Contact allergy to the preservative methyldibromoglutaronitrile

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PREFACE

This thesis is based on the following papers:

I.   Jensen CD, Johansen JD, Menné T and Andersen KE. Methyldibromo glutaronitrile in rinse-off products causes allergic contact dermatitis - an experimental study. *Br J Dermatol* 2004; 150(1): 90-95.

II. Jensen CD, Johansen JD, Menné T, and Andersen KE. Methyldibromo glutaronitrile contact allergy: effect of single versus repeated daily exposure. *Contact Dermatitis* 2005; 52: 88-92.

III. Jensen CD, Johansen JD, Menné T, and Andersen KE. Increased retest reactivity by both patch and use test with methyldibromoglutaronitrile in sensitized individuals. *Accepted for publication*.

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ABBREVIATIONS

Aq	Aqua
CAS	Chemical Abstracts Service
CCET	Cumulative contact enhancement test
D	Day
DNCB	2,4-Dinitrochlorobenzene
EECDRG	European Environmental and Contact Dermatitis Research Group
ESCD	European Society of Contact Dermatitis
Eth	Ethanol
EU	European Union
GPMT	Guinea pig maximisation test
HEMA	2-Hydroxyethylmethacrylate
HRIPT	Human repeat insult patch testing
ICDRG	International Contact Dermatitis Research Group
IPBC	3-Iodo-2-propynyl-butylcarbamate
LC	Langerhans cell
LLNA	Local lymph node assay
Log	Logarithmic
MCI/MI	2-Methyl-5-chloro-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one
MDBGN	Methyldibromoglutaronitrile
MHC	Major histocompatibility complex
NOAEL	No-observed-adverse-effect-level
Pet	Petrolatum
PPD	*p*-phenylenediamine
Ppm	Parts per million
ROAT	Repeated open application test
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
SD	Standard deviation
SLS	Sodium lauryl sulphate

DEFINITION OF TERMS

Cosmetic product: any substance or preparation intended to be placed in contact with the various external parts of the human body (skin, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition.

Rinse-off product: A cosmetic product like a soap, shampoo, cleanser, etc. that is rinsed off the skin immediately after use.

Leave-on product: A cosmetic product like a moisturizer, lipstick, perfume, etc. that is left on the skin for a longer period of time.

Preservative: substances which may be added to cosmetic products for the primary purpose of inhibiting the development of micro-organisms in such products.

BACKGROUND

ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis is an inflammatory skin disease caused by skin contact with sensitizing substances in our environment. Allergic eczema is characterized by itching, erythema, papules, infiltration, vesicles, and scaling. Individuals may become sensitized from a single incidence of skin contact with a substance or from a series of exposures over a period of time. When sensitized, subsequent exposure exceeding a certain threshold level will result in the development of allergic contact dermatitis. Most often the dermatitis is restricted to the site of allergen contact, but systemic reactions may also be induced; for instance by oral ingestion of certain allergens[1,2]. The most common skin sites for allergic contact dermatitis are the exposed areas of the hands and face, but the lower legs are also commonly affected. In Denmark, skin diseases are the third most frequently reported work-related disorder and more than 95% of these are eczemas. Eczema, especially when located to the hands, often results in long-lasting sick leave and it is the most frequently acknowledged occupational disease in Denmark[3]. About 2/3 of these cases are toxic eczemas, while 1/3 have an allergic element. The most common allergens are nickel, fragrance chemicals, preservatives, and rubber compounds.

The diagnosis of allergic contact dermatitis is facilitated by a combination of physical examination, patient history and patch testing with allergens. Clinical relevance of a positive patch test reaction may be confirmed by the exposure history of the patient; or the history may raise suspicions of the causative agent of the dermatitis and motivate patch testing with suspected allergens or products. The most effective treatment of allergic contact dermatitis is allergen avoidance; therefore the identification of the allergen or allergen source is critical to the patient.

Human and animal testing as well as practical experience indicate that threshold levels exist for the induction of sensitization[4]. For instance, in the murine Local Lymph Node Assay (LLNA) it seems that for several allergens there is a concentration level below which no significant response is induced in lymph nodes draining the site of repeated topical application. Also, far from all individuals exposed to nickel via ear-piercing or *p*-phenylenediamine (PPD) through hair dye get sensitized, thus there are inter-individual variations in susceptibility to sensitization and thereby individual sensitization threshold levels. Moss[5] concluded that the population is normally distributed with one "tail" being high responders who are particularly susceptible to sensitization. In a series of important experiments with the potent sensitizer 2,4-dinitrochlorobenzene (DNCB), Friedmann[6] showed that the higher the exposure dose, the greater the number of sensitized individuals (until a plateau is reached).

Correspondingly, thresholds for elicitation exist. Hypersensitivity, as demonstrated by a positive patch test, is often not synonymous with clinical symptoms. In elicitation studies, dose-response relationships have been documented for a number of allergens and concentration levels established at which no sensitized individuals react[7-10]. An example of a dose-response curve is shown in Fig. 1. The elicitation threshold is in experiments dependent on the sensitizing allergen dose. As the sensitization dose is increased, the dose required to elicit a challenge response is decreased[6,11]. The sensitivity to allergen
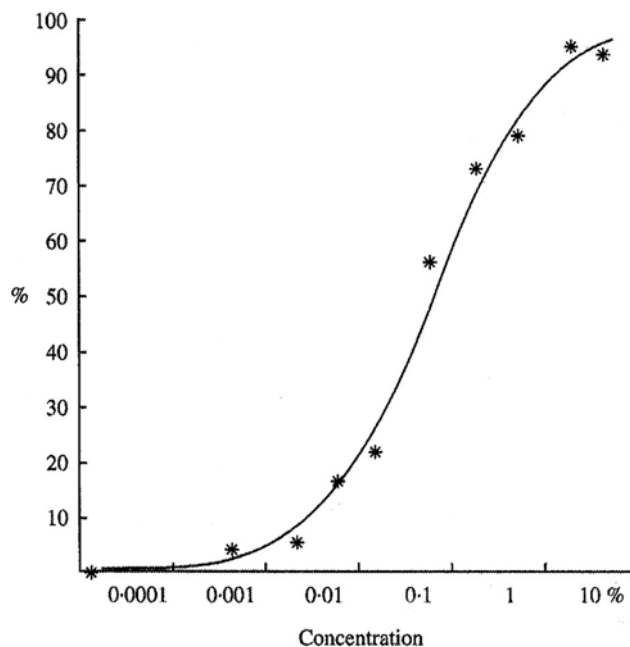
Fig. 1. Example of a dose-response curve obtained by patch testing Lyral (Hydroxyisohexyl 3-cyclohexene carboxaldehyde)-sensitized individuals with a serial dilution of the fragrance Lyral in ethanol from 6% to 0.0006%. The y-axis shows the percentage of the test group that reacted to the exposure dose in question.

exposure may vary considerably over time in a sensitized individual. A variation of as much as 250 times in a patch test experiment has been demonstrated[12].

Sensitization and elicitation threshold values are difficult to establish. Besides the individual variation in susceptibility, the matter is complicated by the dependency of the threshold values on exposure conditions. These are influenced by several factors, such as potency of the allergen, duration and frequency of application[13,14], vehicle and occlusion effects[13,15,16], dose per unit area of skin[17], integrity of skin site[18,19], anatomic site of exposure[20,21], and combination effects with irritants, etc[13,22].

Mechanisms of allergic contact dermatitis (Delayed type IV hypersensitivity)

Allergic contact dermatitis is the manifestation of an overreaction by the immune system where low doses of non-toxic molecules provoke an immunologically T cell mediated, delayed inflammatory reaction. Clinically, the response is typically represented by erythema, itching, vesicles, and scaling. Exposure to an allergen initially causes sensitization, and subsequent above-threshold skin contact with the chemical will elicit the development of allergic contact dermatitis.

Contact sensitizers (or haptens) are most commonly small molecules with a molecular weight of less than 1000 Da that have the ability to penetrate the skin and bind to dermal proteins. An allergen is referred to as a hapten, because they are not antigenic in themselves and need to associate with proteins to be recognized by the immune system[23]. Following rec-

ognition of the hapten-protein complex by antigen-presenting Langerhans cells (LCs), the antigen is processed into smaller peptide fragments in the cell. LCs are epidermal, dendritic cells with the main function of internalizing, processing, transporting, and presenting encountered antigen[24]. The processed hapten then forms complexes with major histocompatibility complex (MHC) proteins and the complex is presented on the surface of the LCs. A hapten-induced cytokine production activates the LCs that, via the afferent lymphatics, migrate to the local lymph nodes presenting the hapten-MHC molecule complexes to naïve T lymphocytes. When a hapten-specific T lymphocyte recognizes the antigen-complex via its T cell antigen receptor, the presenter cell and the T cell bind to each other facilitated by a number of adhesion molecules. The contact between the cells and formation of cytokines stimulates the activation of the T cell and the activated T cell starts proliferating and differentiating[25]. The antigen-specific memory-cell progeny is then released into the blood flow and begins to recirculate. An up-regulation of skin-homing molecules will assist their movement into peripheral tissues such as the skin[26].

Now the person is sensitized and the immune system is prepared to respond to allergen re-exposure. Upon challenge, a stronger and faster secondary immune response is triggered. Allergen-specific memory T cells will be mobilized to the exposure site. The cells will encounter antigen-presenting cells and pro-inflammatory cytokines are released, giving rise to the response clinically recognized as allergic contact dermatitis[23]. The elicitation process is a delayed process compared to immediate allergic reactions, as the accumulation of allergen-specific T cells and the production of cytokines take time.

COSMETIC PRESERVATIVES

Biocides or preservatives are highly protein-reactive chemical substances able to control the growth of microorganisms like bacteria, fungi and yeast by disturbing basic functions of the target organism. They are added to a wide range of water-based products to protect them from the damaging effects of microorganisms.

One product group utilizing preservatives is cosmetics. Legally, in the Cosmetics Directive of the European Union (EU), preservatives are defined as: "substances which may be added to cosmetic products for the primary purpose of inhibiting the development of micro-organisms in such products"[27]. Cosmetic products are expected to keep for a long time and are at the same time often stored in the warm, humid environment of a bathroom for long periods of time, providing ideal growth conditions for microorganisms. Preservatives are added to cosmetic products to protect the consumer from pathogenic microorganisms and to prevent spoilage such as discolouration, formation of malodours, and physical and chemical degradation of the products. An ideal cosmetic preservative would have the following properties[28,29]:

– Effective at low concentrations against a wide spectrum of microorganisms
– Soluble in the formulation at the required concentration
– Non-toxic and non-sensitizing to the consumers at in-use concentrations
– Compatible with other ingredients in the product
– No physical effects on the product
– Stable over a wide range of pH and temperature
– Low toxicity to aquatic organisms

– Easily biodegradable under aerobic and anaerobic conditions
– Low potential for bioaccumulation

However, no single preservative possesses all of these properties for all formulations, so often a combination of two or more preservatives is added.

The use of preservatives in cosmetic products is restricted. For health safety reasons Annex VI of the EU Cosmetics Directive[27] comprises a positive list of the substances permitted as preservatives. Some of the preservatives are listed with restrictions, such as a maximum allowed level of use, a prohibition of use in oral care products, or a restriction of use to rinse-off products only (e.g. shampoos, soaps). All preservatives added to a cosmetic product must be declared on the product labelling by its INCI (International Nomenclature of Cosmetic Ingredients) name.

Contact allergy to cosmetic preservatives

Preservatives are biologically reactive chemicals, not only against microorganisms, but towards the human organism as well and therefore have an expected irritant and allergenic potential. Inherently, the more reactive and thereby efficient a preservative is, the more allergenic it usually is. Also, the greater the exposure to a preservative in dose per unit area of skin is, the greater the number of allergic responses to a product will be. Thus, the lowest possible use concentration with sufficient antimicrobial effect of a preservative is to be preferred as regards safety. As mentioned, two or more preservatives are often combined in cosmetic formulations to obtain the desired properties and biocidal effects from the collective performance of the preservatives. This is also an advantage considering safety, as this allows for the use of lower and thereby safer concentrations of the preservatives.

Following fragrances, preservatives are the most frequently sensitizing ingredients in cosmetic products and 4 preservatives are at this time included in the European standard patch test series: methylchloroisothiazolinone/ methylisothiazolinone (Kathon CG), formaldehyde, parabens, and quaternium 15 (Dowicil 200). As recommended by the European Society of Contact Dermatitis (ESCD), a number of clinics additionally routinely test with a range of other preservatives, such as methyldibromoglutaronitrile (MDBGN), 3-iodo-2-propynyl-butylcarbamate (IPBC), imidazolidinyl urea (Germal 115), diazolidinyl urea (Germal II) to monitor the frequency of sensitivity to these chemicals.

