as fragrance mix II and methyldibromo glutaronitrile were not tested. Thus, contact allergy remains prevalent in the general population.

The prevalence of contact allergy in the general population has previously been estimated.[1–5] Based on 17 studies, the prevalence ranged between 12·5% and 40·6%, with a weighted average of 19·5%.[6] Traditionally, the three most common contact allergens are nickel, fragrances and thimerosal.[6] In 1990,[3] 1998[4] and again in 2006, subjects from the general population in Copenhagen were invited to participate in a general health examination. Patch testing showed that the prevalence of contact allergy was 15·2% among 15–69-year-olds in 1990[3] and 18·6% among 15–41-year-olds in 1998.[4] Among 15–41-year-olds, the prevalence of contact allergy to cosmetic-related haptens increased significantly from 2·4% to 5·8% between 1990 and 1998 whereas the prevalence of thimerosal allergy decreased from 3·4% to 2·1%.[4]

The present study determined the prevalence of contact allergy to allergens from the TRUE-test (panels 1 and 2) among 18–69-year-old Danes in 2006, and compared them with prevalences from the 1990 study. Changes in the prevalence of nickel, fragrance mix I and *Myroxylon pereirae* (Balsam of Peru) allergy have previously been addressed.[7,8] The prevalence of nickel allergy decreased among women aged 18–35 years between 1990 and 2006 whereas the prevalence of fragrance mix I and *M. pereirae* sensitization followed an 'upside down V-shape' pattern among 18–41-year-old women (i.e. an increase from 1990 to 1998, followed by a decrease from 1998 to 2006). The continuous surveillance of contact allergy in the general population remains important in order to establish the burden of chemical exposure and its impact on human health. The current study offers patch test results from the only repeated cross-sectional study among adults in the general population.

Patients and methods

Study populations

In 1990 and 2006, two consecutive cross-sectional studies were organized by the same group of investigators. Danish adults (with Danish citizenship and born in Denmark) living in one of the same 11 municipalities of the Copenhagen County were invited to participate in a general health examination including patch testing. Study populations were random samples obtained from the Danish Central Personal Register, Ministry of Internal Affairs. Participants completed a postal questionnaire using the same questions: 'have you ever had your ears pierced?' and 'have you ever had eczema behind the knees or fronts of elbows?' An affirmative answer to the latter questions was used a marker of atopic dermatitis. The Ethical Committee of Copenhagen County approved all studies (KA-20060011).

Between February 1990 and January 1991, 567 (71·5%) of 793 invited 15–69-year-olds were patch tested (Table 1). In order to compare the results with those from the 2006 study, participants aged 15–17 years were excluded from further analyses. Thus, 543 participants aged 18–69 years were patch tested. Between June 2006 and May 2008, 3471 (43·7%) of 7931 invited subjects aged 18–69 years showed up for health examination and 3460 participants were patch tested (Table 1). For practical reasons, persons below the age of 18 years were not invited in 2006, as an informed, written consent from the parents is now mandatory in Denmark.

Patch tests

Patch testing was performed by using panels 1 and 2 of the standardized ready-to-apply TRUE-test (ALK-Abelló A/S, Hørsholm, Denmark, in 1990; Mekos Laboratories, Hillerød, Denmark, in 2006). The haptens in the TRUE-test differed from the European baseline series by including thimerosal and excluding primine, sesquiterpene lactone mix, tixocortol pivalate, budesonide, hydroxyisohexyl-3-cyclohexene carboxaldehyde (HICC), methyldibromo glutaronitrile (MDBGN) and fragrance mix II. Furthermore, the TRUE-test contained black-rubber mix instead of N-isopropyl-N′-phenylparaphenylenediamine and caine mix (tetracaine, benzocaine and dibucaine) instead of benzocaine. Finally, formaldehyde was not

Table 1 The prevalence of contact allergy in 543 (1990) and 3460 (2006) adults aged 18–69 years from the general population in Copenhagen, Denmark

	Positive reactions % (n)					
	Men		Women		Total	
Allergens and mixtures of allergens	1990 (n = 269)	2006 (n = 1547)	1990 (n = 274)	2006 (n = 1913)	1990 (n = 543)	2006 (n = 3460)
Nickel sulphate	2·2 (6)	1·0 (15)	10·9 (30)	9·9 (189)	6·6 (36)	5·9 (204)
Wool alcohols[a]	0·4 (1)	0	0	0	0·2 (1)	0
Neomycin sulphate	0	0	0	0·1 (2)	0	0·1 (2)
Potassium dichromate	0·7 (2)	0	0·4 (1)	0·3 (5)	0·6 (3)	0·1 (5)
Caine mix	0	0	0	0·1 (2)	0	0·1 (2)
Fragrance mix I	1·1 (3)	1·4 (21)	1·1 (3)	1·8 (34)	1·1 (6)	1·6 (55)
Colophony	0·4 (1)	0·7 (11)	1·1 (3)	0·5 (10)	0·7 (4)	0·6 (21)
Epoxy resin	0·4 (1)	0·8 (12)	0·7 (2)	0·3 (6)	0·6 (3)	0·5 (18)
Quinoline mix[a]	0·4 (1)	0·1 (2)	0·4 (1)	0	0·4 (2)	0·1 (2)
Balsam of Peru[a]	0·7 (2)	0·1 (1)	1·5 (4)	0·1 (2)	1·1 (6)	0·1 (3)
Ethylenediamine dihydrochloride	0·4 (1)	0·1 (2)	0	0·3 (6)	0·2 (1)	0·2 (8)
Cobalt dichloride[a]	0·7 (2)	0·1 (1)	1·5 (4)	0·4 (7)	1·1 (6)	0·2 (8)
p-tert-Butylphenol formaldehyde resin[a]	1·1 (3)	0	1·1 (3)	0·1 (1)	1·1 (6)	0·1 (1)
Paraben mix[a]	0·4 (1)	0	0·4 (1)	0	0·4 (2)	0
Carba mix	0·7 (2)	0·1 (2)	0	0·1 (2)	0·2 (4)	0·1 (4)
Black rubber mix	0·4 (1)	0	0	0·2 (4)	0·2 (1)	0·1 (4)
Cl+ Me– Isothiazolinone[a]	0·4 (1)	0·3 (4)	1·1 (3)	0·1 (2)	0·7 (4)	0·2 (6)
Quaternium-15	0·4 (1)	0·2 (3)	0	0·2 (3)	0·2 (1)	0·2 (6)
Mercaptobenzothiazole[a]	0·4 (1)	0	0	0	0·2 (1)	0
p-Phenylenediamine	0	0	0	0·2 (4)	0	0·1 (4)
Formaldehyde	–	0·1 (2)	–	0·3 (5)	–	0·2 (7)
Mercapto mix[a]	0·7 (2)	0	0	0	0·4 (2)	0
Thimerosal[a]	3·7 (10)	0·4 (6)	3·3 (9)	0·6 (12)	3·5 (19)	0·5 (18)
Thiuram mix[a]	0·7 (2)	0	0·4 (1)	0·2 (3)	0·6 (3)	0·1 (3)
Totals	11·9 (32)	4·7 (73)	19·0 (52)	14·2 (272)	15·5 (84)	10·0 (345)

[a]Allergens that decreased significantly between 1990 and 2006. –, formaldehyde not tested in 1990.

included in the TRUE-test in 1990. Directions to apply the patch test panels to the upper back 2 days before examination were mailed together with the patch tests. They were read and photographed 1–1½ h after removal by N.H.N. in 1990 and by trained health care personnel in 2006 (supervised by J.P.T. and A.L.). Photographs were reviewed by N.H.N. and T.M. in 1990 and by T.M., N.H.N., A.L. and J.P.T. in 2006. This was done to assure that the International Contact Dermatitis Research Group (ICDRG) criteria were used consistently over time.[9] Contact allergy was defined as a positive (at least grade 1+ according to ICDRG) patch test to at least one allergen or mix of haptens. Rubber allergy was defined as a positive patch test reaction to at least one of the following allergens: thiuram mix, mercapto mix, mercaptobenzothiazole, carba mix. When the patch had no skin contact upon patch test reading, or if the subject had removed it prior to testing as a result of known contact allergy, it was regarded as missing data.

Statistical analysis

Characteristics of subjects patch tested in 1990 and 2006 were compared using the χ^2 test. Due to a well-known marked sex difference in the prevalence of allergy to contact allergens, the analyses were stratified by sex. A logistic regression model was performed with 'contact allergy to at least one allergen' as the dependent variable and with sex ('women' vs. 'men'), age group ('18–35 years', '36–55 years', '56–69 years'), response to the question 'have you ever had your ears pierced?' ('yes' vs. 'no') and study year ('2006' vs. '1990') as the independent variables. Furthermore, a similar logistic regression analysis was performed with 'contact allergy to at least one allergen but not nickel' as the dependent variable and with the following independent variables: sex ('women' vs. 'men'), age group ('18–35 years', '36–55 years', '56–69 years') and study year ('2006' vs. '1990'). Finally, two identical analyses were performed with 'contact allergy to at least one allergen but not nickel, fragrance mix I and *M. pereirae*' and 'contact allergy to at least one allergen but not thimerosal' as the dependent variables, respectively. Adjustment for atopic dermatitis did not change the outcome of the above analyses and was therefore left out. Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, U.S.A.) for Windows (release 15.0).

Results

The participation rate among men was 76·5% in 1990 but decreased to 39·1% in 2006 whereas the participation rate among women decreased from 75·6% in 1990 to 48·4% in 2006 as previously reported.[7] The decreases were observed in all age groups.

Patch test results among 3460 subjects aged 18–69 years (1913 women and 1547 men) tested in 2006 are presented in Table 1. In total 345 (10·0%) positive patch test reactions were identified and a higher proportion was observed among women (14·2%) than among men (4·7%). The most common contact allergens in 2006 were nickel (5·9%), fragrance mix I (1·6%), colophony (0·6%) and thimerosal (0·5%). Nickel allergy was more common in women (9·9%) than in men (1·0%). Finally, a positive patch test reaction to more than one allergen was observed among 21 (0·6%) subjects. All were nickel sensitized and the most common combination was allergy to fragrance mix I and nickel (42·9%) whereas concomitant nickel and cobalt allergy was less common (14%).

Patch test data from the 1990 study revealed that 84 (15·5%) positive reactions were identified among 543 18–69-year-olds (Table 1). Thus, the overall prevalence of contact allergy decreased from 15·5% in 1990 to 10·0% in 2006. This decrease was observed in men (1990 = 11·9% and 2006 = 4·7%) and women (1990 = 19·0% and 2006 = 14·2%). Furthermore, a decrease was observed in all male age groups and among young women ('18–35 years' and '36–55 years') whereas an increase was observed among older women ('56–69 years') (Table 2). The prevalence of thimerosal allergy demonstrated a large overall decrease from 3·5% in 1990 to 0·5% in 2006. The decrease of thimerosal allergy was observed in all age groups except among 56–69-year-old women where a slight increase was registered (Table 2). If thimerosal allergy was left out of the analyses, an overall decrease of contact allergy to at least one allergen was observed from 12·7% in 1990 to 9·6% in 2006 ($P < 0·03$). Other prevalent allergens also showed decreasing prevalences between 1990 and 2006 (Table 1): cobalt (from 1·1% to 0·2%), *p*-tert-butylphenol (PTBP) formaldehyde resin (from 1·1% to 0·1%), Cl+ Me– isothiazolinone (MCI/MI) (from 0·7% to 0·2%) and rubber-related allergens (from 1·5% to 0·2%). No significant increases were observed between 1990 and 2006.

A logistic regression analysis with 'contact allergy to at least one allergen' as the dependent variable was performed (Table 3). It showed that 'contact allergy to at least one allergen' was associated with 'female sex' (OR = 2·11; CI = 1·58–2·82) and that a positive trend test was identified for age (OR = 1·18; CI = 1·01–1·37). Finally, it showed that study year '2006' was inversely associated with contact allergy (OR = 0·55; CI = 0·42–0·78). A similar logistic regression analysis with 'contact allergy to at least one allergen but not nickel' as the dependent variable and with sex, age and study year as the explanatory variables showed that 'contact allergy to at least one allergen but not nickel' was also associated with female sex (OR = 1·33; CI = 1·00–1·76) and inversely associated with study year '2006' (OR = 0·43; CI = 0·31–0·60). No association with age group was identified. The outcome of the analysis did not change if the dependent variable was replaced with 'contact allergy to at least one allergen but not nickel, fragrance mix I and *M. pereirae*' or with 'contact allergy to at least one allergen but not thimerosal'. Thus, a decrease in the prevalence of contact allergy was observed between

Table 2 The prevalence of contact allergy[a] among adults from the general population in Copenhagen, Denmark (1990 and 2006)

	Study year					
	Men			Women		
Age group (years)	1990 (n = 269) % (n)	2006 (n = 1547) % (n)	Crude OR with 95% CI[b]	1990 (n = 274) % (n)	2006 (n = 1913) % (n)	Crude OR with 95% CI[b]
18–35 (n = 837)						
Contact allergy to at least one allergen[a]	13·1 (11)	4·9 (13)	0·34 (0·15–0·80)	24·5 (26)	15·2 (58)	0·55 (0·33–0·93)
Contact allergy to at least one allergen but not nickel[a]	8·5 (8)	4·2 (11)	0·47 (0·18–1·06)	9·9 (11)	5·2 (20)	0·50 (0·23–1·08)
Contact allergy to thimerosal	4·8 (4)	0·4 (1)	0·08 (0·01–0·72)	3·8 (4)	0	–
36–55 (n = 1917)						
Contact allergy to at least one allergen[a]	9·9 (12)	4·1 (30)	0·39 (0·19–0·78)	18·5 (23)	17·2 (161)	0·91 (0·56–1·48)
Contact allergy to at least one allergen but not nickel[a]	8·6 (11)	2·8 (21)	0·31 (0·15–0·66)	13·2 (17)	6·1 (57)	0·42 (0·24–0·76)
Contact allergy to thimerosal	3·3 (4)	0·3 (2)	0·08 (0·02–0·45)	4·0 (5)	1·2 (11)	0·29 (0·01–0·84)
56–69 (n = 1249)						
Contact allergy to at least one allergen[a]	14·1 (9)	5·5 (30)	0·35 (0·16–0·59)	6·8 (3)	8·9 (53)	1·33 (0·40–4·45)
Contact allergy to at least one allergen but not nickel[a]	12·3 (8)	5·1 (28)	0·38 (0·17–0·89)	4·3 (2)	4·3 (26)	1·00 (0·23–4·35)
Contact allergy to thimerosal	3·1 (2)	0·6 (3)	0·18 (0·03–1·07)	0	0·2 (1)	–

CI, confidence interval; OR, odds ratio. [a]In 1990, formaldehyde was not included in the TRUE-test. [b]OR (95% CI) comparing the prevalence of contact allergy in 2006 with that in 1990.

Table 3 Logistic regression analysis with the outcome 'contact allergy' and with different explanatory variables performed in 4003 subjects patch tested in 1990 (n = 543) and 2006 (n = 3460)

Explanatory variables	Adjusted OR[b] with 95% CI
Study year	
1990	1 (reference)
2006	0·55 (0·42–0·78)
Sex	
Male	1 (reference)
Female	2·11 (1·58–2·82)
Age (years)	
18–35	1 (reference), [a]P < 0·04
36–55	0·97 (0·76–1·26)
56–69	1·42 (1·04–1·94)
Ear piercing	
No	1 (reference)
Yes	1·62 (1·22–2·16)

CI, confidence interval; OR, odds ratio. [a]P-value of trend test. [b]Mutually adjusted for variables shown in table.

1990 and 2006 for the categories 'thimerosal' as well as 'contact allergens from the TRUE-test (panels 1 and 2) but not nickel, fragrance mix I and M. *pereirae*'.

Discussion

This study showed that the overall prevalence of contact allergy to the allergens of the TRUE-test (panels 1 and 2) decreased in Denmark from 15·5% in 1990 to 10·0% in 2006 (Table 1). Stratification by sex and age group revealed decreasing prevalences in all male age groups and in the young and middle aged female age groups (18–55 years) whereas increasing prevalences were observed among middle-aged and older women (56–69 years) (Table 2). The diverging trend observed in young and middle-aged vs. older women was probably explained by a cohort effect due to a change in the prevalence of nickel allergy as it was decreasing in young women ear-pierced later than 1990 (i.e. after the Danish nickel regulation was passed) but increasing in women ear-pierced before 1990.[8] However, when nickel allergy (and also fragrance mix I and M. *pereirae*) was omitted from the analysis, a significant decrease was still observed in most age groups between the two study years that was due to decreasing prevalences of other allergens (Tables 1 and 2). Of note, female sex remained associated with contact allergy even when nickel (and also fragrance mix I and M. *pereirae*) was omitted from the logistic regression analysis. This finding suggests that women may have a heavier exposure to chemicals than men and therefore a higher prevalence of contact allergy.[10]

A weakness of our study was the significantly decreasing rate of participation in all age groups from 1990 to 2006.[7] To limit the influence of bias, our analyses were stratified by age group and sex, and therefore the observed trends were unlikely to be explained by differences in these variables between the studies. However, it cannot be ruled out that other characteristics differed between participants in the two studies (leading to an over- or under-representation of sensitized subjects). Although

we were unable to stratify for these unknown factors, we believe that data on the prevalence of contact allergy in repeated cross-sectional general population studies are likely to show more reliably the development of time trends of contact allergy than data obtained in series of patients admitted to specialized clinics. Of note is that the development, e.g. in the prevalence of nickel and fragrance contact allergy,[7,8,11,12] observed in the present study was in line with clinical data supporting the validity of the observed trends. However, it should be acknowledged that increasing rates of nonparticipation in general population studies represents an important limitation.