RISK ASSESSMENT OF COSMETIC PRODUCTS

As consumers are becoming more aware of issues like health, product quality, animal testing and environmental safety, there is an increasing requirement for preservatives to be proven safe as well as effective in use. Also, according to the EU Cosmetics Directive, cosmetic products must not cause harm to human health when used under normal and foreseeable conditions. Therefore products must be demonstrated to be essentially safe prior to release. The extrapolation from small scale tests to exposure of a large population of consumers will, nevertheless, most often result in some complaints of unwanted effects. Some temporary health effects, such as slight irritation, may be acceptable, whereas adverse and permanent effects are not. For instance, the ability of chemicals to sensitize

and induce allergic contact dermatitis is a major regulatory concern.

Prior to the introduction of new cosmetic products in the market place, tests are performed to identify the sensitizing capacity of the ingredients and the formulation. Methods used are animal tests like the LLNA, the guinea pig maximisation test (GPMT), the Buehler Test, and clinical testing, e.g. human repeat insult patch testing (HRIPT). Also computer-based structure activity analysis of chemicals is being developed, where molecular and physiochemical properties are used to predict the allergenic potential of a molecule. However, this method is still rather crude and is mainly used in hazard identification. A lot of research is performed in the development of hazard identification and risk assessment methods due to a future ban of the use of animals in cosmetics testing in the EU. A replacement method for skin sensitization evaluation is far from being realized, because of the difficulty in imitating the complicated immunological mechanisms, fundamental for an allergic response, outside a living organism. Tests based on cell cultures and computer models are being investigated and consistently improved, but they are still far from being safe replacements of the animal tests.

For allergens showing even a strong sensitizing potential, there might be a threshold surface concentration below which the allergens can be used in consumer products without eliciting significant contact allergy. Skin sensitization risk assessment is based on both the potency of the chemical in question and on the expected exposure from the product type in which the use of the substance is intended. Usually a no-observed-adverse-effect-level (NOAEL) is established using experimental data from exposure studies like predictive animal tests, HRIPTs or clinical elicitation studies. The NOAEL is the greatest concentration of a substance that causes no detectable adverse effects. The robustness of established NOAEL values is however often questionable. Extrapolation from the experimental studies takes into consideration a number of uncertainty factors, such as unintended use, inter-individual and regional skin differences, vehicle and product matrix effects, and product usage patterns such as single or multiple daily exposures[30]. Furthermore, the expected skin exposure from the finished product is calculated, because this will vary considerably according to the formulation being a leave-on or rinse-off product or a body lotion or mascara, etc. Comparative benchmarking, where already available data of known safe or unsafe chemicals are utilized in the evaluation, is also a very important step of the risk assessment process.

Despite the efforts to ensure the safety of cosmetic products, safety evaluation failures of cosmetic ingredients are not few with resulting high frequencies of sensitization among consumers. Of preservatives, formaldehyde and formaldehyde-releasers have caused problems with sensitization for many years and in Europe in 2000 the average frequency of eczema patients tested positive to formaldehyde was around 2–2.5%[31]. Another famous example of a risk assessment failure is the preservative 2-methyl-5-chloro-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (MCI/MI), also known by the trade name Kathon CG. Kathon CG was introduced as a cosmetic preservative in Europe in the mid-70's and due to its antimicrobial efficiency in very low concentrations against a broad range of microorganisms it soon became popular and widespread. In the mid-80's, the first of numerous reports describing allergic contact dermatitis from a Kathon CG-preserved cosmetic product appeared[32,33], and as the use of Kathon CG increased, so did the number of reports and the controversy regarding the safety of the preservative. Kathon CG is a strong sensitizer and the allowed use-concentration of the preservative in cosmetic products at the time was too high with regard to safety. The allowed use-levels were evaluated and down-regulated to 0.0015% (15 ppm) and today the use of Kathon CG in leave-on products is much rarer and the use of the preservative in rinse-off products is considered safe[34,35]. Like formaldehyde, the level of sensitivity to Kathon CG of patch tested eczema patients is now stabilized, although at the rather high level of 2-2.5%[31].

A more recent and still ongoing case of a failed detection of significant allergenic potential is the case of the preservative MDBGN.

THE METHYLDIBROMOGLUTARONITRILE STORY

In the mid-80's, a new compound for preservation of cosmetic products was introduced in Europe. The compound, Euxyl K 400 (Schülke & Mayr, Hamburg), was a combination of the two preservatives MDBGN (CAS No: 35691–65–7) and phenoxyethanol (CAS No: 122–99–6) in a ratio of 1:4. The combination of the two chemicals was very effective against a broad spectrum of bacteria and fungi in very low use-concentrations, and consequently, Euxyl K 400 grew increasingly popular. The preservation compound is now being used in a wide range of cosmetic products, like moisturizers, shampoos, soaps, sunscreen lotions, hair-care products, and make-up, but is also used in cleaning agents and various industrial products. 2-Phenoxyethanol is a rare sensitizer and only few cases of hyperreactivity to the chemical have been published[36-38]. MDBGN is synonymous with 1,2-dibromo-2,4-dicyanobutane and the INCI name to be declared on the labelling of cosmetic products is Methyldibromo Glutaronitrile. The structure of MDBGN is shown in Fig. 2. According to the EU Cosmetics Directive[27], MDBGN is allowed at a maximum concentration of 0.1% (Euxyl K 400 0.5%) in both leave-on and rinse-off
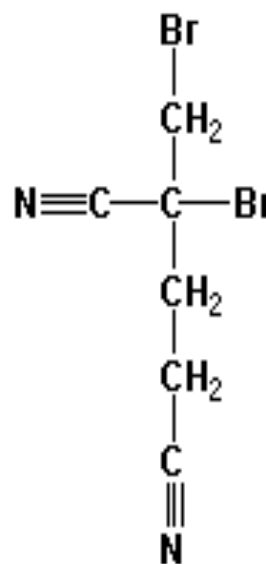

Fig. 2. Chemical structure of MDBGN (CAS No 35691–65–7)

Table 1. Case reports of allergic contact dermatitis from MDBGN.

No of cases	Product(s) causing allergic contact dermatitis	Localization of dermatitis	Reference
1	Soap	Genital area	45
23	Body lotion, facial cream, hand cream, liquid hand soap (1 case occupational)	Hands, arms, legs, face, trunk	44
1	Permanent wave solution protection cream	Forehead	46
3	Hand cleanser and barrier cream (occupational)	Hands and forearms	47
1	Deer-fat cream	Axilla spreading to trunk and limbs	48
1	Ultrasonic gel	Abdomen	49
1	Hair mousse and leave-on hair conditioner	Scalp	50
1	Sunscreen cream	Face	51
2	Hand cleansing agent (occupational), dishwashing liquid detergent (occupational)	Hands	52
12	Moistened toilet paper, night cream	Perianal, face, hands, axilla	53
1	Anti-aging day cream	Face	54
1	Ultrasonic gel	Abdomen	55
1	Cucumber eye gel	Face	56
1	Night cleansing cream	Face and neck	57
2	Cucumber eye gel	Eye lids	58
8	Soap, cleansing face lotion, foaming soap, moisturizing cream	Face, hands, widespread	59
2	Wrinkel-removal lotion, massage lotion	Face and neck	42
1	Paste glue formulation (occupational)	Hands and forearms	43
1	Facial cleansing milk and body milk	Face and hands	60
3	Hand soap (occupational)	Hands	61

Table 2. Reported frequencies of consecutive eczema patients patch test positive to MDBGN and/or Euxyl K 400.

Country	Period of testing	Test material	Frequency of positives	Reference
11 European countries	1991–2000	MDBGN 0.1–0.3% pet.	1521/48485 (3.1%)(0.7% in 1991, 3.5% in 2000)	31
UK	1989–2000	MDBGN 0.3% pet. (1998–2000)	88/12704 (0.7%)	62
Spain	1998–1999	Euxyl K 400 0.5% pet.	5/528 (0.9%)	63
UK	1998–1999	MDBGN 0.3% pet.	1.4%	64
	1991–1997	Euxyl K 400 0.5% pet.	0.3%	
Germany	1997–1998	Euxyl K 400 1% pet.	160/4615 (3.5%)	65
		Euxyl K 400 0.5% pet.	105/4615 (2.3%)	
		MDBGN 0.3% pet.	33/988 (3.3%)	
		MDBGN 0.1% pet.	14/988 (1.4%)	
USA	1996–1998	Euxyl K 400 2.5% pet.	308/4054 (7.6%)	66
		Euxyl K 400 1% pet.	109/4053 (2.7%)	
USA	–	Euxyl K 400 2.5-1% pet.	19/163 (11.7%)	67
USA	1994–1996	Euxyl K 400 1% pet.	61/3074 (2.0%)	68
The Nederlands	1993–1995	MDBGN 0.1% pet.	24/1307 (2.4%)	69
		Euxyl K 400 0.5% pet.	24/1307 (2.4%)	
The Nederlands	1993–1994	MDBGN 0.1% pet.	16/809 (2.0%)	53
USA	1992–1994	Euxyl K 400 1% pet.	52/3481 (1.5%)	70
Italy	1991-1994	Euxyl K 400 2.5% pet.	99/3455 (2.8%)	37
		Euxyl K 400 0.5% aq.	54/3022 (1.8%)	
		Euxyl K 400 1.5% aq/PG	5/729 (0.7%)	
		MDBGN 0.5% pet.	21/919 (2.3%)	
Italy	–	Euxyl K 400 2% PG	21/1033 (2.0%)	71
		Euxyl K 400 1.5% PG	18/1033 (1.7%)	
France	1988–1994	Euxyl K 400 2% pet.	26/1217 (2.1%)	72
The Nederlands	1994	MDBGN 0.3–0.05% pet.	119/2943 (4.0%)	73
The Nederlands	1991	MDBGN 0.05% pet.	6/1142 (0.5%)	38
Italy	1988–1990	Euxyl K 400 2.5% pet.		
		Euxyl K 400 2.5% eth.	24/2057 (1.2%)	59
UK	1989	Euxyl K 400 1% aq.	0/>1800 (0%)	58

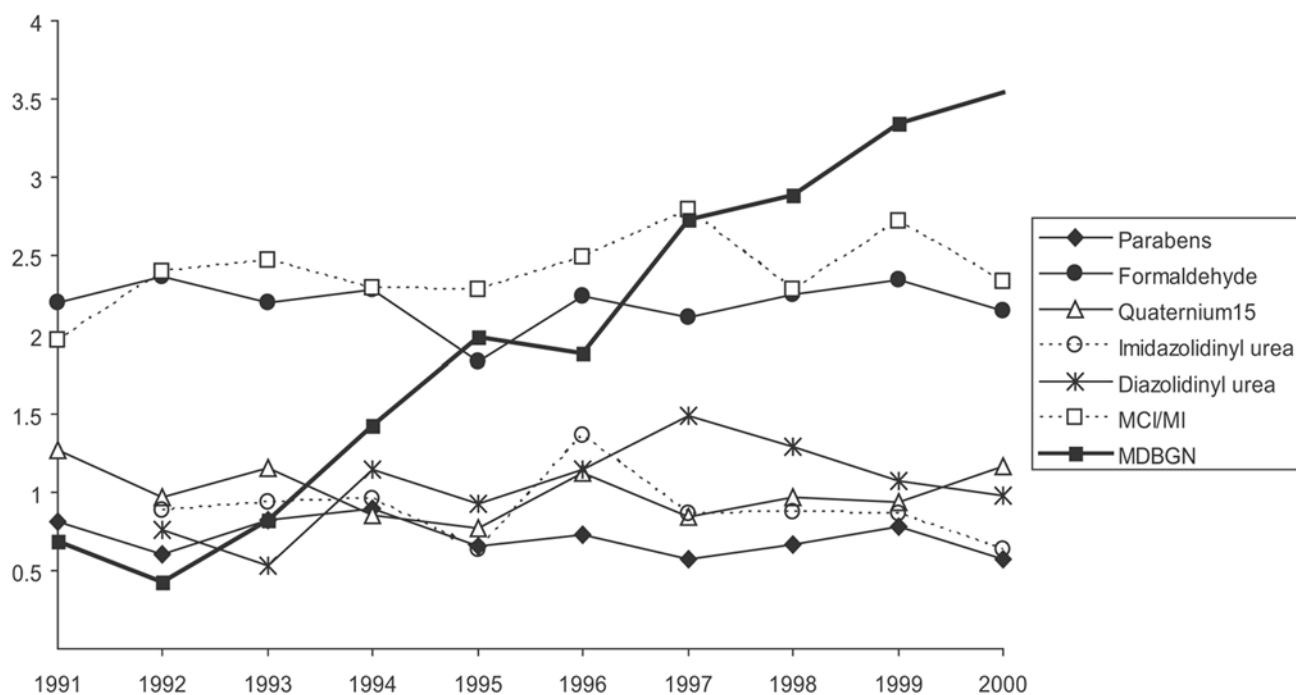Pet = petrolatum, aq = water, eth = ethanol, PG = propylene glycol

Fig. 3. The figure shows the average sensitivity rates of 7 cosmetic preservatives from 1991 to 2000 calculated by combining all the positive patch test results of 16 European clinics. The data for MDBGN is based on 48,485 individuals tested over the decade.

cosmetic products with the exception of sunscreen products where the concentration must not exceed 0.025%. The reason for this different level in sunscreen products is that when the restriction of 0.1% was established, no skin penetration data was available for sunscreens and the use of MDBGN herein was therefore initially banned. In 1992, data from an *in vitro* penetration study was available and the use of MDBGN in sunscreens was re-considered and a concentration limit of 0.025% was reached[39].

In previous predictive animal tests and HRIPTs, MDBGN appeared to be a weak sensitizer[39–41] and as it moreover was a very efficient cosmetic preservative, Euxyl K 400 became increasingly popular; in part as an alternative to the problematic Kathon CG. However, the first report of allergic contact dermatitis from Euxyl K 400 in a cosmetic product was published in 1989[42], and since, numerous cases of allergic contact dermatitis has been reported from MDBGN in products such as soaps, moisturizers, cleansing milk, eye gel, ultrasonic gel, moistened toilet paper, sunscreen lotion, cleansing agent, and detergent (summarized in Table 1). The very first report of allergic contact dermatitis from MDBGN was published in 1983. Here a factory worker had developed eczema from using a paste glue formulation containing the MDBGN-containing biocide, Tektamer 38[43]. Allergic contact dermatitis caused by MDBGN as a cosmetic ingredient is often more severe than what is usually seen for cosmetic dermatitis, which may be an indication of use of a potent allergen in too high concentrations[44].

As the use of MDBGN became increasingly widespread, several dermatological clinics in Europe reported of a rising number of patients found patch test positive to the chemical (summarized in Table 2). In 2000, a 10-year analysis of the level of reactivity to common cosmetic preservatives in Europe was published by the European Environmental and Con-

tact Dermatitis Research Group (EECDRG)[31]. This analysis showed the average sensitivity rates to 7 cosmetic preservatives calculated by combining all the positive patch test results of consecutive patients with eczematous skin conditions from 16 dermatology clinics in 11 European countries (Fig. 3). This included data for MDBGN based on 48,485 individuals tested over a decade. The report revealed a rise in the average frequency of sensitivity to MDBGN in eczema patients from 0.7% in 1991 to 3.5% in 2000 in the participating clinics. These observations promoted MDBGN to an important contact allergen in Europe and urged a safety re-evaluation of the use of the chemical.

The current regulation of MDBGN is based on 11 predictive animal tests (GPMTs) and 7 HRIPTs[39] and all indicated that MDBGN was non-sensitizing. In light of the high number of cases of contact allergy to MDBGN reported, Hausen[41] in 1993 published a sensitization study of MDBGN using a modified Freund's complete adjuvant test which is considered as sensitive as the GPMT. He found MDBGN to be a distinct, but weak sensitizer with a low risk of sensitization and welcomed it as a replacement for Kathon CG. Further, a few years later Wahlquist *et al*[74] conducted sensitization studies of MDBGN using the GPMT, the Cumulative Contact Enhancement Test (CCET) (another guinea pig test method using Freund's adjuvant)[75], and the LLNA. Again, the GPMT did not demonstrate any significant difference between the MDBGN-exposed and control animals, but the CCET and the LLNA both classified MDBGN as a sensitizer. It was speculated that the different results of the predictive tests may be due to the number of topical applications in the tests; maybe multiple applications of MDBGN is necessary for sensitization. The GPMT has only a single topical application, while the CCET and LLNA have multiple topical applications. It was, however, questioned why

the HRIPTs then failed to demonstrate the sensitizing capacity of MDBGN. This, though, may be explained by the rather low test concentrations of 0.0012% to 0.0396% MDBGN[76]. The fact that MDBGN is labile in biological systems, as it is debrominated to 2-methyleneglutaronitrile[77,78], and/or that the chemical may be a pro-hapten have been mentioned as explanations for the possible requirement of multiple applications[74], but the reason for the different test outcomes has yet to be clarified.

In 2002, a mandate was presented to the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) of the EU, requesting an evaluation of the safety of the current use of MDBGN in light of the published EECDRG data[31]. The SCCNFP is the scientific advisory body of the European Commission in matters of consumer protection with respect to cosmetics and non-food products. In the following Opinion of the SCCNFP[79] it was recommended: "Until appropriate and adequate information is available to suggest a level of the preservative in leave-on products that poses an acceptable risk to the consumer (compared with the risk to the consumer from other preservatives), restricting its use to rinse-off products at the current maximum permitted level of 0.1%". The 7th amendment of the Cosmetics Directive will become effective in 2005, and in this, the regulation of MDBGN is modified and its use now restricted to rinse-off products at a maximum concentration of 0.1%.

MDBGN PATCH TEST CONCENTRATION AND VEHICLE

An important diagnostic tool for allergic contact dermatitis is the epicutaneous patch test. Eczema patients are exposed to common and suspected allergens under controlled conditions and the skin response to the allergens is registered. To get reliable readings, it is important to expose the patients to concentrations of allergen that do not miss clinically relevant allergies and will not frequently produce false-positive irritant reactions. A commonly used MDBGN patch test concentration is 0.3% in petrolatum (pet). In recent experiments, however, the patch test concentration appears to miss clinically relevant cases of contact allergy (personal communication). The higher concentration of 0.5% MDBGN, though, often produces false-positive irritant reactions, which means that some patients will be falsely diagnosed with contact allergy. But because of the risk of false-negative reactions it is being considered to recommend the use of a patch test concentration of 0.5% MDBGN in pet.

EXPERIMENT EXAMINING THE MDBGN PATCH TEST VEHICLE

The preferred MDBGN patch test vehicle is pet. Pet. is popular as vehicle because of its ability to provide efficient occlusion, keep allergens stable and, moreover, it is easy to work with. It is, however, difficult to dose pet. accurately and therefore it was decided to use an ethanol/water (eth./aq.) mixture as vehicle in the experiments of this thesis. Ethanol was added to ensure that the MDBGN was properly dissolved, as its solubility in water is limited.

To examine and compare the patch test response to MDBGN in eth./aq. and MDBGN in pet., a small experiment was performed. 20 consecutive patients were patch tested with MDBGN in 3 concentrations: 0.1%, 0.3% and 0.5% in both pet. and a 50:50 mixture of eth./aq. The patch test results are presented in Fig. 4. 19 of the tested patients had a patch test response on D3 to at least one of the patches with at least one doubtful, positive or irritative reaction. The test size is limited, but some tendencies were observed: a) MDBGN in eth./aq. caused significantly more visibly irritative reactions than MDBGN in pet. at the concentrations of 0.3% and 0.5%. Frequent irritative reactions from Euxyl K 400 in ethanol have been reported earlier[37]. b) With eth./aq. as vehicle, 3 patients had a positive reaction (+) to at least one of the patch test concentrations compared to 1 person with pet. as vehicle. c) Doubtful reactions were frequent for all patches except 0.1% pet. Most of these reactions are probably weak irritative reactions.

In general, stronger patch test responses with frequent irritative reactions were seen to MDBGN in eth./aq. than to MDBGN in pet. This was supported by patch test results from Trial I of this project. The patients in Trial I were patch tested with a dilution series of MDBGN (0.2%–0.001%) in eth./aq. (50:50) along with a patch of 0.3% MDBGN pet. to compare the patch test responses. (The highest concentration chosen in the dilution series was 0.2% MDBGN eth./aq. to avoid most irritative reactions). 0.2% MDBGN eth./aq. generally produced a patch test response equal to or stronger than 0.3% MDBGN pet. (Table 3). Feasible explanations for the augmented response with the eth./aq. vehicle are a greater penetration of
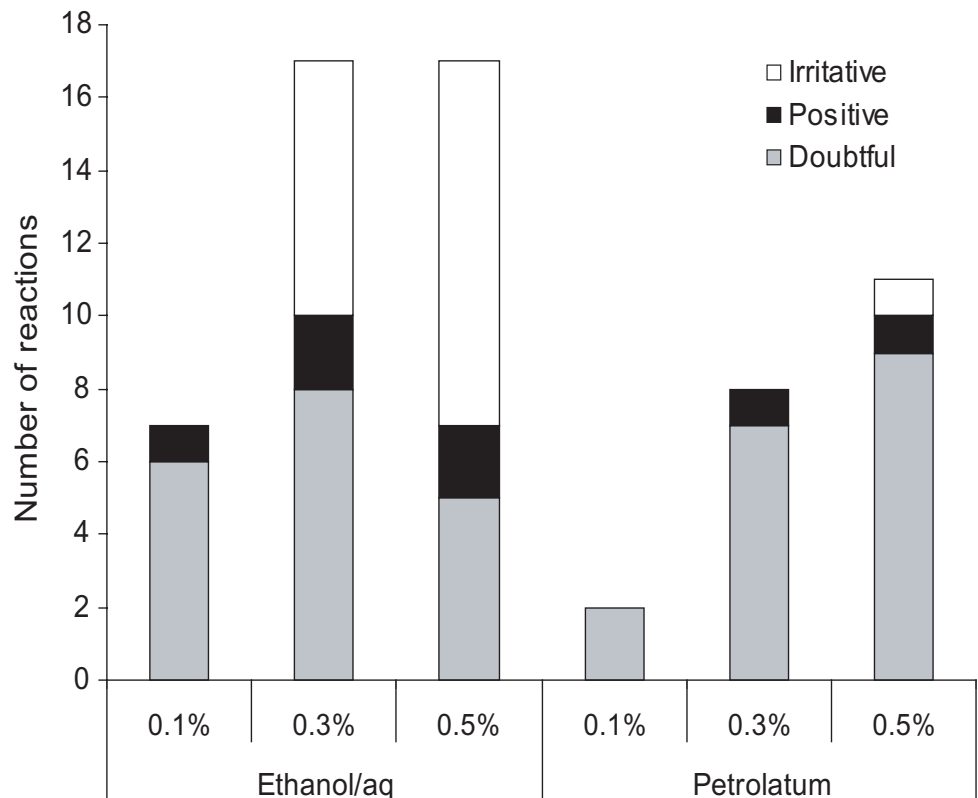
Fig. 4. Patch test reactions of 19 consecutive patients to MDBGN in pet. and eth./aq. Some patients had reactions to several of or all test solutions.

Table 3. Comparison of patch test reaction to 0.2% MDBGN eth./aq. and 0.3% MDBGN pet. from trial I of this thesis.

Pt.	0.2% eth./aq. (D3)	0.3% pet. (D3)
1	++	++
2	+	+
3	+	+
4	++	++
5	+++	++
6	++	++
7	+	+
8	++	++
9	NT	+++
10	++	++
11	+	+?
12	+	Neg
13	+++	++
14	++	+
15	+	+
16	++	++
17	++	+?
18	NT	++
19	+	neg

NT = not tested, Neg = negative

allergen into the skin when dissolved in eth./aq. than when dispersed in pet. Another explanation may be the irritant properties of ethanol contributing to the inflammatory response.

As MDBGN is easily dissolved in 20% ethanol solution, it was decided to change the eth./aq. ratio from 50:50, which was the mixture used in Trial I of this thesis, to 20:80 in Trial II and III to ensure a minimization of the number of irritant and doubtful reactions caused by the ethanol content.

AIMS OF THE THESIS

The aim of the thesis was to characterize the allergic response to MDBGN in pre-sensitized individuals. During 3 clinical elicitation studies, sensitized subjects were exposed to the preservative in experimental use tests designed to resemble the actual use-situation of cosmetic products. Exposure conditions in the trials were varied in order to examine different aspects of the allergic response.

The focus of this thesis was decided based on the alarming rise in the frequency of eczema patients sensitized to MDBGN in Europe and the urgency of a safety re-evaluation of MDBGN. Results obtained from the experimental studies were intended to contribute to a risk assessment and an evaluation of the existing threshold limiting values of MDBGN in cosmetic products. However, in the initial stages of this project a decision was made to ban the use of MDBGN in leave-on cosmetics in Europe. This ban will be effective "until appropriate and adequate information is available to suggest a level of the preservative in leave-on products that poses an acceptable risk to the consumer…"[79]. Following this development, it was decided in the first trial of this thesis to investigate the risk of utilizing MDBGN in rinse-off products, as the preservative will continue to be allowed in this type of products at the current permitted level of 0.1%. In the second trial, the influence of the frequency of applications on the elicitation potential was examined, and in the third trial, the significance of previous allergic contact dermatitis when re-exposed was investigated.

AIM OF TRIAL I – ELICITATION STUDY OF MDBGN IN A RINSE-OFF PRODUCT

The aim of the trial was to investigate the allergic response elicited in pre-sensitized individuals from exposure to a rinse-off product preserved with the maximum permitted level of MDBGN, 0.1%. The results may be used in a risk assessment of MDBGN and in an evaluation of the existing threshold limiting value of the preservative in rinse-off cosmetic products.