The overall decrease of contact allergy to the allergens in the TRUE-test (panels 1 and 2) was mainly explained by a significant decrease in the prevalence of thimerosal allergy (Table 1). The decrease of thimerosal allergy between 1990 and 2006 was observed in all age groups among men whereas a slight increase was observed among older women (56–69 years), perhaps owing to a cohort effect. Thimerosal allergy is known to be prevalent in the general population due to its use as a preservative in vaccines and ophthalmic solutions.[13] Furthermore, thimerosal has traditionally been widely used in biologics and vaccines in the U.S.A., which has resulted in a significant increasing prevalence of thimerosal allergy among North American dermatitis patients between 1984 and 1994.[14] However, positive patch test reactions to thimerosal are generally very poor predictors of dermatitis reactions to thimerosal-containing vaccines.[15] In Denmark, thimerosal allergy mainly derives from childhood vaccination as it has been an ingredient in vaccines since the 1950s and until March 1992. Because the Danish childhood vaccination programme is voluntary and free of charge, the vast majority of Danish children have been exposed to thimerosal through four decades. Furthermore, thimerosal may be an ingredient in vaccines against hepatitis and influenza virus, for example. However, steps have been taken in Denmark and in the rest of the world to strongly reduce or totally remove thimerosal from vaccines as thimerosal is a mercuric compound that may be nephrotoxic and neurotoxic at high doses. Furthermore, it has been suspected of causing neurodevelopmental disorders such as autism, although this association has been rejected.[16] The observed decrease of thimerosal allergy in Denmark is interesting as it shows that when exposure to a contact allergen (whether nickel, thimerosal or other) is strongly reduced by an administrative initiative, a clear decrease of contact allergy can be registered in the general population.

Besides the decrease of thimerosal allergy, the overall decrease of contact allergy between 1990 and 2006 was explained by a decrease in the prevalence of allergy to nickel, cobalt, MCI/MI, PTBP formaldehyde resin and rubber-related allergens. The observed decreases were all significant but it may of course be a result of random error. Furthermore, one should remember that the sample size was small when compared with clinical databases. It is possible that the Danish general population is less exposed to contact allergens today than almost 20 years ago as a result of personal precautions and protection in an increasingly educated population. Cobalt is a hard metal that is common in combination with other metals such as nickel, chromium and tungsten to increase hardness and durability. Because it is often mixed with, or is an impurity in other metals, cobalt allergy may go along with nickel allergy in women or chromate allergy in men.[17] It has been suggested that nickel sensitivity and irritant hand eczema precede cobalt allergy in metal workers whereas cross-sensitization is rare.[18,19] Ear-piercing has been associated with cobalt allergy in the general population.[20] Some 11 (79%) of 14 positive cobalt reactions were observed in women in 1990 and 2006. However, seven women and two men were sensitized to cobalt only. The findings therefore suggest that combined cobalt and nickel allergy is not that prevalent in the general population. It has been speculated whether cobalt has replaced nickel in jewellery after the introduction of the nickel regulation.[21] In comparison, the prevalence of concomitant patch test reactivity to cobalt and nickel is much higher among patients with dermatitis.[22,23] This area needs further attention to clarify the significance of these findings.

The decrease of allergy to rubber-related allergens may possibly be a result of the focus on rubber gloves during the 1990s.[24] Rubber manufacturers reduced the content and use of accelerators as they are considered to be the most frequent contact sensitizer in rubber gloves. Hence, the use of thiurams was strongly reduced in single-use natural rubber latex gloves.[24] The decrease observed in the general population in Denmark parallels the decrease observed among dermatitis patients from the Gentofte University Hospital between 1995 and 2004.[24] Also, the prevalence of thiuram mix, mercapto mix, mercaptobenzothiazole and carba mix allergy has decreased recently among dermatitis patients in the U.S.A.[14,25] The prevalence of chromate allergy in men decreased from 0·7% in 1990 to 0% in 2006 whereas it remained stable in women with 0·4% and 0·3% positive patch test reactions in 1990 and 2006, respectively. The decrease in the prevalence of chromate allergy in men could possibly be explained by an effect of the cement chromate regulation in Denmark[26] whereas the persistence of chromate allergy in women may be explained by continuous exposure to chromate in leather goods.[27] Finally, as patch test readings were performed only on day 2, the prevalence of allergy to late-reacting allergens such as p-phenylenediamine and neomycin may be underestimated. To study such allergens properly, later readings should be carried out.

This study showed that the prevalence of contact allergy to allergens of the TRUE-test (panels 1 and 2) decreased modestly to 10·0% in Denmark and that it was mainly explained by a lower prevalence of thimerosal allergy but also that cobalt, chromate (in men) and rubber allergy decreased. A recent study using the clinical epidemiology and drug utility research (CE-DUR) method estimated that 7·3–12·9% of the Danish population (> 18 years) was contact sensitized.[28] The CE-DUR estimate was based on allergens from the European baseline series and therefore thimerosal was not included.

Despite the prevalence of contact allergy showing a decrease in the general population in Denmark, one should be aware

that only day 2 readings were performed in this study. Thus, the true prevalence of contact allergy is expected to be higher as 27% of positive patch test reactions to nickel are missed when readings are not performed beyond day 2 in a general population.[29] Similarly, patch test studies performed among dermatitis patients have suggested that 24–34·5% of positive patch test reactions potentially are missed when readings are not performed beyond day 2.[30–32] Furthermore, important high-prevalence contact allergens such as HICC, fragrance mix II and MDBGN are not included in the TRUE-test. The true prevalence of contact allergy in the general population is likely to be higher than 10%. Future studies are necessary to monitor the prevalence of contact allergy in the general population in Denmark.

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The association between alcohol consumption and contact sensitization in Danish adults: the Glostrup Allergy Study

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Conflicts of interest
None declared.

Summary

Background Population-based epidemiological studies have indicated that alcohol consumption is associated with IgE-mediated immune diseases (i.e. allergic rhinitis, asthma and urticaria). These studies have been strongly supported by several immunological studies. Furthermore, an inhibitory effect of alcohol consumption on the development of delayed-type hypersensitivity has been shown in healthy controls. However, a possible association between contact sensitization and alcohol consumption in a general population has never been reported.

Objectives To investigate whether alcohol consumption is associated with contact sensitization in a general population.

Methods In 1990, self-reported consumption of alcohol and patch testing results were assessed in 1112 subjects, aged 15–69 years, participating in a population-based cross-sectional study in Glostrup, Denmark. In 1998, they were invited to a follow-up and 734 were re-examined (participation rate 69·0%). Adjustment for potential confounders was performed by using logistic regression analyses.

Results Women who reported no consumption of alcoholic drinks per week were more likely to develop contact sensitization (adjusted odds ratio 2·12, 95% confidence interval 0·98–4·61) during a 8-year follow-up period. A positive trend among women was detected ($P = 0·045$).

Conclusions These data support the hypothesis that alcohol consumption leads to IgE-mediated immune responses rather than delayed-type hypersensitivity reactions. It is probable that alcohol consumption prevents the development of contact sensitization. Further epidemiological studies are warranted.

In Denmark, a general increase in the consumption of alcohol has been observed during the second half of the 20th century.[1,2] Excess alcohol intake may lead to liver cirrhosis, acute and chronic pancreatitis, hypertension, cardiomyopathy, some types of cancers and death.[3] There is substantial evidence that alcohol consumption has an effect on the immune system: alcohol leads to impaired T-helper 1 (Th1) lymphocyte-regulated cell-mediated immune responses favouring a Th2 lymphocyte deviation of the immune system.[4–8] Furthermore, in alcohol abstinence syndrome, an increased level of Th2 lymphocyte-related cytokines has been demonstrated in comparison with healthy controls.[9] Thus, alcohol consumption is associated with changes in the cytokine profile consistent with a relative Th2 lymphocyte deviation of the immune system.

The Th1/Th2 dichotomy has for some years been the cornerstone of immunological thinking.[10,11] The basic idea is that naïve precursor CD4+ T cells differentiate to either Th1 or Th2 cells under the influence of cytokines.[12] Th1 cells are crucial for the host to control the replication of intracellular pathogens but also contribute to the pathogenesis of autoimmune diseases and contact sensitization. Th2 cells support the development of humoral immunity but are, furthermore, associated with IgE-mediated allergic diseases (i.e. allergic rhinitis, asthma and urticaria). The reciprocal downregulation of Th1 cells by Th2 cytokines and Th2 cells by Th1 cytokines is a key element in this hypothesis and accordingly IgE-mediated immune diseases result from a predominance of Th2 cells and are, furthermore, negatively regulated by Th1 cells. Contact sensitization, asthma and allergic rhinitis are all common conditions of which increasing prevalences have been reported.[13–15] Recently, population-based epidemiological studies have indicated that alcohol consumption is associated with IgE-mediated immune diseases, probably due to an imbalance in favour of Th2 cell predominance.[16–19] From

these findings, it is likely that alcohol consumption may also have an influence on the development of contact sensitization where a Th1-dominated immune response plays a major role in the pathogenesis. Hence, we decided to investigate whether alcohol consumption is associated with contact sensitization in a general population.