AIM OF TRIAL II – EFFECT OF SINGLE VERSUS REPEATED DAILY EXPOSURE OF MDBGN

This trial examined whether one high-dose exposure a day of MDBGN is more or less potent than several low-dose exposures with regard to elicitation of allergic contact dermatitis in pre-sensitized individuals. This knowledge may be useful in the risk assessment and regulation of products potentially used multiple times a day.

AIM OF TRIAL III – INVESTIGATION OF RE-TEST REACTIVITY BY BOTH PATCH AND USE TEST WITH MDBGN IN SENSITIZED INDIVIDUALS

It has been shown that skin with healed allergic contact dermatitis is re-test hyperreactive for up to several months after the dermatitis has healed. This trial aimed to investigate whether skin with previous dermatitis elicited by MDBGN showed an augmented response compared to normal and irritated skin when re-exposed by both a patch test challenge and in a use test with a liquid soap preserved with MDBGN.

METHODS

In this section the populations and methods used in this project are described. Detailed descriptions of the study design and methodology of the individual trials are given in the next section and in the manuscripts of which this thesis is based.

POPULATIONS

The test populations in all 3 trials of this project were eczema patients previously tested positive to MDBGN in routine diagnostic patch testing at the Department of Dermatology, Odense University Hospital. Since 1998, all patients have routinely been patch tested with 0.3% MDBGN pet. and at least a + reaction representing erythema and infiltration to this patch was required for participation. Additionally, if a positive patch test reaction to MDBGN could not be reproduced during the trial the subject was excluded. Further exclusion criteria were age below 18, pregnancy, and dermatitis on the test areas. All participants provided written informed consent and the study was performed according to the Helsinki Declaration II. Approval was obtained from the local ethical committees.

PATCH TESTING

The Finn Chamber patch testing technique (Epitest Oy, Helsinki, Finland) was used. 15µ l of each patch test solution was micropipetted onto filter paper discs of Finn Chambers (Epitest Oy, Helsinki, Finland) on Scanpor tape (Alpharma A/S, Vennesla, Norway) and mounted on the subject's back. The patches were removed on day 2 (D2) by the patient and readings were done on D3 and D7 and classified according to the International Contact Dermatitis Research Group (ICDRG) as +?, +, ++, +++, and IR[80]. The patch testing technique was among other uses employed to determine the patch test threshold values of the individual patients by patch testing with a dilution series of MDBGN. The patch test threshold value is the lowest patch test concentration to produce a positive reaction in an individual and is a measure of the sensitivity of the patient to the allergen. As patch test vehicle an eth./aq. mixture and not pet. was used to enable accurate dosage of the patch test material with a micropipette.

USE TEST

Variations of the use test or the repeated open application test (ROAT)[81] was used to imitate real-life use of cosmetic products and examine the elicitation potential of MDBGN in different exposure situations. The test material was applied to a marked area of skin on a daily basis for 3-4 weeks and it was registered if dermatitis appeared within the test period. A cut-off value of a positive use test was erythema covering at least 25% of the test area and infiltration represented by papules regardless of number.

ELICITATION OF EXPERIMENTAL DERMATITIS

A method described by Hindsén *et al*[19] was used with a few small alterations in Trial III to produce an area of homogeneous dermatitis on the skin of pre-sensitized volunteers. A 5×8 cm filter paper was saturated in 800 µl of MDBGN in eth./aq. (20:80) of a concentration equal to the lowest concentration of MDBGN to produce a ++ patch test reaction in the subject. Another identical filter paper was saturated in 800 µl of the vehicle solution to be used as a control. The filter papers were placed symmetrically in a randomized manner on either side of the spine on the lower back of the patients. To ensure a degree of occlusion hydrocolloid dressings (Duoderm, Convatec, Denmark) were used to fix the filter papers onto the skin. The patches were removed by the patient on D2, and on D3 the dermatitis was evaluated at our clinic.

EXPERIMENTAL WORK –
STUDY DESIGN, RESULTS AND DISCUSSIONS

In the following section, the findings of the thesis are described. Due to the division of the project into 3 separate clinical trials, it would be inconvenient to compile all in a common section. To ensure clarity, a description of each trial, including a discussion of the findings, is given separately. A general discussion and future perspectives will follow.

TRIAL I – ELICITATION STUDY OF MDBGN IN A RINSE-OFF PRODUCT

A double-blind, randomized experimental model imitating the use of an MDBGN-containing rinse-off product was developed based on the ROAT method. 19 subjects with a positive patch test reaction to MDBGN were asked to wash areas of skin on their forearms measuring 5×10 cm with liquid soaps. The test area on one arm would be washed with a liquid soap containing 0.1% MDBGN and the other with a soap without MDBGN. This would be done twice a day for up to 4 weeks or until dermatitis developed on the test area. A control group of 9 individuals with negative patch test reactions to MDBGN also performed the use test.

Of the 19 participants, 7 (37%) developed dermatitis on the test area after 6 to 34 days. 2 individuals agreed to continue the use test for a fifth week, because they described a slight itching on one test area on the final day of the test. One of these subjects developed a positive reaction after 34 days on the MDBGN-exposed arm. The other developed a few square centimetres of weak dermatitis, but did not fulfil the criteria for a positive response. An example of a positive use test is shown in Fig. 5 (p. 17), and test results for all participants can be seen in Table 4. All 9 controls had negative use tests.

The frequency of positive use test reactions to the MDBGN-containing soap was significantly different between the test group and the control group (one-sided Fischer's Exact Test, $p = 0.04$). The average amount of soap used per application varied considerably among the test subjects with the range 0.019g to 0.29g (Table 5). However, the corresponding use in the control group was similar with the range 0.018g to 0.28g. Considerable inter-individual variation in exposure was noted because some participants deliberately widened the opening of the soap container to ease the application of the soap resulting in application of larger amounts.

The dose per unit area of exposure in the test group was estimated to a mean of 2.2 µg/cm² MDBGN per application, and the 7 subjects with a positive response averagely applied a summed total of 35 µg/cm² in the test period (Table 4). For comparison, in a time-dose-response study in sensitized volunteers with the potent fragrance allergen isoeugenol, the total amounts of allergen applied in a use test before positive responses developed were averagely 66 and 144 µg/cm² for a low and a high dose of isoeugenol, respectively[8]. It must be taken into account that this was leave-on test material with a greater skin contact than in the current experiment, where some of the soap and allergen was washed off the skin shortly after application. However, it is also important to consider the presence of detergents in the soap as they may influence the elicitation and induction thresholds and the tendency of the skin to develop contact dermatitis[22,82]. Soaps have irritating properties, for instance due to the detergent sodium lauryl sulphate (SLS), and may cause unspecific inflammatory reactions and increase penetration into the skin. Pedersen *et al*[83] demonstrated an augmented allergic response in sensitized patients when patch tested with MDBGN combined with SLS com-

Table 4. Test results of use test with rinse-off product containing 0.1% MDBGN.

Pt	Patch test threshold concentration (MDBGN eth./aq.)	Patch test reaction to MDBGN 0.3% pet.	Day of positive ROAT	Average amount of MDBGN per application(µg/cm²)	MDBGN applied until positive ROAT (µg)
1	0.02%	++	-	4.3	-
2	0.1%	+	-	4.6	-
3	0.2%	+	-	4.2	-
4	0.005%	++	-	1.3	-
5	< 0.001%	++	11	1.0	20
6	< 0.001%	++	-	0.74	-
7	0.1%	+	-	0.38	-
8	< 0.001%	++	15	1.0	29
9	< 0.001%	+++	6	1.0	11
10	< 0.001%	++	-	4.2	-
11	0.2%	+?	-	2.6	-
12	0.2%	neg	23	3.9	171
13	0.003%	++	12	0.54	12
14	< 0.001%	+	-	1.0	-
15	0.05%	+	-	0.89	-
16	0.05%	++	13	2.8	66
17	0.01%	+?	-	5.5	-
18	< 0.001%	++	34	0.83	55
19	0.2%	neg	-	1.4	-

Neg = negative, Pt = patient, < = below.

Table 5. The table shows the amount of soap used per application and the MDBGN exposure per unit test area for the test group and the controls.

	Average amount of soap used per application (g) (mean ± SD) (range)	Allergen-dose per application (μg/cm²) (mean ± SD) (range)
Test subjects	0.11 ± 0.08 [0.019,0.29]	2.2 ± 1.7 [0.37,5.81]
Controls	0.10 ± 0.07 [0.018,0.28]	2.1 ± 1.5 [0.36,5.59]

SD=standard deviation

pared to MDBGN alone. Together with the difficulties of determining the actual allergen exposure, combination effects complicate the estimation of the inflammatory impact of rinse-off products.

In addition to the use test, the subjects were patch tested with a dilution series of MDBGN (0.2%, 0.1%, 0.05%, 0.03%, 0.02%, 0.01%, 0.005%, 0.003%, 0.002%, and 0.001% in eth./aq. (50:50)) to determine their patch test threshold values. An example of patch test reactions is seen in Fig. 6. 7 test subjects had a strong positive patch test reaction to the lowest patch test concentration of 0.001% and thus their patch test threshold values were below 0.001%. The test group was inhomogeneous with sensitivities to MDBGN ranging from very low patch test threshold values below 0.001% up to 0.2% MDBGN eth./aq. (Table 4). Most subjects with a positive use test response were highly sensitive to MDBGN-exposure with low patch test threshold values. 4 had a patch test threshold value below 0.001%; one a value of 0.003%, and 2 had somewhat higher threshold values of 0.05% and 0.2% MDBGN eth./aq., respectively. These 2 used a larger amount of soap per application (0.14 and 0.19g) compared to the 5 other subjects who used an average of 0.044g of soap per application.

Besides the content of allergenic ingredients, the use-pattern and type of cosmetic product are important factors in the evaluation of sensitization risk and in the determination of use-concentrations of cosmetic ingredients. For instance, application to a more penetrable and sensitive skin area, like the face, may increase the risk of sensitization or elicitation, as may frequent use of a product. Here we utilized liquid hand soap as the test product. Hand soap is applied to an often compromised skin barrier and this type of product is also commonly used several times a day, especially in occupational use-situations. Therefore the allergen exposure from a liquid hand soap may be more potent than e.g. exposure from a body or hair shampoo. Facial rinsing creams are also rinse-off products with an increased risk of sensitization or elicitation, as the facial skin is delicate.

A few cases of allergic contact dermatitis from rinse-off products have been described. However, often it has not been possible to establish whether the product was also the primary sensitizer. Zachariae et al [44] recently presented a number of cases of allergic contact dermatitis from cosmetic products preserved with MDBGN; in some of the cases rinse-off products containing MDBGN were involved. In 4 cases, liquid hand soaps containing MDBGN were found among the patient's cosmetic products and in 2 of these cases the soaps were believed to have played a part in sensitization. In another case, a facial cleansing cream had elicited hand and facial dermatitis in a pre-sensitized patient. Fernandez et al [60] presented a case of elicitation of allergic contact dermatitis from a facial cleansing cream as well. In a recent communication by Diba [61], 3 cases of allergic contact dermatitis from occupational uses of hand soaps containing MDBGN were described. Whether the soaps were responsible for the sensitization of the patients was not discussed in the article. Tosti et al [59] reported 4 cases of allergic contact dermatitis from MDBGN with rinse-off products as the only apparent sources of exposure to the allergen. Two patients were using a soap containing MDBGN, one was using a facial cleansing cream and one was using a foaming soap. The dermatitis cleared once the use of these products was discontinued. In a recent case, a man developed allergic contact dermatitis in the genital area and MDBGN in a hand soap was found to be the causative agent [45]. However, the man described vigorous use of the soap to cleanse his skin after intercourse, implying that irritation of the sensitive genital skin was an important contributing factor.

Rinse-off exposure studies with MCI/MI have been performed. In one use test, 4/4 pre-sensitized subjects developed reactions to a shampoo containing 25 ppm (0.0025%) MCI/MI [34]. In a double blind, randomized parallel study in 27 MCI/MI-allergic subjects, no statistically significant difference was found between reactions to a shampoo preserved with 15 ppm
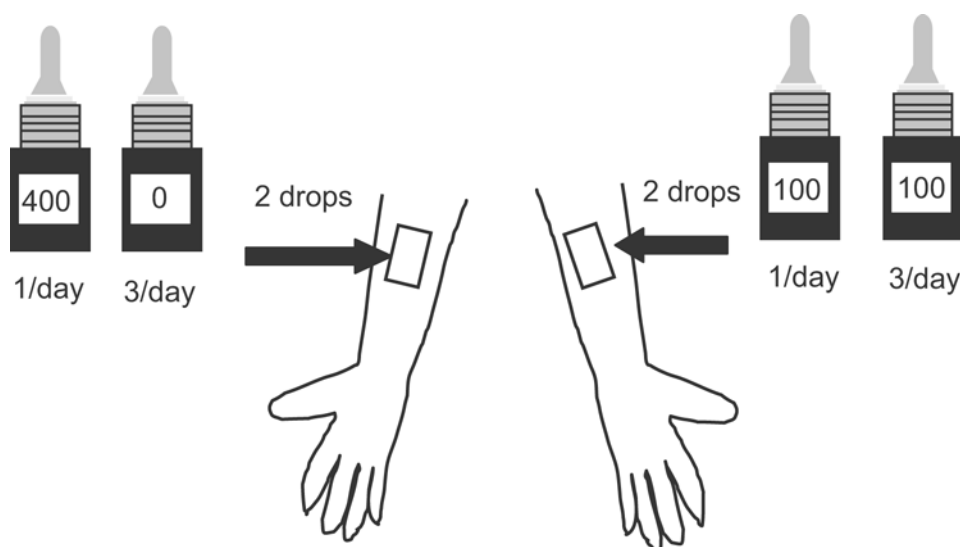

Fig. 7. Illustration of the study design of Trial II.

MCI/MI and a shampoo preserved with 0.3% imidazolidinyl urea[35]. In a third study, 6 rinse-off product types (4-6 ppm MCI/MI) were used by 18 subjects for 3-6 weeks[84]; all but one subject used several product types concurrently. No reactions were seen. MCI/MI is allowed in rinse-off products at a maximum level of 15 ppm which, as mentioned earlier, is regarded as safe.

One provocation study with MDBGN in a rinse-off product has been published[85]. The 11 subjects who completed the study were required to shampoo their hair at least three times a week for a period of 9-13 weeks with a shampoo containing 0.02% MDBGN. This level of exposure did not provoke elicitation in any of the test subjects.

In conclusion, this trial shows that exposure to a rinse-off product, containing the maximum permitted level of MDBGN, can elicit an allergic response in pre-sensitized individuals. Thus, it will be relevant to advise patients with a positive patch test reaction to MDBGN, to also avoid rinse-off products containing the preservative. Case reports imply that MDBGN in rinse-off products may also sensitize, particularly when present in hand soaps. The results of this trial coupled with the case reports indicate that the permitted level of MDBGN in rinse-off products is too high.

TRIAL II – EFFECT OF SINGLE VERSUS REPEATED DAILY EXPOSURE OF MDBGN

Some types of cosmetic products such as hand soaps and hand-care products are not uncommonly used several times a day both in the home and at the work place. For instance, in the health care sector it is not unusual to wash the hands 20-30 times a day[86]. Little has been shown experimentally of how the daily frequency of application of an allergen in a product influences the allergic response. We wanted to examine the effect of single versus multiple daily exposures with regard to

Fig. 5. Example of a positive use test from a liquid soap containing MDBGN.

elicitation of allergic contact dermatitis in pre-sensitized individuals.

An experimental model based on the ROAT method was developed and 19 subjects with a positive patch test reaction to MDBGN participated in the use test. They were provided with randomized sets of 2×2 eth./aq. (20:80) solutions in droplet bottles containing different amounts of MDBGN, to be applied to test areas of 3×4 cm on the inside of their forearms for up to 3 weeks. The subjects were instructed to apply 2 solutions to each arm, one of the solutions once daily and the other thrice daily. The study design is illustrated in Fig. 7. The 2 solutions for one arm contained 0.04% MDBGN (to be applied once a day) and no MDBGN, respectively. The solutions

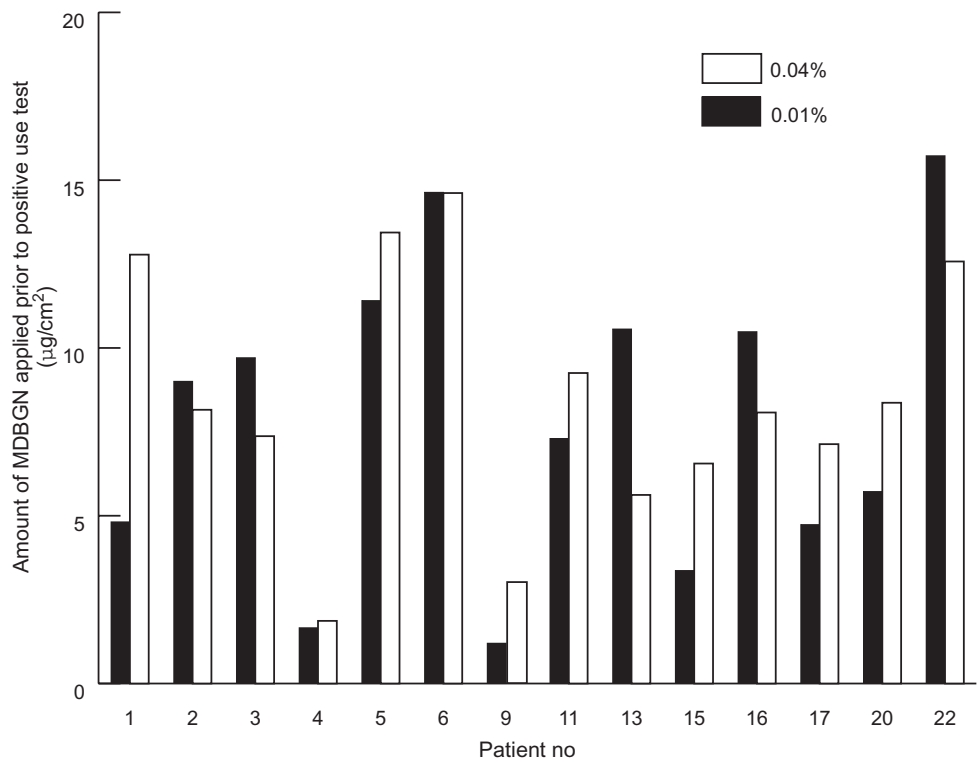*Fig. 6.* Example of patch test reactions to dilution series of MDBGN.

Fig 8. Amount of MDBGN applied until an allergic response was seen from the 4 times 0.01% MDBGN applications and the single 0.04% MDBGN application a day.

for the other arm both contained 0.01% MDBGN. This resulted in an application of approximately equal amounts of MDBGN on both arms, applied either in one application of 0.04% or distributed over four applications of 0.01%. This design was necessary to ensure a blinded, randomized experiment. A control group of 9 individuals, with negative patch test reactions to 0.3% MDBGN pet., also performed the use test.

Of the 19 trial participants, 14 developed dermatitis on both arms from the use test exposure, while 5 were completely negative on both arms at the termination of the trial. None of the controls showed any skin changes during the use test. The total amount of MDBGN applied and the number of days of exposure until a positive use test, is illustrated in Figs. 8 and 9 for the two exposure modes for each subject. In most cases, only 1–2 days separated the development of dermatitis to the two concentrations of MDBGN. In 7 of the 14 reacting patients, the total amount of allergen applied to provoke dermatitis was lowest for the 0.01% MDBGN-solution compared to the 0.04% solution, while the opposite was found in 6 patients. For one patient equal amounts of MDBGN had been applied

to both arms when dermatitis appeared. The 14 subjects developed dermatitis on the test areas after an average application of almost equal amounts of MDBGN: 7.9 $\mu g/cm^2$ from the 0.01% solution and 8.3 $\mu g/cm^2$ from the 0.04% solution (Table 6). The amounts of MDBGN were delivered to the skin in an average of 15.0 applications (4 daily) of the 0.01% MDBGN solution and 3.9 applications (1 daily) of the 0.04% MDBGN solution. Thus, the two modes of exposure had approximately equal capabilities of producing allergic reactions in pre-sensitized individuals. Accordingly, the accumulated total dose from multiple exposures over short time seems to be of considerable importance.

In addition to the use test, the subjects were patch tested with a dilution series of MDBGN (0.2%, 0.1%, 0.05%, 0.025%, 0.0125%, 0.0063%, 0.0031%, 0.0016%, 0.0008%, 0.0004%, 0.0002%, 0.0001%, 0.00005%, 0.000025%, 0.000013%, and 0% in eth./aq. (20:80)) to determine their patch test threshold values. The threshold values ranged from 0.0016% to 0.05% MDBGN. The dose-response curve is shown in Fig. 10. The dose eliciting a response in 10% of the test group was around 0.001% (10 ppm) and the dose eliciting in 50% of individuals was approximately 0.008% (80 ppm). This corresponds to an exposure of 0.3 $\mu g/cm^2$ and 2.4 $\mu g/cm^2$, respectively, given the application of 15 μl solution in a 0.5 cm^2 patch test Finn Chamber. The required total exposure in the open use test to produce a positive response in 50% of the whole test group can be determined to be close to 10 $\mu g/cm^2$ MDBGN for both of the concentrations (Fig. 11). As expected, the occluded, continuous patch test exposure was more potent than the exposure from the open applications.

A correlation between the patch test threshold value and the total amount of MDBGN applied to produce a positive provocative use test was demonstrated. This dose-response relationship shows that the more sensitive the subject is, according to patch test data, the less allergen was needed to elicit a

Table 6. Average amount of MDBGN applied and average number of applications of the 2 solutions prior to an allergic response in the use test.

	0.01% solution 4 applications a day (mean ± SD) (range)	0.04% solution 1 application a day (mean ± SD) (range)
Average amount of MDBGN applied in total ($\mu g/cm^2$)	7.9 ± 4.5 [1.2,15.7]	8.3 ± 4.1 [0.8,14.6]
Average number of applications	15.0 ± 10.2 [2,34]	3.9 ± 2.0 [1,7]

SD=standard deviation

Fig 9. Number of days until an allergic response was produced from the 4 times a day applications of 0.01% MDBGN and the single application a day of 0.04% MDBGN.
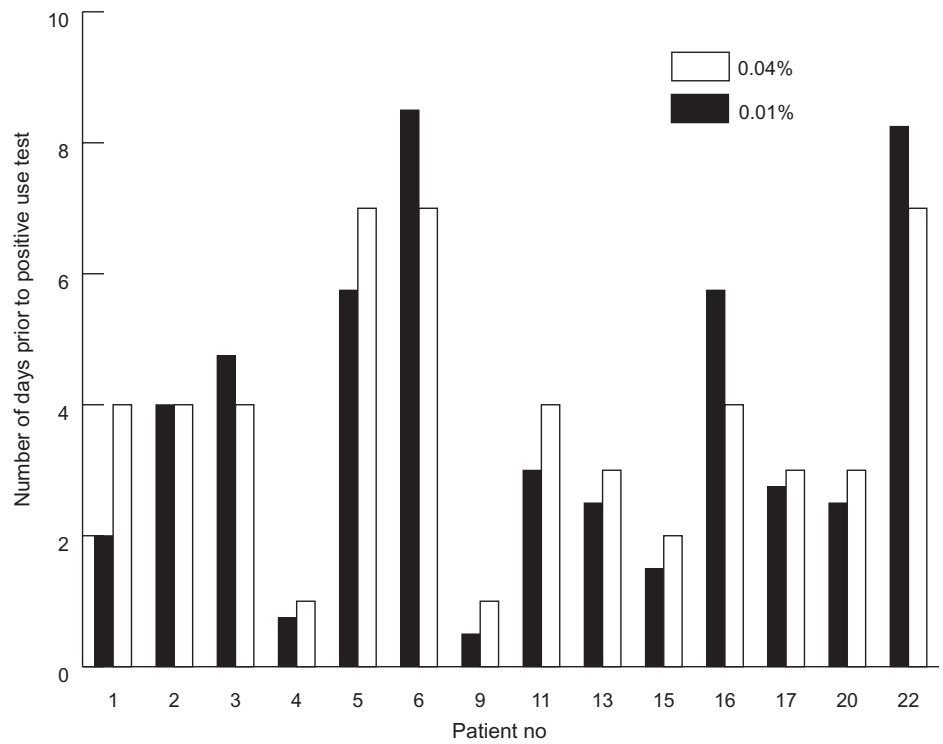
positive use test. A Spearman rank correlation was statistically significant for both the 0.01% (*p* = 0.006) and 0.04% (*p* = <0.0001) solutions and the almost coinciding trend lines (Fig. 12) emphasize the equal elicitation potencies of the two exposure modes using the present test design.

More experiments are needed before any general conclusions can be made. The results from this study may be allergen-dependent, and it cannot be determined from this experiment how the time span between applications and the duration of the exposure period affects the cumulative elicitation potential. Also, in this experiment, most patients had positive

use tests on both arms within only 4 days. Lower test concentrations resulting in later use test reactions would have been preferable, as this would have made a difference in the reaction time to the two solutions, if present, more easily distinguishable. The concentrations of MDBGN used in this experiment are however of a level that may be found in marketed products.