Materials and methods

Baseline study

The study was conducted according to a two-stage protocol. In the first stage a screening questionnaire on respiratory symptoms was mailed to a sample of 15- to 69-year-olds (n = 8000) living in the Western part of Copenhagen, the capital of Denmark. The sample was drawn randomly from the Civil Registration System. A total of 6998 or 87·5% responded to the screening questionnaire. In the second stage, a random group and a symptom group selected among the respondents were invited to a health examination. The symptom group comprised all respondents (n = 788) who had reported in the screening questionnaire upper ('itchy or stuffy nose or sneezing') or lower ('shortness of breath or trouble breathing') respiratory symptoms on exposure to either pollen or furry animals.[20] The random group comprised 647 subjects who were randomly selected from among the remaining respondents. Subsequently, 635 (participation rate 80·6%) and 477 (participation rate 75·5%) subjects were examined in the symptom and random group, respectively. Thus, in total 1112 subjects (overall participation rate 77·5%) were examined, and of these 1056 were patch tested. On the test day, all participants filled out another questionnaire with a variety of health questions including questions on eczema, atopic dermatitis and exposure to allergens. The invitational procedure and characteristics of both participants and nonparticipants have previously been described in more detail.[20] Examinations took place between February 1990 and January 1991. The Ethical Committee of Copenhagen County approved the study. A written, informed consent form was obtained from all participants prior to the beginning of the study.

Follow-up study

At the time of follow-up, 28 subjects had died, eight had emigrated and 12 could not be located. Thus, 1064 of the participants in the baseline study were invited to the follow-up study; 734 subjects were examined (participation rate 69%). We invited eligible subjects in the same months as they had been examined in the baseline study to avoid potential seasonal differences. Hence, 469 of 734 (63·9%) participants in the follow up-study were examined on a date within 2 months (62 days) of the date of examination in the baseline study. The median follow-up time was 7 years and 10 months (range 6 years and 10 months–8 years and 8 months). The participants were examined between October 1997 and November 1998. The study was approved by the Ethics Committee of Copenhagen County. A comparison of characteristics among participants and nonparticipants has previously been presented[21] and revealed that male sex and high educational level at baseline were significantly associated with participation in the follow-up study.

Patch tests

The patch tests used were the ready to apply TRUE-Test (ALK-Abelló A/S, Hørsholm, Denmark).[22,23] The haptens differed from the European Standard Series (Hermal-Chemi, Reinbek, Germany) by including thiomersal and excluding primine and formaldehyde. Directions to apply the patch tests to the upper back 2 days before the examination were mailed with the patch test. The patch tests were read 30–90 min after removal. Reactions were classified according to the International Contact Dermatitis Research Group.[24] A positive reaction (+) was defined as at least homogeneous redness and palpable infiltration in the test area. Reactions not fulfilling these criteria, whether these were follicular reactions, faint erythema or typical irritant reactions, were classified as nonallergic reactions. Contact sensitization was defined as a positive patch test to at least one of 23 allergens. In 1990/1991, N.H.N. read all reactions. In 1997/1998, A.L. read all reactions. In both studies, all photographs of the test sites were reviewed by the same professor in dermatology, and in case of disagreement consensus was reached by discussion. If the tests were not applied 2 days before attending, or if the contact with the skin was poor, a new appointment was made when possible.

Questionnaire

A questionnaire about health, lifestyle and socioeconomic factors was sent with the standard invitation letter to the participants. The participants were asked about their average weekly consumption of beer (bottles), wine (glasses) and spirits (standard drinks), respectively, during the last 12 months ('During the last 12 months, how many of the following have you been drinking on average per week? Number of beers per week? Number of glasses of wine per week? Number of glasses/units of spirits per week?'). It was assumed that one bottle of beer, one glass of wine and one serving of spirits contain 12 g/15 mL ethanol. The total weekly consumption was calculated by adding the intake of beer, wine and spirits. The total alcohol consumption was categorized as 0, 1–7, 8–14 and ≥ 15 drinks per week for the prevalence calculations and as 0, 1–7 and ≥ 8 drinks per week for the incidence calculations. The questions used for assessment of alcohol consumption have previously been validated against increased levels (≥ 80 IU L^{-1}) of serum γ-glutamyltransferase (GGT), a marker of alcohol exposure.[25] The results revealed that self-reported total alcohol intake (total number of drinks per week) was significantly and positively associated with increased levels of GGT.[26] Information about tobacco exposure was obtained in the questionnaire in as much detail as possible and has been described previously.[24,27] Ear piercing was

defined as an affirmative answer to the question 'Have you ever had your ears pierced?' Questions about eczema and hand eczema included: 'Have you ever had eczema from exposure to earpins or earrings, under the tightener of your watch strap or under the button of your jeans?' and 'have you ever had hand eczema?' The following socioeconomic variables were defined: vocational training (yes, no), educational level (≤ 9, 10–11, 12–13, ≥ 14 years), type of residence (house, apartment, other), ownership of residence (yes, no) and social group (self-employed, white-collar workers, skilled blue-collar workers, unskilled blue-collar workers, others).

Statistics

Potential differences in alcohol consumption between groups with different baseline characteristics were examined by a 2×2 frequency table using the χ^2 test. The association between alcohol consumption (independent variable) and contact sensitization (dependent variable) was analysed by logistic regression models and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). In this model, the following potential confounders (independent variables) were considered: sex, age (15–34, 35–49, 50–69 years), smoking (ex-smoker, never-smoker, ≤ 15 g daily, > 15 g daily) and ear piercing (yes, no). In further analyses possible confounding by socioeconomic variables were investigated but revealed essentially similar results and were excluded from the final analysis. *A priori* it was decided to analyse the results separately for each gender as both contact sensitization and alcohol consumption are known to be strongly related to gender. All data were analysed with the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, U.S.A.) for Windows (release 15.0).

Results

Tables 1, 2 show the baseline characteristics among 1112 participants in the 1990 study. The prevalence of contact allergy and nickel contact allergy was substantially higher among women than among men (Table 1). Alcohol abstinence as compared with drinking at least one drink per week was associated with female sex ($P < 0{\cdot}001$), a history of ear piercing ($P < 0{\cdot}001$) and never-smoking status ($P < 0{\cdot}02$), whereas no significant age differences were detected (Table 2). Similarly, consumption of one to seven alcoholic drinks per week was associated with female sex ($P < 0{\cdot}001$), age 15–34 years ($P < 0{\cdot}001$), a history of ear piercing ($P < 0{\cdot}01$) and never-smoking status ($P < 0{\cdot}01$). Finally, heavy drinking (≥ 15 drinks per week) as compared with drinking < 15 drinks per week was associated with male sex ($P < 0{\cdot}001$), age 50–69 years ($P < 0{\cdot}001$), no history of ear piercing ($P < 0{\cdot}001$) and heavy smoking (> 15 g daily) ($P < 0{\cdot}001$).

Table 3 shows the association between alcohol consumption and the prevalence of contact sensitization to at least one of 23 allergens in 1990. Among men, no statistical significant association was found and the adjusted ORs were almost similar for drinkers and nondrinkers. Among women, the prevalence of contact sensitization was significantly lower among nondrinkers (adjusted OR 0·53, 95% CI: 0·31–0·94) in comparison with women who consumed one to seven drinks per week (reference group). However, the prevalence of contact sensitization was not increased among women drinking 8–14 or ≥ 15 drinks per week. No relation between the type of alcoholic drink and contact sensitization was found.

Table 4 shows the association of alcohol consumption with the incidence of contact sensitization between 1990 and 1998. In total, 573 (292 men and 281 women) persons with

Table 1 The prevalence of contact sensitization (to at least one of 23 allergens), nickel contact allergy, allergic nickel contact dermatitis, a history of ear piercing, skin prick test reactivity and smoking among women and men

	Women	Men	
	n/total (%)	n/total (%)	P-value[a]
Contact sensitization	133/574 (23·2)	50/482 (10·4)	< 0·001
Nickel contact sensitization	87/574 (15·2)	7/482 (1·5)	< 0·001
Allergic nickel contact dermatitis[b]	79/573 (13·8)	4/482 (0·8)	< 0·001
Ear piercing	419/595 (70·4)	73/517 (14·1)	< 0·001
Skin prick test reactivity	226/593 (38·1)	248/517 (48·0)	< 0·001
Smoking status			
Never	210/595 (35·3)	158/517 (30·6)	< 0·001 (3 d.f.)
Previously	99/595 (16·6)	122/517 (23·6)	
≤ 15 g daily	199/595 (33·4)	106/517 (20·5)	
> 15 g daily	87/595 (14·6)	131/517 (25·3)	
Lifetime smoking			
0 pack-years	227/589 (38·5)	171/513 (33·3)	< 0·001 (2 d.f.)
≤ 15 pack-years	238/589 (40·4)	158/513 (30·8)	
> 15 pack-years	124/589 (21·1)	184/513 (35·9)	

[a]P-value of χ^2 test for the comparison of women and men. [b]Persons with a history of eczema from wearing earpins or earrings, under the tightener of one's watch strap or under the button of one's jeans and a positive patch test to nickel were defined as cases of allergic nickel contact dermatitis.