Speculations concerning the influence of the time span between applications and the exposure period duration on the cumulated elicitation potential are interesting with regard to the risk assessment of cosmetic products, as cosmetics are of-

Fig. 10. The dose–response curve obtained by patch testing with a serial dilution of MDBGN in eth./aq. from 0.2% to 0.000013%. The y-axis shows the percentage of the test group that reacted to the exposure dose in question. Some plotted points represent more than one patient. The dose eliciting a response in 10% of the test group was around 0.001% and the dose eliciting in 50% of individuals was approximately 0.008%.
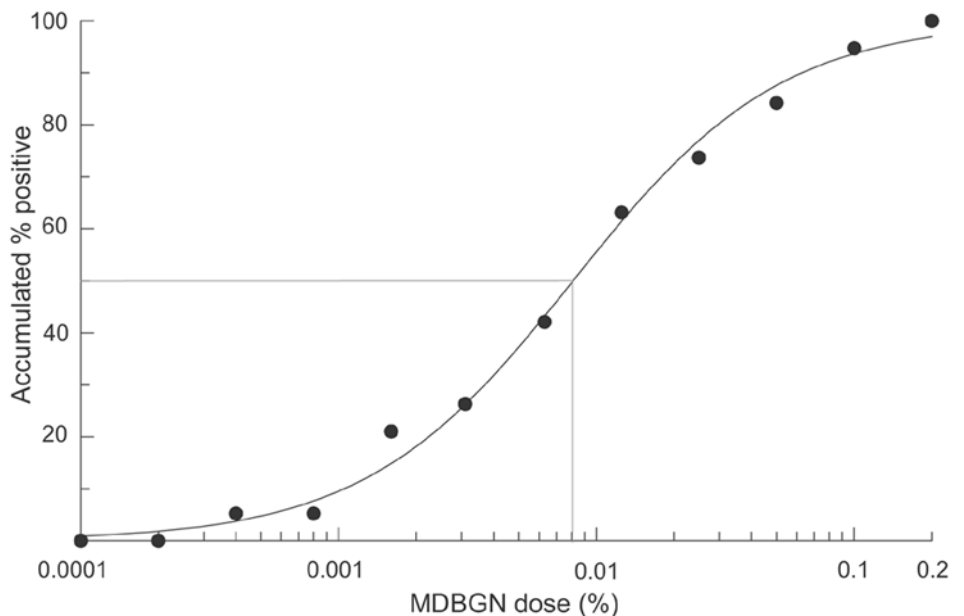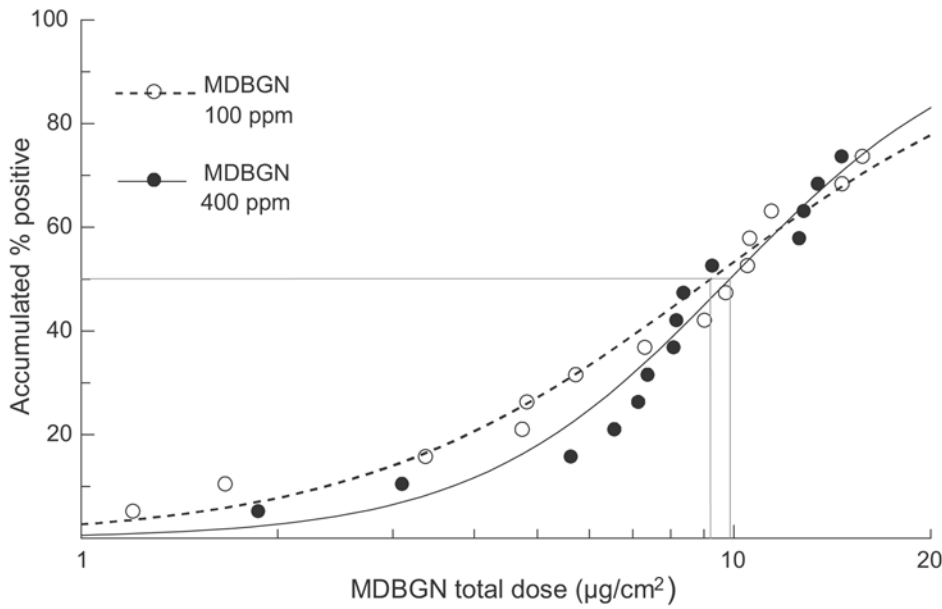
Fig. 11. Dose-response curve obtained from a use test with solutions containing 0.01% and 0.04% MDBGN. The y-axis represents the percentage of the test persons with a positive use test at the relevant exposure. It was calculated that a total dose of 9.2 and 9.9 $\mu g/cm^2$ MDBGN of the 0.01% and 0.04% solutions, respectively, was needed to produce a positive use test in 50% or the test group.

ten used frequently over long periods of time. It would seem feasible that elicitation simulates the reaction pattern of cumulative irritation, as seen in Fig. 13. Repeated skin exposure to irritants can be tolerated for a while until the regeneration ability of the skin has been transcended and eczema develops. The latency period may be short or long and if the tolerance limit is not exceeded no eczema will develop. In this experiment 4 applications in a day seemed sufficiently frequent to build an application response on the response of the previous application.

Few studies have been published regarding cumulative exposure of allergens. One study investigated exposure duration of the allergen PPD. In a use test with a 5 minute daily exposure to 1% PPD, the percentage of subjects who responded in 6 days was comparable to the responses observed to a single 30 minute exposure to the equivalent concentration of PPD indicating cumulative behaviour[14].

Risk assessment of cosmetic products is usually performed using experimental data from exaggerated exposure studies not directly comparable to actual use-situations. Extrapolation from the experimental studies involves a number of 'uncertainty factors' that must be considered, such as unintended use, interindividual and regional skin differences, vehicle and product matrix effects, and also product usage patterns, such as single or multiple daily exposures[30]. According to this study, particular care must be taken in the risk assessment of products containing MDBGN that may be applied to the skin several times in one day, as for instance hand soaps and hand-care products. A daily accumulated exposure may elicit eczema in allergic individuals even when low concentrations of the allergen are used. More experiments are required to determine whether this also applies to other allergens.

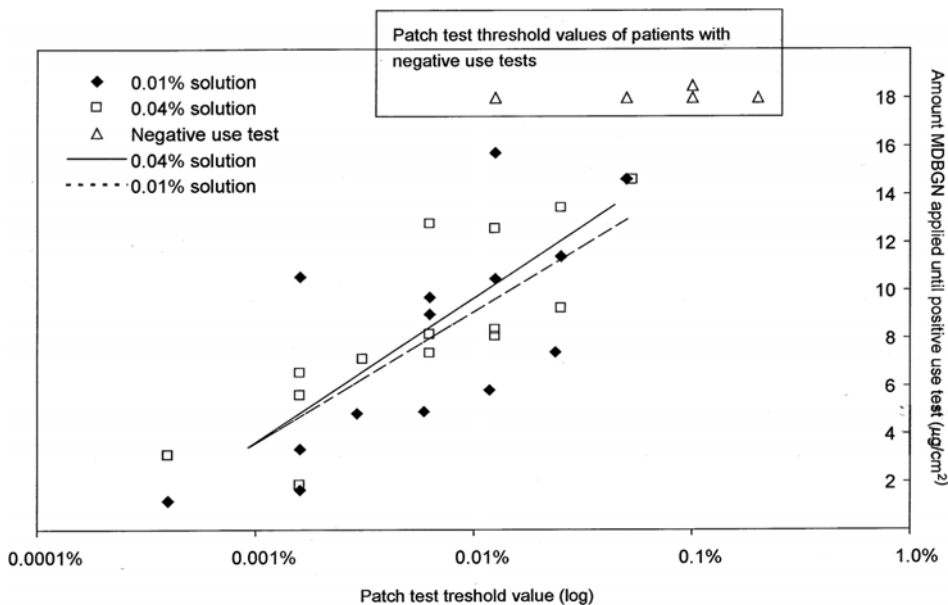*Fig. 12.* Patch test threshold value plotted against the amount of MDBGN applied to elicit dermatitis in the use test from the 0.01% and 0.04% solutions. Trend lines have been drawn. The triangles represent the patch test threshold values of the patients that did not react in the use test, and as seen they have mostly the largest threshold values which fits nicely into the dose-response relationship.
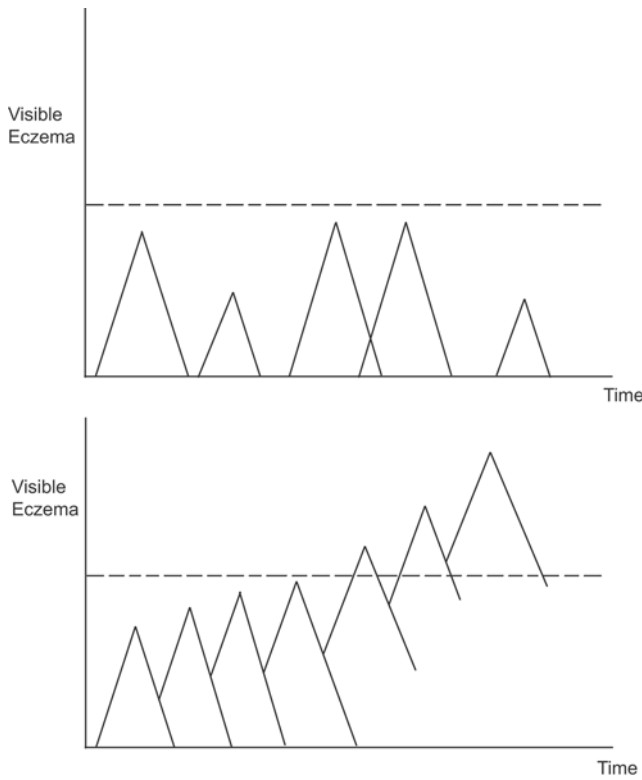
Fig. 14. Example of area of provoked MDBGN-dermatitis on the lower back of a subject.

Fig. 13. Illustration of skin irritation. The top shows subclinical irritation responses that do not develop into a visible eczema. Below is illustrated cumulated irritation that results in the development of clinical eczema.

patient (Table 8). 11 patients had a higher summed score for the pre-treated skin site and 4 had the highest summed score for the normal skin site. Two subjects had equal summed scores for the two areas. The differences in the summed scores for the pre-treated area and the area of normal skin are illustrated for all subjects in Fig. 16, and the highest total score was given to the formerly eczematous area. A statistically significant dif-

TRIAL III – INVESTIGATION OF RE-TEST REACTIVITY BY BOTH PATCH AND USE TEST WITH MDBGN IN SENSITIZED INDIVIDUALS

Hindsén and co-workers showed in clinical trials that skin with healed nickel-induced allergic contact dermatitis was re-test hyperreactive to nickel for several months after the dermatitis was clinically healed[19,87]. Accordingly, animal studies have shown increased re-test reactivity to the allergens DNCB and 2-hydroxyethylmethacrylate (HEMA)[88-90]. In this study, we investigated the re-exposure response of skin with previous eczema from MDBGN.

17 MDBGN-sensitized subjects participated in this trial. An area of homogeneous dermatitis was produced on the lower back of the test patients by exposing a 5×8 cm skin site to MDBGN (see the Methods section). The dermatitis was at the least characterized by erythema and infiltration. An example of experimental eczema on the back of a subject is shown in Fig 14. As control site, a corresponding area was treated with vehicle and no skin changes developed from this exposure.

One month later, when the dermatitis had healed, the test sites were challenged. This was done by patch testing with 6 consecutive dilutions of MDBGN with concentrations within a range determined according to the sensitivity of the patient found one month earlier. In Fig. 15 the study design is illustrated. The scoring system described in Table 7 was used to get a more detailed patch test reading than with the ICDRG scoring system[91]. The scores for the 6 patch test reactions were summed for each test site, resulting in a total score for both the previously eczematous area and the control area for each

Fig. 15. Diagram of study design.

Table 7. Recording of challenge patch test reactions on back.

0	negative
½	doubtful
1	erythema and infiltration
1½	erythema, infiltration and a few papules
2	erythema, infiltration, papules
2½	erythema, infiltration, papules and a few vesicles
3	erythema, infiltration, papules, vesicles

Table 8. Sums of the 6 patch test reactions on the areas with previous MDBGN-eczema and normal skin for each patient.

Patient no	Previous eczema	No previous eczema
1	10.5	7.5
2	15.5	12
3	10	5
4	5	5
5	11	10
6	13	13.5
7	8.5	8
8	16	10
9	16.5	12
10	5	5.5
11	9.5	10.5
12	5.5	7.5
13	7	4.5
14	6	2.5
15	3	3
16	6	5
17	18	17.5
Total	166	139

ference was found between the summed scores of the pre-treated and the normal skin ($p=0.02$, Wilcoxon matched-pairs signed-ranks test (two-tailed)).

Increased re-test reactivity was also seen in a use test challenge of skin with previous MDBGN-dermatitis. The same 17 subjects, along with 10 controls with a negative patch test reaction to 0.3% MDBGN pet., participated in a use test with a liquid soap. Prior to the test, areas of dermatitis were produced on the 5×10 cm marked test areas on the forearms by patch testing. 50 μl of 4 different solutions, two for each arm, were applied to the test areas using large Finn Chambers (12 mm) on Scanpor tape. The 4 solutions applied were: MDBGN eth./aq. (20:80) in a high concentration (range 0.0016% to 0.2%) and a low (range 0.0004% to 0.05%) concentration, 1% SLS aq, and a vehicle control of eth./aq. (20:80) (see Fig. 15). They were applied in a randomized fashion. The high and low concentrations of MDBGN were chosen with regard to the sensitivity of the patients with a factor of 4 of difference between the two concentrations in order to get a stronger and a weaker reaction. All test subjects and no controls developed dermatitis from the two MDBGN-patches on the arms and both test and control subjects developed irritant contact dermatitis from the SLS-patch. An example of the reactions on the arms of a subject is shown in Fig. 17.

After one month of healing of the dermatitis areas, the test and control subjects participated in a provocative use test. They were provided with a liquid soap containing 0.1% MDBGN with which they were asked to wash the marked test areas on the arms twice a day for up to 3 weeks or until dermatitis appeared. 6/17 test patients and all control subjects did not respond to the washing with the MDBGN-containing soap. 2/17 test patients had a doubtful response to the soap with an early appearance of small patches of weak erythema with few papules that did not develop any further during the use test period. 9/17 patients had a positive response to the use test with dermatitis developing on the test areas on both arms (results are summarized in Table 9). Of these 9 subjects, 8 developed earlier
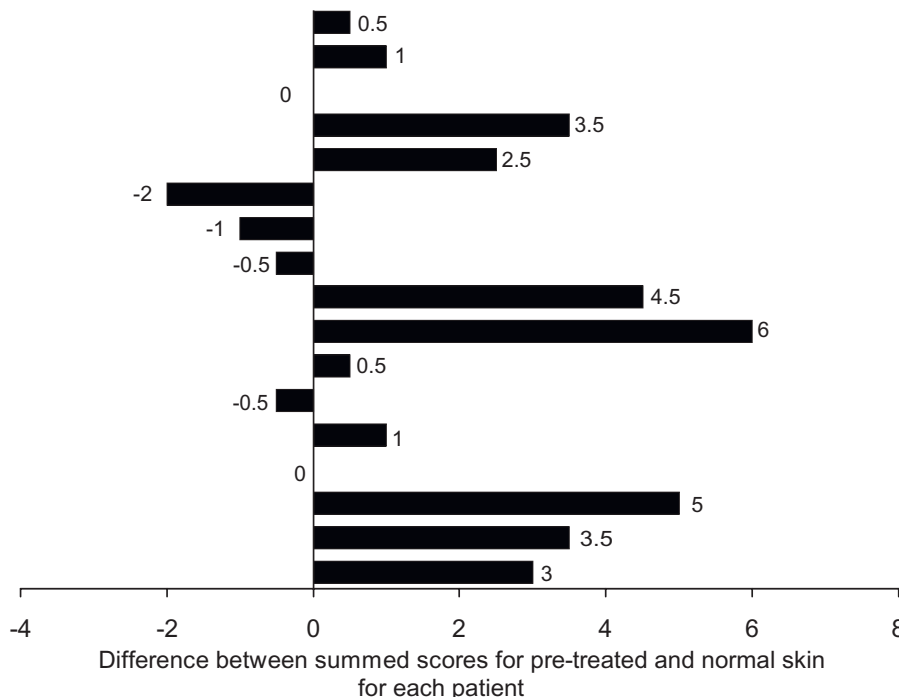

Fig. 16. Difference between the summed patch test scores for the pre-treated and the normal skin areas. A positive value signifies a higher score for the area with previous MDBGN-eczema.

Difference between summed scores for pre-treated and normal skin for each patient

and/or stronger dermatitis at the location of the formerly positive MDBGN-patch on both arms (see Fig. 18). In 1 patient, a moderate dermatitis developed on the entire test site without an augmented development at the areas with previous MDBGN-dermatitis. No increased response was seen at the patch test area with previous irritant dermatitis. The dermatitis on the MDBGN pre-treated areas appeared stronger on one of the arms in 7 of the subjects with an augmented response. In 6 of these, this concurred with the arm previously patch tested with the high concentration of MDBGN, thus the stronger the previous patch test reaction to MDBGN, the stronger the response at challenge. A correlation between the strength of previous dermatitis and the intensity of flare-up reactions on oral challenge has been described[87]. In the control group all use tests were negative.

Skin areas that one month prior had an inflammatory reaction to MDBGN-exposure, exhibited enhanced re-exposure reactions to both patch tests and use tests with a rinse-off product containing MDBGN. According to our knowledge, this is the first time that challenge of previously inflamed skin is performed with a rinse-off product in a use test. Pre-irritated skin did not exhibit an augmented response to allergen exposure, thus the increased sensitivity to MDBGN seems to be specific to allergic eczema. These findings document the necessity for careful avoidance of exposure to MDBGN-containing household and cosmetic products in patients previously sensitized.

Our results are in agreement with the findings of Hindsén *et al* who performed a series of important studies investigating the significance of previous dermatitis for the allergic response to nickel exposure[19,87,91]. They observed an increased reactivity similar to our results when previous nickel-eczema sites were challenged with nickel. Moreover, these studies examined the response to nickel exposure of skin with prior eczema elicited by an allergen other than nickel. They found that skin with prior cobalt-eczema had a response to nickel comparable to non-pre-treated skin[91]. Also, in oral challenge studies, it has been observed that flare-up reactions were localized to sites of previous dermatitis caused by the challenging allergen only[92,93]. In guinea pig studies, similar results have been observed as increased re-test reactivity to HEMA was only observed at former HEMA-skin test sites and not at previous DNCB-test sites, while likewise for DNCB re-test hyperreactivity was only found at former DNCB-test locations and not at previous HEMA-test sites[88]. Also, in these studies, sites of previous HEMA-dermatitis were re-tested with the irritant croton oil without resulting in hyperreactivity. These findings, together with the lack of persisting hyperreactivity of pre-irritated skin on allergen exposure, indicate that the enhanced response of earlier eczematous skin is an allergen-specific immunological phenomenon. It cannot be explained by increased penetration and bioavailability due to a compromised skin barrier, instead it seems likely that an immunological local memory function is responsible. Experiments have shown that hapten-specific T lymphocytes may persist for several weeks at former inflammatory sites, and probably cause local hyperreactivity at chal-

Table 9. Results of use test challenge with an MDBGN-containing soap on test areas on the arms previously patch tested with MDBGN.

	Subjects	Controls
Positive use test	9	0
(Augmented response 8)		
Doubtful use test	2	0
Negative use test	6	10

lenge with hapten[89,94]. Moreover, it has recently been shown that expression of the homing chemokine CCL27 and the receptor chemokine CCR10 remains increased 3 weeks after inflammation, while neither could be detected in normal or previously irritated skin after 3 weeks. As CCL27 is involved in the recruitment of T lymphocytes during an allergic reaction it is speculated that CCL27 might also play a part in retaining CCR10+ T cells at the previously exposed area of skin[94].

Skin irritated with 1% SLS 1 month before re-exposure did not exhibit an enhanced response to the use test. Hyporeactivity to exposure from nickel on pre-irritated skin has been demonstrated after 1 month while after 6-30 hours an augmented response was seen[91,95]. When challenged with an irritant, both hyper- and hyporeactivity have been described on previously irritated skin[95-99]. The reactivity is possibly dependent on the time period allowed between treatments as, generally, hyperreactivity has been described when re-test was performed up to 2-3 weeks after first treatment and hyporeactivity has been described for re-test after 2 weeks or more. The increased reactivity succeeding the development of irritant dermatitis may partly be due to an enhanced penetration because of a damaged skin barrier, but probably also arises from induction of immunological mechanisms like expression of cytokines or increased density of Langerhans cells from the previous irritant reaction[100]. The following hyporeactivity has been hypothesized to occur because of other mechanisms as for instance thickening of the skin from hyperkeratosis and/or changes in the composition of strateum corneum lipids[97,101].

The results from this study could constitute the basis for the development of a standardized model for evaluation of rinse-off products, for instance to be used to compare the effects of different product formulations. Exposure from rinse-off products may be difficult to predict, as the products are washed off the skin and they contain irritant ingredients that may produce unpredictable combination effects with allergens[22]. Also, long-time exposure is often necessary to observe the effects of the products because of a limited exposure. Alternatively, the augmented response of pre-treated skin in a use test, as observed here, may be exploited in this regard.

Experimental studies have shown that previously eczematous skin, although appearing healed, may be susceptible to certain exposures for several months. This is important information to patients suffering from dermatitis and enables them to take the best possible precautions.

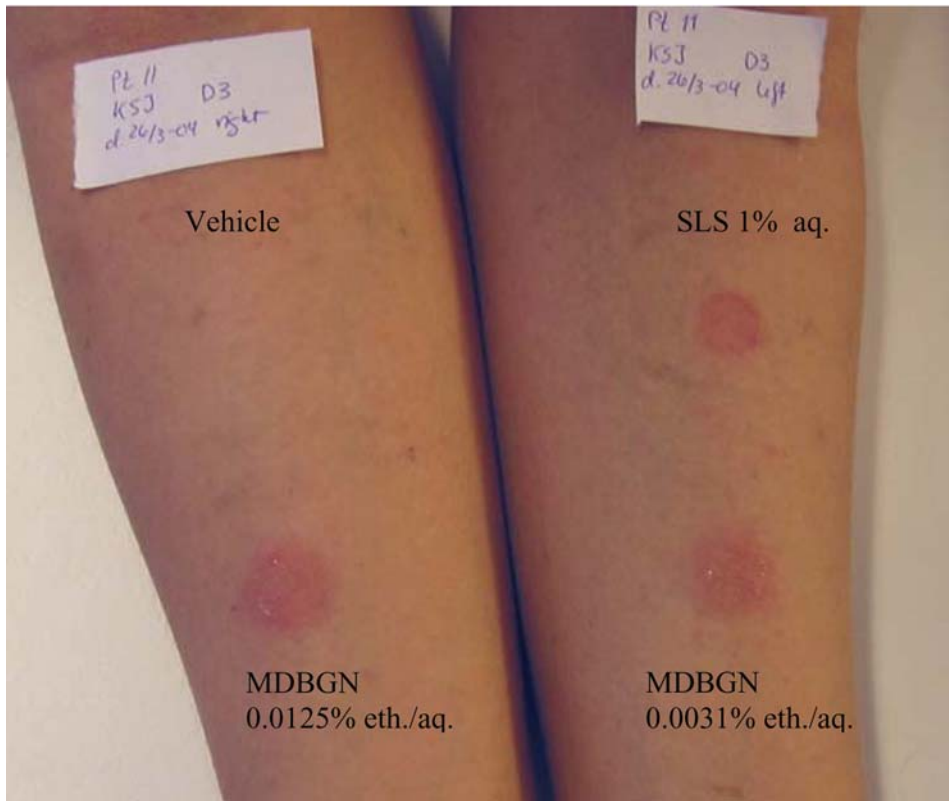*Fig. 17.* Example of provoked eczema on the arms of a subject.

Fig. 18. Example of an increased use test response on an arm with a previous positive patch test reaction to MDBGN. Dermatitis first appeared on D2 on the site of the previous patch test reaction and spread to the entire test area on D4. The dermatitis is clearly stronger localized to the area of the prior patch test reaction.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The more we know of the mechanisms of allergic contact dermatitis, the better the disease may be managed and prevented. The overall purpose of this thesis was to study some aspects of allergic contact dermatitis and contribute to the safe use of new and existing chemicals utilized in cosmetics and household products. The study consisted of 3 clinical elicitation trials with the preservative and contact allergen MDBGN. Elicitation studies on sensitized patients are a very important means of determining safe use-levels and investigating the behaviour of allergens without sensitizing healthy volunteers. If an allergen level is safe for the majority of sensitized individuals it may also be assumed safe with regard to induction[102]. In parts of this study the results were intended for use in a re-evaluation of the use-levels of MDBGN, while in others MDBGN was used as a model allergen and parallels may be drawn to other allergens.

In the trials of this thesis, different features of MDBGN were examined. In summary, it was, based on the results, concluded that:

– MDBGN in rinse-off products can elicit allergic contact dermatitis in sensitized individuals.
– multiple daily exposures to MDBGN appear to accumulate.
– skin areas with previous allergic contact dermatitis from MDBGN are hyperreactive to re-exposure both by patch testing and in a use test even though the skin appears clinically healed.

Parts of the results may be allergen-dependent, while others may apply to allergens in general.