Table 2 Baseline characteristics of the participants. Weekly alcohol consumption (drinks per week) stratified by gender, age, ear piercing and smoking

Alcohol consumption (drinks per week)	Gender		Age (years)			Ear piercing		Smoking			
	Male, n (%)	Female, n (%)	15–34, n (%)	35–49, n (%)	50–69, n (%)	No, n (%)	Yes, n (%)	Never smoker, n (%)	Ex-smoker, n (%)	≤ 15 g daily, n (%)	> 15 g daily, n (%)
0	33 (6·4)	126 (21·2)	73 (16·9)	34 (8·9)	52 (17·4)	64 (10·3)	95 (19·3)	66 (17·9)	19 (8·6)	44 (14·5)	30 (13·8)
1–7	246 (47·6)	359 (60·4)	276 (63·7)	192 (50·5)	137 (46·0)	311 (50·2)	294 (59·9)	226 (61·4)	120 (54·3)	176 (57·9)	83 (38·1)
8–14	108 (20·9)	85 (14·3)	55 (12·7)	92 (24·2)	46 (15·4)	121 (19·5)	72 (14·7)	48 (13·0)	41 (18·6)	62 (20·4)	42 (19·3)
≥ 15	130 (25·1)	24 (4·0)	29 (6·7)	62 (16·3)	63 (21·1)	124 (20·0)	30 (6·1)	28 (7·6)	41 (18·6)	22 (7·2)	63 (28·9)
Total	517 (100)	594 (100)	433 (100)	380 (100)	298 (100)	620 (100)	491 (100)	368 (100)	221 (100)	304 (100)	218 (100)
χ^2	146·5		68·9			62·2		88·4			
P-value	< 0·001		< 0·001			< 0·001		< 0·001			

negative patch test results in 1990 were patch tested again in 1998. Of these, 69 (12%) developed a positive patch test reaction. No men tested positive among nondrinkers and it was therefore impossible to calculate ORs for this category. Among women, individuals who reported no consumption of alcohol were more likely to develop contact sensitization (adjusted OR 2·12, 95% CI: 0·98–4·61) during the 8-year follow-up period. A positive trend test among women was detected (P = 0·045). No relation between the type of alcoholic drink and contact sensitization was found.

Discussion

This investigation revealed an inverse dose–response relationship between alcohol consumption and incident contact sensitization among women (i.e. women who consume alcohol are less likely to develop contact sensitization than nondrinkers) (Table 4). A possible association could not be evaluated among men as the number of incident positive patch test reactions was too low. Furthermore, it appeared that alcohol abstinence was associated with a lower prevalence of contact sensitization among women (Table 3). The findings of the cross-sectional and prospective analyses appear to be contradictory as a nondrinking status (among women) was associated with a lower prevalence of contact sensitization (Table 3) but a higher incidence of contact sensitization (Table 4). The reason for this discrepancy is not clear. In general, the results of prospective analyses are considered as more reliable when determining cause–effect relationships and as less prone to bias and confounding. Confounding seems unlikely as independent well-known determinants of contact sensitization were included in the analyses (i.e. female sex, history of ear piercing and cigarette smoking).[27–29] A possible source of bias in the study could be that persons with excess alcohol consumption were less likely to participate. Similarly, it is possible that persons with a history of eczema were more likely to participate in the study as its focus was on allergy. Random error cannot be ruled out as this is the first epidemiological study on this topic. Previous population-based epidemiological studies have indicated that contact sensitization and IgE-mediated immune diseases are independent[30] and that alcohol consumption is associated with IgE-mediated allergic diseases.[16,18] Furthermore, the Th1/Th2 dichotomy would suggest an inverse relation between alcohol consumption and contact sensitization. Our prospective analyses indicated that the well-documented alcohol-induced impairment of Th1 immune response found in immunological studies also can be demonstrated in population-based epidemiological studies. Finally, it has been hypothesized that the effect of alcohol consumption on Th2-mediated immune responses is due to ethanol itself and not to the nonethanol content of alcoholic drinks.[26,31] In line with this, the present results did not reveal any significant effects of the type of alcoholic drink. However, the study size may not have been sufficient to examine for this issue properly.

The Th1/Th2 hypothesis was introduced in the mid 1980s and has been applied as an immunological model since then.[10]

Table 3 The association between alcohol consumption and the prevalence of contact sensitization (a positive patch test to at least one of 23 allergens) in 1990

	Men			Women		
Alcohol consumption (drinks per week)	Contact sensitization, n (%)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	Contact sensitization, n (%)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)
0	2 (6·7)	0·52 (0·12–2·23)	0·62 (0·13–2·83)	19 (15·3)	0·54 (0·31–0·95)	0·53 (0·31–0·94)
1–7	28 (12·2)	1·00	1·00	85 (24·9)	1·00	1·00
8–14	9 (9·1)	0·72 (0·33–1·59)	0·67 (0·30–1·51)	23 (27·7)	1·16 (0·67–1·99)	1·20 (0·68–2·20)
≥ 15	11 (8·9)	0·71 (0·34–1·47)	0·67 (0·30–1·44)	6 (25·0)	1·00 (0·38–2·62)	0·90 (0·33–2·48)
Total	50/482 (10·4)			133/573 (23·2)		

[a]Adjusted for age, ear piercing and smoking. CI, confidence interval.

Table 4 The effect of alcohol consumption on the incidence of contact sensitization between 1990 and 1998

	Men			Women		
Alcohol consumption (drinks per week)	Contact sensitization, n (%)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	Contact sensitization, n (%)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)
0	0 (0)	–	–	14 (23·0)	1·69 (0·82–3·48)	2·12 (0·98–4·61)
1–7	13 (9·4)	1·00	1·00	27 (15·0)	1·00, P = 0·071[b]	1·00, P = 0·045[b]
≥ 8	11 (8·1)	0·94 (0·38–2·23)	0·84 (0·37–1·96)	4 (12·5)	0·72 (0·23–2·26)	0·91 (0·28–2·89)
Total	24/292 (8·2)			45/281 (16·0)		

[a]Adjusted for age, ear piercing and smoking. [b]P-value for test for trend. CI, confidence interval.

It is becoming evident that allergic immune responses are not always as strongly polarized as dictated by the Th1/Th2 dichotomy,[11,32] as allergen-specific Th1 and Th2 cells have been isolated from skin biopsies[33] and coexpression of Th1 and Th2 cytokines following sensitization has been observed.[34] Furthermore, it appears that the secreted Th2 cytokines are independent of the type of sensitizer.[35] Recently it was demonstrated that a subgroup of T cells, 'T-regulatory' (Treg) cells, suppresses both Th1- and Th2-mediated immune responses[36,37] and, furthermore, that Th17 cells secrete interleukin (IL)-17 that provides defence against extracellular bacteria and is involved in the inflammatory process of cancer and autoimmune diseases.[38–40] The differentiation of Th17 cells is inhibited by cytokines from both Th1 and Th2 cells [interferon (IFN)-γ and IL-4] and is stimulated by transforming growth factor-β1 and IL-23.[40] Similar to the reciprocal interaction of Th1 and Th2 cells, Th17 cells are involved in reciprocal interaction with Th1 cells (i.e. Th1 cells inhibit the inflammatory damage caused by Th17 cells via the secretion of IFN-γ).[11] Finally, Th17 cells and Treg cells show reciprocal interactions through the action of IL-6.[11]

The exact mechanism played by alcohol consumption in allergic skin diseases has only partly been elucidated, whereas the effect of alcohol on pulmonary host defence has been investigated to a greater extent.[41] Alcohol mainly displays its effect on antigen-presenting cells (APCs) (both *in vitro* and *in vivo*) where it leads to a decreased T-cell activation.[42] It can inhibit the antigen-presenting capacity of these cells for nearly 7 days.[42] Glutathione levels in APCs may influence whether a Th1 or Th2 response will develop.[43] Glutathione inhibition prevents IL-12 synthesis in APCs and leads to an increased production of IL-4 and thus a Th2-mediated immune response.[44,45] As alcohol is an inhibitor of glutathione synthesis, consumption may lead to IgE-mediated allergic diseases and possibly prevent contact sensitization. Furthermore, ethanol leads to increased gut permeability which in turn leads to increased absorption of endotoxins (lipopolysaccharides).[31] Monocyte CD14 receptors may then interact with absorbed lipopolysaccharides and indirectly favour IgE synthesis.[31] In addition, it has been indicated that alcohol consumption inhibits Th1-mediated immune responses both *in vitro* and *in vivo* (and in acute and chronic alcohol intake).[46–48] Smith *et al.*[48] showed that alcohol intake reduced the probability of developing a delayed-type hypersensitivity in 166 healthy individuals. Apparently, alcohol interferes with early cell surface-associated signal transduction phosphorylation events leading to impaired IFN-γ and IL-12 secretion, whereas IL-2 synthesis is almost unaffected.[49–51] Administration of IL-12 can restore IFN-γ levels and delayed-type hypersensitivity reactions.[5] A Th2-mediated immune response is caused by the synthesis of IL-4 that leads to elevated levels of serum IgE.[44] At present,

little is known about the effect of alcohol on Treg cells and Th17 cells but it has been demonstrated that alcohol can suppress IL-17 synthesis in the lungs.[52]

Despite its shortcomings, the Th1/Th2 hypothesis remains illuminating. It is acknowledged that no single cytokine can regulate a vital process such as tissue damage and that a refinement of the model is necessary. However, it demonstrates that a reciprocal interaction between whole subsets of T cells (i.e. Th1 and Th2) is a key point in inflammatory responses. It therefore continues to guide our perspective on allergic immune responses to a great extent. There is evidence to support that alcohol leads to a Th2 deviation of the immune system and that a Th1-mediated response is impaired.[16,18] For the first time ever, we are able to show that it is probable that alcohol consumption prevents the development of contact sensitization. This finding supports previous immunological studies but also raises important questions for future allergy testing (i.e. should patient medical histories contain information about alcohol consumption in order to interpret the outcome of patch testing or skin prick testing better?). Recently, we initiated the third consecutive Glostrup Allergy study. It will hopefully bring us another chance to investigate what role is played by alcohol in the development of contact sensitization and IgE-mediated allergic diseases.