The EU Commission has prohibited the use of MDBGN in leave-on cosmetics until a safe use-level can be determined. This has by industry been suggested to be in the range of 0.005-0.010% (50–100 ppm) MDBGN[44]. However, in a recent clinical elicitation study a moisturizer containing 0.005% MDBGN easily elicited dermatitis in more than half of the sensitized test persons[103]. As the allergenic potency of this chemical has become apparent, the existence of an acceptable use concentration of MDBGN in leave-on products with adequate antimicrobial effect is being questioned. Thus, a future unconditional prohibition of MDBGN in leave-on products is probable. A safe use of MDBGN in rinse-off products needs further investigation.

The results presented here, along with the reported cases of allergic contact dermatitis from rinse-off products containing MDBGN, indicate that the currently permitted level of MDBGN in rinse-off products is too high. The probability of sensitization occurring in a use-situation with prolonged, frequent use of a rinse-off product containing MDBGN cannot be disregarded. MDBGN in liquid hand soaps is of special concern. This is for several reasons. 1) In Denmark and probably the EU in general, MDBGN is widely used as preservative in liquid hand soaps. Liquid hand soaps have a high water content and hence require an efficient preservation. This is obtained with MDBGN explaining its popularity of use in these products. 2) The hands may be susceptive to exposure as it is not uncommon for the skin of the hands to be damaged to some degree from repeated exposure to irritants and water from frequent hand washing, house work, or various occupational exposures. 3) Hand soaps contain detergents which have been shown to increase the exposure response. 4) It is not unusual to use a hand soap multiple times in a day and according to our results this appears to generate a cumulated exposure. 5) We have shown in two studies that MDBGN in the maximum allowed concentration in a liquid soap can easily elicit allergic contact dermatitis in sensitized individuals, and case reports indicate that MDBGN-containing hand soaps may also sensitize. 6) Moreover, hand eczema is a potentially very problematic condition that in severe cases may be accompanied by long-term sick leave and forced job rehabilitation. On the basis of these results and considerations, we recommend a re-evaluation of the existing threshold limiting value of MDBGN in rinse-off products

The determination of safe exposure levels of chemicals in contact with the skin and an implementation of these in the legislation is an essential means to prevent allergic contact dermatitis in the population. Examples of allergens where legislative regulation have had a clearly preventive effect are nickel, potassium chromate and Kathon CG. In 1992 a nickel-regulation was implemented in Denmark reducing the allowed nickel-release threshold from nickel-containing alloys and coatings that are in prolonged contact with the skin. This threshold was determined at a level where only a minority of nickel-sensitized individuals will react with dermatitis. In a study of ear-pierced school girls, a decrease in nickel-sensitization was seen when the piercing had been performed after the regulation was implemented[102]. A similar regulation has now been adopted by the EU. Chromate allergy is frequently caused by occupational exposure to cement. In Denmark it has been mandatory to add ferrous phosphate to cement since 1983 in order to reduce the amount of water-soluble chromate and this has lead to a decrease in chromate sensitivity in workers exposed to cement from 8.9% in 1981 to 1.3% in 1987[104]. Another example is the previously mentioned cosmetic preservative Kathon CG that caused an epidemic of allergic contact dermatitis in the 80's and early 90's. A down-regulation of the allowed use-level of the active ingredient in cosmetic products induced a significant decline in the frequency of sensitivity to the substance.

Fragrances are, following nickel, the second most common cause of allergic contact dermatitis in Europe[105] and regulatory action is urged to improve this circumstance. A regulatory measure will come into effect in March 2005 which requires manufacturers to list 26 fragrance chemicals with known allergenic potential on the product label if present above a certain concentration in the product. Currently, it is sufficient to declare that the product contains perfume. This prophylactic measure allows sensitized persons to avoid the relevant fragrance chemicals and prevent outbreaks of allergic contact dermatitis. A primary prophylaxis is however preferable and it should be attempted to avoid or minimize sensitization. In the effort to determine safe use concentrations, several elicitation studies have been performed with known allergenic fragrance chemicals in recent years[8,9,106-109].

The establishment of safe threshold levels for allergenic cosmetic ingredients, like preservatives and fragrance chemicals, is an extensive task requiring a lot of resources. Additionally, acceptance and implementation of safe use levels into legislation is a long and slow process. This is however an important objective that will be accompanied by significant social and economical benefits.

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SUMMARY IN ENGLISH

In recent years the cosmetic preservative methyldibromoglutaronitrile (MDBGN) has gained notice due to a significant increase in the frequency of contact allergy to the chemical in Europe. A 10-year analysis, involving 16 dermatological clinics in 11 European countries, revealed a rise in the average frequency of sensitivity to MDBGN from 0.7% in 1991 to 3.5% in 2000 in consecutive eczema patients. These observations promote MDBGN to an important contact allergen in Europe.

The aim of this project was to characterize different aspects of the allergic response to MDBGN in previously sensitized individuals. During 3 clinical elicitation studies, sensitized subjects were exposed to the preservative in experimental use tests designed to resemble the actual use-situation of cosmetic products among consumers. Aspects examined were: the allergic response to MDBGN in a rinse-off product, the implication of application frequency, and the significance of previous exposure.

Some types of rinse-off products, e.g. liquid soaps, are potentially used many times a day and may thus carry a similar risk of causing contact allergic contact dermatitis as leave-on products (moisturizers, lotions etc.) used only once or a few times a day. The EU Commission service has requested a revision of the use-level of MDBGN in leave-on products, while the use of MDBGN in rinse-off products is unchanged. The first experiment investigated the allergic response elicited in pre-sensitized individuals from exposure to a rinse-off product preserved with the maximum permitted level of MDBGN. 19 contact-allergic individuals participated in a double-blind, randomized repeated open application test (ROAT) using two coded liquid soaps with and without MDBGN. 7/19 subjects (37%) developed allergic contact dermatitis from the MDBGN-containing soap. The experiment showed that the exposure to a rinse-off product containing the maximum permitted level of MDBGN easily can elicit allergic contact dermatitis in pre-sensitized individuals and indicates that the permitted level of MDBGN in rinse-off products may be too high. It is recommended that the currently allowed level of MDBGN in rinse-off products is re-evaluated.

The second experiment examined whether one high-dose exposure a day of MDBGN is more or less potent than several low-dose exposures with regard to elicitation of allergic contact dermatitis in pre-sensitized individuals. This knowledge may be useful in the risk assessment and regulation of cosmetic ingredients in products potentially used several times a day. MDBGN-sensitized individuals were exposed to MDBGN in a double-blind, randomized manner both with a single high-dose application a day and with 4 low-dose applications a day. The daily high-dose and multiple low-dose exposures had approximately equal capabilities of eliciting allergic contact dermatitis. According to this study, particular care must be taken in the risk assessment of products containing MDBGN that may be applied to the skin several times in one day. Examples of this could be occupationally used products, like soaps and hand-care products.

The aim of the third trial was to investigate whether skin with previous dermatitis elicited by MDBGN showed an augmented response when re-exposed to the allergen. Challenge was performed both by patch testing and in a use test with an MDBGN-containing soap. Areas of allergic contact dermatitis were elicited on the back and on the arms of 17 MDBGN-sensitized individuals. After one month, the previously eczematous areas were challenged with MDBGN. On the back of the subjects, the test sites were patch tested with a serial dilution of MDBGN and a use test was performed on the arms with an MDBGN-containing soap. A statistically significant augmented patch test response was seen on the back areas with previous dermatitis. 9 patients developed dermatitis on the arms from exposure to the MDBGN-containing soap in the use test. Of these, 8 had an augmented response on the areas with prior allergic contact dermatitis. Even though allergic contact dermatitis appears healed, an increased reactivity to exposure from the allergen that elicited the pre-existing dermatitis is present.

In summary it was concluded that 1) MDBGN in rinse-off products can elicit allergic contact dermatitis in sensitized individuals, 2) multiple daily exposures to MDBGN appear to accumulate, and 3) skin areas with previous allergic contact dermatitis from MDBGN are hyperreactive to re-exposure both by patch testing and in a use test even though the skin appears clinically healed.

SUMMARY IN DANISH (RESUMÉ PÅ DANSK)

I de senere år er man blevet opmærksom på konserveringsmidlet methyldibromoglutaronitril (MDBGN), på grund af en betydelig stigning i hyppigheden af overfølsomhed overfor kemikaliet i Europa. En 10 års analyse på 16 dermatologiske klinikker i 11 lande har vist en stigning fra 0,7% i 1991 til 3,5% i 2000 i den gennemsnitlige hyppighed af eksempatienter der er overfølsomme overfor MDBGN. Disse observationer har gjort MDBGN til et vigtigt kontaktallergen i Europa.

Formålet med dette projekt er at karakterisere det allergiske respons overfor MDBGN hos personer der er overfølsomme overfor stoffet. I tre kliniske eksponeringsstudier er overfølsomme personer blevet eksponeret for konserveringsmidlet i eksperimentelle brugstests designet til at efterligne den reelle brugssituation af kosmetikprodukter. De aspekter der er blevet undersøgt er det allergiske respons over for MDBGN i et skyl-af produkt, betydningen af applikationshyppighed, og implikationen af tidligere eksponering.

Nogle typer skyl-af produkter, såsom f.eks. håndsæber, anvendes potentielt mange gange om dagen og vil muligvis udgøre en ligeså stor risiko for allergi som en begrænset brug af produkter der ikke skylles af huden efter brug, såsom cremer og lotioner. Imidlertid har EU Kommissionen kun påkrævet en evaluering af brugen af MDBGN i produkter som bliver siddende på huden i længere tid efter brug, mens anvendelsen af MDBGN i skyl-af produkter vil fortsætte som hidtil. I dette projekts første eksponeringsforsøg blev det allergiske respons, fremkaldt hos overfølsomme personer ved eksponering med et skyl-af produkt, konserveret med den højest tilladte mængde af MDBGN, undersøgt. 19 kontaktallergiske personer deltog i en dobbeltblindet, randomiseret åben applikationstest med to flydende sæber henholdsvis med og uden MDBGN. 7/19 (37%) af deltagerne udviklede allergisk kontakteksem fra sæben indeholdende MDBGN. Dette forsøg viser at eksponering med et skyl-af produkt indeholdende den maksimalt tilladte mængde MDBGN let kan fremkalde allergisk kontakteksem hos overfølsomme personer og indicerer at den tilladte mængde MDBGN i skyl-af produkter er for høj.

I det andet eksponeringsforsøg blev det undersøgt om én højdosis eksponering om dagen er mere eller mindre potent end flere lavdosis eksponeringer med hensyn til fremkaldelse af allergisk kontakteksem hos overfølsomme personer. Denne viden vil være brugbar i risikovurderingen og reguleringen af kosmetikingredienser i produkter der muligvis vil blive brugt hyppigt dagligt, som f.eks. håndsæber og håndplejeprodukter. MDBGN-overfølsomme personer blev eksponeret for MDBGN i et dobbeltblindet, randomiseret design. To hudområder blev påført lige store mængder MDBGN dagligt. På det ene hudområde blev det påført af én gang, mens det på det andet blev påført i løbet af dagen delt ud på 4 mindre doser. Den enkelte højdosis og de flere lavdosis eksponeringer dagligt havde tilnærmelsesvis det samme potentiale for at fremprovokere allergisk kontakteksem. Ifølge dette forsøg skal der tages særligt hensyn ved risikovurderingen af produkter indeholdende MDBGN som potentielt anvendes flere gange om dagen, såsom sæber og håndplejeprodukter.

Det tredje forsøg havde til formål at undersøge om hud med tidligere MDBGN-eksem har en øget reaktivitet hvis det senere gen-eksponeres med allergenet. Gen-eksponering blev i dette forsøg foretaget både ved lappetestning og med en brugstest med en sæbe indeholdende MDBGN. Der blev fremprovokeret områder med kontakteksem på ryggen og på armene af de 17 MDBGN- overfølsomme deltagere. Efter 1 måned, hvor eksemet fik lov til at hele, blev disse tidligere inflammerede områder igen provokeret med MDBGN. På ryggen blev teststederne lappetestede med en fortyndingsrække med MDBGN, mens teststederne på armene blev vasket med en sæbe indeholdende MDBGN. På de tidligere eksematiserede områder på ryggen sås statistisk signifikant øgede lappetestreaktioner sammenlignet med normal hud. 9 testpersoner udviklede allergisk kontakteksem fra sæbevask på armene og heraf havde 8 en øget allergisk respons på de hudområder der tidligere havde været eksematiseret. Det kan konkluderes at selvom allergisk kontakteksem synes helet, vil huden for en tid have en øget følsomhed overfor gen-eksponering med allergenet.

Sammenfattet blev det konkluderet at 1) eksponering med et skyl-af produkt indeholdende den maksimalt tilladte mængde MDBGN kan fremkalde allergisk kontakteksem hos overfølsomme personer, 2) flere daglige eksponeringer med MDBGN ser ud til at akkumuleres, og 3) hudområder med tidligere allergisk kontakteksem forårsaget af MDBGN vil have en øget følsomhed over for gen-eksponering med MDBGN både ved lappetest og via en brugstest, selvom huden fremstår klinisk helet.