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INVESTIGATIVE REPORT

The Effect of Tobacco Smoking and Alcohol Consumption on the Prevalence of Nickel Sensitization and Contact Sensitization

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Running Head: Contact allergy and lifestyle factors

ABSTRACT

There is evidence to support that stimulants such as alcohol consumption and tobacco smoking have an effect on the immune system but little is known about how these life-style factors affect the prevalence of contact sensitization. This study investigated whether smoking and alcohol consumption were associated with contact sensitization and nickel sensitization. A random sample of adults (n=3,460) from the general population of Copenhagen was invited to participate in a general health examination including patch testing. Alcohol consumption was not associated with nickel sensitization whereas a significant trend ($p<0.05$) was identified between smoking status and nickel sensitization in an adjusted model (i.e. nickel sensitization was higher among both previous smokers (OR=1.19; CI=0.81-1.76), current light smokers (OR=1.50; CI=0.94-2.37) and current heavy smokers (OR=1.56; CI=0.87-2.80) as compared to never smokers). This study confirmed that smoking is associated with nickel sensitization but rejected an association with alcohol consumption.

Key words: alcohol drinking; contact sensitization; general population; public health; tobacco smoking.

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INTRODUCTION

Contact sensitization affects 10-20% of the general population (1;2). It is mainly caused by exposure to nickel, fragrances, and preservatives whereas genetic susceptibility seems to be of limited importance (3;4). There is substantial evidence to support that alcohol consumption and tobacco smoking have an effect on the immune system (5-9) but little is known about how these life-style factors affect the prevalence of contact sensitization. Recently, a prospective Danish population based study revealed contradictory results regarding a possible association between alcohol consumption and contact sensitization (10). Furthermore, three general population studies have examined whether tobacco smoking is associated with contact sensitization (11-13): among 1,056 adult Danes, a significant dose-response relationship was identified between smoking and contact sensitization (11). Furthermore, current smoking was significantly associated with contact sensitization among 690 Norwegian adult women (12) whereas no association was identified among 520 young Swedish young men doing military service (13). Thus, it remains to be convincingly determined whether alcohol consumption and tobacco smoking affect the prevalence and risk of contact sensitization.

The present study aimed to re-investigate a possible association between these lifestyle factors and contact sensitization. A random sample of adults from the general population in Copenhagen was invited to participate in a general health examination including patch testing. A focus was made on nickel sensitization as it is by far the most prevalent contact sensitization in the general population (1). Furthermore, data from previous cross-sectional studies have suggested that the association between tobacco smoking and nickel sensitization was slightly stronger than the association between smoking and contact sensitization to at least one allergen (11). The current study is of relevance as smoking and drinking is prevalent in many countries and as an association may have clinical implications (e.g. the interpretation of patch test reactions in smokers).

MATERIALS AND METHODS

Study population

A cross-sectional study was performed in the general population in Copenhagen, Denmark. A random sample of 7,931 subjects aged 18-69 years was obtained from the Danish Central Personal Register. All were Danish adults with Danish citizenship and born in Denmark. A total of 3 471 (43.7%) subjects participated in a general health examination and 3,460 were patch tested. The participation rate was higher among older age-groups than among younger age-groups in both genders (14). The Ethical Committee of Copenhagen County approved the study (KA-20060011). A written informed consent form was obtained from all participants prior to the beginning of the study.

Patch tests

Patch testing was performed by using panel 1 and 2 from the standardized ready to apply TRUE-test® (Mekos Laboratories, Hillerød, Denmark). Directions to apply the patch test panels to the upper back 2 days before examination were mailed together with the patch tests. All testing was performed between June 2006 and May 2008. At the day of examination, they were read and photographed 1-1½ hour after removal by trained health care personnel (supervised by Thyssen and Linneberg). Photos were reviewed by Menné, Nielsen, Linneberg and Thyssen. This was done to secure that the International Contact Dermatitis Research Group (ICRDG) criteria were used consistently over time. Contact sensitization was defined as a positive (at least grade 1+ according to ICRDG) patch test to at least one allergen or mixes of haptens. It has been estimated that approximately 18-29% of positive patch test reactions to nickel are missed when patch test readings are only performed on day 2 and not also on day 4 (15). In case the patch had no skin contact upon patch test removal, or if the subject had removed it prior to testing as a result of known contact sensitization, it was regarded as missing data.

Measurement of Immunoglobulin E antibodies

Venous blood was taken on the day of examination and was left to coagulate for 2 h. The serum was then separated by centrifugation at 3000 r.p.m. for 10 min and frozen immediately afterward. The serum samples were analyzed for immunoglobulin (Ig) E specific to birch, grass (timothy), cat, and mite (Dermatophagoides

pteronyssinus) with the ADVIA Centaur IgE antibody assay system (Bayer Corporation) (16). The analysis for IgE antibodies was judged to be positive if the measurement was in excess of 0.35 kU/l. Specific IgE positivity was defined as a positive test to at least one of the four allergens tested.

Questionnaire

Table I presents questions used for the present study. Participants were asked about smoking and drinking habits as well as about vocational training, social status and ear-piercing status. Occasional smokers (3.3%) were excluded from the analyses. The amount of tobacco in grams among current smokers were calculated for cigarettes, cheroots, cigars and pipe tobacco by equating one cigarette/one gram of pipe tobacco to 1 g tobacco, one cheroot to 3 g tobacco and one cigar to 5 g tobacco. The information was used to define smoking status ("never smokers", "previous smokers", "current light smokers: ≤15 g/day", "current heavy smokers: >15 g/day"). It was assumed that one normal beer, one glass of wine, and one serving of spirits equalled one drink (each contained 12 g/1.5 cl ethanol) whereas one strong beer was assumed to equal 1½ drink (each contained 18 gram/1.5 cL ethanol). The total weekly consumption was then calculated by adding the number of drinks of beer, wine, and spirits. The total alcohol consumption was categorized as 0, 1-7, 8-14, ≥15 drinks per week for the prevalence calculations. The questions used for assessment of alcohol consumption has previously been validated against increased levels (≥80 IU/L) of serum γ-glutamyl transferase (GGT), a marker of alcohol exposure (17). The results revealed that self-reported total alcohol intake (total number of drinks/week) was significantly and positively associated with increased levels of GGT (18).

Statistical analysis

Characteristics of participants were compared using the chi-square (χ^2) test. A logistic regression model was performed with nickel sensitization as the dependent variable, and sex, age-group ("18-35 years", "36-55 years", "56-69 years"), and smoking status ("never smokers", "previous smokers", "current light smokers: ≤15 g/day", "current heavy smokers: >15 g/day") as the independent variables. In this model, a test for interaction between sex and smoking status was performed by using a log-likelihood ratio test. In order to examine the potential confounding effects of selected variables, we performed several logistic regression models adding one variable at a time while observing changes in the risk estimates for the exposure variables (smok-

ing and alcohol consumption). These analyses were performed with nickel sensitization as the dependent variable and with sex, age-group ("18-35 years","36-55 years", "56-69 years"), smoking status ("never smokers", "previous smokers", "current light smokers: ≤15 g/day", "current heavy smokers: >15 g/day"), ear piercing ("yes", "no"), alcohol consumption ("0", "1-7", "8-14", "≥15"), and educational level ("skilled or unskilled blue-collar workers", "short-cycle higher education", "medium higher education", "long-cycle higher education", "other education") as the explanatory variables. In further analyses, possible confounding by other socio-economic variables such as "self-estimated social status", "vocational training" and "type of residence" were investigated and revealed essentially similar results as adjustment with the variable for educational level. In fact, an analysis adjusted for "self-estimated social status" instead of educational level revealed a much stronger association between tobacco smoking and nickel sensitization. Also, adjustment for the occurrence of IgE antibodies was performed but this did not change the results. Finally, similar logistic regression analyses were performed with "contact sensitization to at least one allergen" and "contact sensitization to at least one allergen but not nickel", respectively, as the dependent variables and with the explanatory variables listed in table III. Associations were expressed as odds ratios (ORs) with confidence intervals (CIs) of 95%. Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, USA) for windows (release 15.0).

RESULTS

Characteristics of the study population according to gender are presented in Table II. The prevalence of contact sensitization to at least one allergen, nickel contact sensitization, and ear piercing was markedly higher among women than among men whereas men consumed significantly more alcohol than women. The prevalence of never smokers and previous smokers was nearly identical among women and men whereas the prevalence of current light smokers (≤15 g/day) was higher among women than men (16.3% versus 9.5%) and the prevalence of current heavy smokers (>15 g/day) was higher among men than women (12.6% versus 7.9%).

Table III shows the baseline characteristics of participants stratified by smoking status: The proportion of current light smokers (≤15 g/day) was higher among subjects who were ear-pierced or were nickel sensitized in comparison to subject who were not ear-pierced and who were not nickel sensitized. Alcohol consumption tended to increase with smoking status and the proportion of current heavy smokers (>15 g/day) was higher among subjects with a short education.

Crude data analyses without adjustment for potential confounders showed that nickel sensitization was significantly associated with female sex, ear-piercing, alcohol consumption (≥15 drinks per week), and tobacco smoking (Table IV). The relationship between nickel sensitization and educational level revealed no clear pattern except a higher prevalence of nickel sensitization among subjects with a short-cycle higher education. We evaluated whether it could be assumed that the effects of smoking were independent of gender. Thus, a logistic regression model was performed with nickel sensitization as the dependent variable, and with sex, age-group (“18-35 years”,”36-55 years”, “56-69 years”), smoking status (“never smokers”, “previous smokers”, “current light smokers ≤15 g/day”, “current heavy smokers >15 g/day”), and an interaction term between sex and smoking status as the independent variables. It did not reveal any significant interaction between sex and smoking status ($p=0.97$) which means that the possible effect of smoking status on the prevalence of nickel sensitization did not differ between men and women. In order to examine possible confounding, several logistic regression models were performed in which one variable where added at a time while observing changes in the risk estimates for the exposure variables (smoking and alcohol consumption) (Table IV). The regression analyses revealed that ear-piercing was an important confounder which indicates that nickel sensitization to a high degree is an environmental disorder. Furthermore, the analyses showed that

alcohol consumption was not associated with nickel sensitization whereas a significant trend ($p<0.05$) was identified between smoking status and nickel sensitization in the fully adjusted model (Table IV). Finally, similar logistic regression analyses were performed with “contact sensitization to at least one allergen” and “contact sensitization to at least one allergen but not nickel”, respectively, as the independent variable and with the explanatory variables listed in Table IV. These analyses did not show any significant associations between contact sensitization on one hand and alcohol consumption or smoking status on the other hand. Thus, the fully adjusted regression analysis with contact sensitization to at least one allergen as the dependent variable revealed a non-significant trend test for smoking status ($p<0.6$) (data not shown).

DISCUSSION

This study showed that nickel sensitization was significantly associated with tobacco smoking. This association was dose-dependent and independent of gender. The results are in line with those from another cross-sectional population-based study performed in 1,056 Danish adults (11) and are also supported by a Norwegian patch test study in which a significant association was identified in adult women (12).

It is important to evaluate to which extent confounding by other factors could explain the positive association observed between smoking and nickel sensitization (Table III). The association remained relatively unchanged after adjustment for confounders by multivariable regression analyses (Table IV) although it can not be ruled out that residual confounding (insufficient adjustment) or confounding by factors not included in this study could play a role. When the logistic regression analysis was adjusted for educational level, the association between smoking and nickel sensitization was weakened. Thus, it is possible that we were not able to sufficiently adjust for social status in our analyses as an association between nickel sensitization and socio-economic status has been suggested previously (19). In Malmö, Sweden, the prevalence of nickel sensitization was significantly higher among immigrants, unemployed, and patients on social security than among patients from higher socio-economic groups (19). Furthermore, a German study showed that the prevalence of nickel sensitization was higher among nurses (24.9%) and receptionist (29.3%) than among physicians (12.1%), indicating that nickel sensitization may be less prevalent in high-income groups (20). Despite the suggested association between nickel sensitization and socio-economic status, no association was identified between educational level and nickel sensitization in both an adjusted and an unadjusted analysis in this study (Table IV). We cannot exclude that the association between nickel allergy and tobacco smoking to some degree was explained by ear piercing as it was more frequently reported among current light smokers (Table III).

This study did not identify any significant associations between smoking status and ”contact sensitization to at least one allergen but not nickel” and “contact sensitization to at least one allergen”, respectively. It should be emphasized that the prevalence of contact sensitization to other contact allergens than nickel was low in this general population (Table II). Also, since patch test readings were only performed on day 2 in this study, a lower prevalence of contact sensitization was expected (15;21). The low prevalence estimates will necessarily lead to reduced statistical power in the regression analyses which may hide associations. However, a

previous Danish study also showed that nickel sensitization had a slightly stronger association with smoking than contact sensitization to at least one allergen (11). The stronger association observed for nickel sensitization may be explained by the fact that nickel is found in tobacco plants as a result of absorption from soil, fertilizing products or pesticides. Furthermore, the nickel content in cigarettes and tobacco is high regardless of its kind and origin (22). One study examined the nickel concentration in 123 blood samples and 147 urine samples from smokers and non-smokers. It revealed a significantly higher concentration of nickel in the urine but not in the blood of smokers in comparison to non-smokers (22). It is therefore possible that T cells in smokers are exposed to nickel in concentrations that may lead to nickel sensitization. However, nickel exposure from cigarettes is probably of minor importance in terms of inducing nickel contact sensitization as the prevalence of nickel sensitization in men was approximately 1% whereas nearly 50% of men reported current or previous smoking. Finally, the findings in this study (i.e. a stronger association for nickel sensitization than contact sensitization) could be coincidental or a result of confounding as nickel sensitization may have a stronger association with lower social groups than e.g. fragrance and preservative sensitization.
Contact sensitization and autoimmune conditions have traditionally been regarded as T-helper 1 (Th1) mediated immune responses whereas sensitization to aeroallergens, as observed in allergic asthma and rhinitis, has been regarded as a T-helper 2 (Th2) mediated condition (23;24). The Th1/Th2 dichotomy was for many years the cornerstone of immunological thinking and dictated that Th1 cells were down regulated by cytokines released from Th2 cells and vice versa. As it was recently demonstrated that a subgroup of T cells, "T-regulatory" (Treg), may suppress both Th1 and Th2 mediated immune responses, the dichotomy may only partially explain the development of various immune responses (25;26). However, tobacco smoking has been causally linked to the development of autoimmune diseases such as systemic lupus erythematosus, multiple sclerosus, Grave's hyperthyroidism, rheumatoid arthritis (27), and contact sensitization (11;12) whereas prospective population based studies have suggested that tobacco smoking may decrease the risk of IgE-mediated allergic sensitization to aeroallergens (28;29). Also, cross-sectional population based studies have demonstrated a lower prevalence of sensitization to common aeroallergens among smokers and ex-smokers than among non-smokers (30;31). Thus, it seems plausible that tobacco smoking favours Th1 mediated immune responses and suppresses Th2 mediated immune responses. These immunological perspectives support the findings from this study although it should be recognized that humane immune responses are very com-

plex as demonstrated by contact sensitization being inversely related to type I diabetes and inflammatory bowel diseases (32;33).

This study did not identify any association between alcohol consumption and the prevalence of nickel sensitization (or contact sensitization) although nickel allergy seemed lower for individuals who reported alcohol abstinence in the adjusted analysis (table IV). However, as participants were only asked about alcohol consumption within the past 12 months, it is possible that we did not accurately assess the cumulated alcohol exposure. Also, the limitations of day two patch test readings reduced statistical power in our analysis which may hide an association (15;21). We are only aware of one previous study that also addressed the association between contact sensitization and alcohol consumption (10). It did not identify any association between the prevalence of alcohol consumption and contact sensitization whereas it suggested that the 8-year incidence of contact sensitization was significantly higher among non-drinking women (10). In general, a prospective incidence-based analysis is considered more reliable than cross-sectional studies when determining the cause-effect relationship. A follow-up of the present study population would be of interest to further investigate this issue. Furthermore, it may be of interest to take into account genetic variations in alcohol metabolism as certain genetic variations may influence both alcohol drinking behaviour and susceptibility to the immunological effects of alcohol (34). Such genetic influence would tend to bias associations between alcohol and immune effects.

In conclusion, this general population study confirmed our previous finding that smoking is associated with nickel sensitization. The possible biological mechanisms underlying this association remain to be elucidated. We could not confirm the previously reported negative association between alcohol consumption and the development of contact sensitization. In future prospective studies, it could be of interest to investigate whether tobacco-smoking leads to a poor prognosis of allergic nickel contact dermatitis in comparison to non-smokers.

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Table I. Questions used in the questionnaire.

Question category	Group questioned	Question	List of answers
Smoking	All participants	Do you smoke?	Yes, daily
			Yes, occasionally (less than 1 cigarette, or 1 cheerot, or 1 pipe of tobacco per day)
			No, but previously
			No, never
	Daily smokers only	Please indicate how much tobacco you smoke on average per day?	Number of cigarettes
			Number of cheroots
			Number of cigars
			Grams of pipe tobacco
Alcohol consumption	All participants	Have you consumed any alcoholic drinks during the past 12 months?	Yes
			No
	Drinkers within the past 12 months	How many of the following drinks have you had on average per week during the past 12 months?	Number of normal beer
			Number of strong beer
			Number of glasses of wine (1 bottle of wine equals 6 glasses)
			Number of glasses/units of spirits (standard drinks)
Ear piercing	All participants	Have you ever had your ears pierced?	Yes
			No
Vocational training	All participants	Have you ever had vocational training?	Yes
			No
	All participants	What is your educational level?	Skilled or unskilled blue-collar workers
			Short-cycle higher education (<3 years, e.g. dental technician and nursing assistants)
			Medium higher education (3-4 years, e.g. nurse, school teacher, and physiotherapist)
			Long-cycle higher education (>4 years, e.g. medical physician, psychologist, and engineer
			Other education
Social status	All participants	What is your self-estimated social status based on education, job, income, etc.	High

			Middle
			Below middle
			Low
			Very high
Type of residence	All participants	What kind of residence do you live in?	House
			Apartment
			Other

Table II. Gender specific characteristics regarding contact sensitization (to at least one of 24 allergens), nickel contact sensitization, a history of ear piercing, specific immunoglobulin (Ig)E status, alcohol consumption, smoking status, and educational level. Data was based on a general health examination including patch testing performed among 3471 18-69 year old participants from a cross-sectional study performed in Copenhagen, Denmark between 2006 and 2008.

	Men % (n/total)	**Women** % (n/total)	**P-value †**
Contact sensitization to at least one allergen	4.7 (73/1547)	14.2 (272/1913)	0.001
Nickel contact sensitization	1.0 (15/1495)	10.3 (189/1913)	0.001
Ear piercing	17.0 (261/1538)	82.2 (1564/1902)	0.001
Specific immunoglobulin E‡	27.3 (418/1531)	20.0 (378/1889)	0.001
Alcohol consumption (drinks per week within past 12 months)			
0	9.0 (138/1532)	19.2 (367/1912)	< 0.001
1-7	33.7 (516/1532)	53.0 (1013/1912)	
8-14	24.3 (372/1532)	17.7 (338/1912)	
≥ 15	33.0 (506/1532)	10.1 (194/1912)	
Smoking status			
Never smokers	43.3 (640/1478)	43.1 (795/1846)	< 0.001
Previous smokers	34.6 (512/1478)	32.7 (604/1846)	
Current light smokers <=15 g/day	9.5 (140/1478)	16.3 (301/1846)	
Current heavy smokers >15 g/day	12.6 (186/1478)	7.9 (146/1846)	
Educational level			
Skilled or unskilled blue-collar workers	44.8 (602/1345)	37.3 (609/1633)	< 0.001
Short-cycle higher education	14.1 (189/1345)	20.1 (328/1633)	
Medium higher education	17.4 (234/1345)	26.1 (426/1633)	
Long-cycle higher education	13.4 (180/1345)	7.5 (122/1633)	
Other	10.4 (140/1345)	9.1 (148/1633)	

† = P-value of Chi-square test for the comparison of women and men.

‡ = Analysis for IgE specific to birch, grass (timothy), cat, and mite (Dermatophagoides pteronyssinus). The analysis was judged to be positive if the measurement was in excess of 0.35 kU/l.

Table III. Characteristics of 3471 participants from a cross-sectional study performed in Copenhagen grouped by smoking status.

	Smoking status				**P-value†**
	Never smokers % (n/total)	**Previous smokers** % (n/total)	**Current light smokers ≤15 g/day** % (n/total)	**Current heavy smokers >15 g/day** % (n/total)	
Age (years)					
18-35 (n=593)	57.8 (343)	20.4 (121)	14.2 (84)	7.6 (45)	0.001
36-55 (n=1613)	39.7 (641)	35.0 (565)	13.9 (224)	11.3 (183)	
56-69 (n=1118)	40.3 (451)	38.5 (430)	11.9 (133)	9.3 (104)	
Ear-piercing					
Yes (n=1752)	38.7 (678)	34.1 (598)	17.1 (300)	10.0 (176)	0.001
No (n=1563)	48.0 (751)	33.0 (516)	9.0 (140)	10.0 (156)	
Nickel sensitization					
Yes (n=1752)	31.4 (678)	36.6 (598)	21.1 (300)	10.8 (176)	0.001
No (n=1563)	44.4 (751)	33.2 (516)	12.6 (140)	9.7 (156)	
Alcohol consumption (drinks/week within past 12 months)					
0 (n=472)	43.2 (204)	31.8 (150)	12.5 (59)	12.5 (59)	0.001
1-7 (n=1484)	50.1 (743)	30.1 (447)	12.7 (188)	7.1 (106)	
8-14 (n=673)	39.5 (266)	36.4 (245)	15.5 (104)	8.6 (58)	
≥15 (n=668)	31.0 (207)	40.0 (267)	13.0 (87)	16.0 (107)	
Educational level					
Skilled or unskilled blue collar worker (n=1174)	38.4 (451)	35.2 (413)	14.1 (166)	12.3 (144)	0.001
short cycle higher education (n=499)	37.7 (188)	36.5 (182)	14.0 (70)	11.8 (59)	
Medium cycle higher education (n=631)	46.0 (290)	37.1 (234)	11.6 (73)	5.4 (34)	
Long cycle higher education (n=289)	64.7 (187)	24.9 (72)	6.2 (18)	4.2 (12)	
Other education (n=275)	44.4 (122)	35.3 (97)	12.7 (35)	7.6 (21)	

† = P-value of Chi-square test for the comparison of different categories of smoking status.

Table IV. The relationship of different potential risk factors to the prevalence of nickel sensitization.

	Nickel sensitization % (n/total)	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted OR†† (95%)	Adjusted OR††† (95%)	Adjusted OR†††† (95%)
Smoking status						
Never smokers	4.4 (61/1397)	1.00	1.00, * p<0.001	1.00, * p<0.005	1.00, * p<0.009	1.00, * p<0.05
Previous smokers	6.6 (71/1071)	1.56 (1.09-2.21)	1.60 (1.11-2.91)	1.45 (1.00-2.09)	1.41 (0.98-2.05)	1.19 (0.81-1.76)
Current light smokers <=15 g/day	9.7 (41/421)	2.36 (1.57-3.57)	1.91 (1.25-2.31)	1.72 (1.13-2.63)	1.65 (1.08-2.53)	1.50 (0.94-2.37)
Current heavy smokers > 15 g/day	6.7 (21/313)	1.58 (0.94-2.63)	1.97 (1.15-3.35)	1.78 (1.04-3.05)	1.73 (1.01-2.98)	1.56 (0.87-2.80)
Sex						
Men	1.0 (15/1495)	1.00	1.00	1.00	1.00	1.00
Women	10.3 (189/1843)	11.27 (6.63-19.16)	11.03 (6.36-19.16)	5.50 (2.95-10.2)	5.83 (3.10-10.97)	5.55 (2.85-10.81)
Age (years)						
18-35	7.2 (45/622)	1.00	1.00	1.00	1.00	1.00
36-55	7.9 (128/1625)	1.10 (0.77-1.56)	1.04 (0.71-1.52)	1.09 (0.75-1.60)	1.03 (0.70-1.52)	0.99 (0.65-1.51)
56-69	2.8 (31/1091)	0.38 (0.24-0.60)	0.41 (0.25-0.64)	0.48 (0.29-0.79)	0.45 (0.27-0.74)	0.41 (0.24-0.73)
Ear piercing						
No	1.2 (19/1567)	1	-	1.00	1.00	1.00
Yes	10.6 (184/1741)	9.63 (5.97-15.52)	-	3.35 (1.89-5.96)	3.44 (1.93-6.13)	3.01 (1.66-5.46)
Alcohol consumption (drinks/week within past 12 months)						
0	7.6 (36/475)	1.00	-	-	1.00	1.00
1-7	6.7 (98/1472)	0.87 (0.59-1.29)	-	-	1.02 (0.67-1.56)	0.96 (0.61-1.52)
8-14	6.4 (44/683)	0.84 (0.53-1.32)	-	-	1.42 (0.86-2.34)	1.33 (0.77-2.29)
>= 15	3.8 (26/682)	0.48 (0.29-0.81)	-	-	1.34 (0.77-2.37)	1.05 (0.56-1.97)
Educational level						
Skilled or unskilled blue collar worker	5.8 (67/1151)	1.00	-	-	-	1.00
Short cycle higher education	9.3 (46/495)	1.66 (1.12-2.45)	-	-	-	1.16 (0.76-1.76)
Medium cycle higher education	6.4 (41/638)	1.11 (0.74-1.66)	-	-	-	0.87 (0.57-1.33)
Long cycle higher education	4.0 (12/297)	0.68 (0.36-1.28)	-	-	-	0.71 (0.33-1.49)
Other education	5.3 (15/281)	0.91 (0.51-1.62)	-	-	-	0.99 (0.54-1.84)

† = Logistic regression analysis adjusted for sex, age and smoking.
†† = Logistic regression analysis adjusted for sex, age, smoking, and ear- piercing.
††† = Logistic regression analysis adjusted for sex, age, smoking, ear-piercing, and alcohol consumption.
†††† = Logistic regression analysis adjusted for sex, age, smoking, alcohol consumption, ear- piercing and educational level.
* = Trend test
OR = Odds ratio
CI = Confidence interval