

**The Critical Review of Methodologies
and Approaches to Assess the Inherent Skin Sensitization
Potential (skin allergies) of Chemicals**

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Executive Agency for Health and Consumers

Purpose and context of contract

Aims

The objective of the contract is to increase the knowledge base for a systematic approach to the issue of skin allergies by conducting the following work:

1. Critically review currently available methods, or methods under development (*in vivo*, *in vitro*, *in silico*, etc.) used in the evaluation of skin sensitization potential and their applicability in the derivation of quantitative “safety thresholds”.
2. Identify specific cases, classes or specific use situations of chemicals for which “safety thresholds” or “safety limits” were set (in regulations, standards, in scientific research/clinical work, etc.) and critically review the scientific and methodological parameters used to set those limits.
3. For those chemicals identified in point 2 above, collect and critically analyse clinical and statistical evidence on the incidence and morbidity (clinical picture) of skin contact allergies (contact dermatitis) cases in the European Union before (at least 3 years) and after the limits were set so as to allow an assessment of the possible effect of the limits in the reduction/prevention of the incidence and morbidity of contact dermatitis.

Abbreviations

AEL	Acceptable exposure level	IPPD	N-isopropyl-N'-phenyl-p-phenylenediamine
APC	Antigen presenting cell	ISO	International Organization for Standardization
CASE	Computer automated structure evolution program	ITS	Integrated Testing Strategy
CCET	Cumulative contact enhancement test	IVDK	Information Network of Departments of Dermatology
CEL	Consumer exposure level	LLNA	Local lymph node assay
CEN	Comité Européen de Normalisation (European Committee for Standardization)	MAK	Maximale Arbeitsplatz Konzentration (threshold limit value)
DCDG	Danish Contact Dermatitis Group	MEC	Minimal elicitation concentration
DfW	Derek for Windows expert system	MEST	Mouse ear swelling test
DPRA	Direct peptide reactivity assay	MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone
DMG	Dimethylglyoxime	MDBGN	Methyldibromoglutaronitrile
DST	Dermal sensitization threshold	MUST	Myeloid U937 skin sensitization test
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals	NESIL	No expected sensitization induction level
ECVAM	European Centre for Validation of Alternative Methods	NOEL	No effect level
ED	Efficient dose	PPD	p-Phenylenediamine
EECRDG	European Environmental Contact Dermatitis Research Group	QMM	Quantitative mechanistic models
EINECS	European Inventory of Existing Commercial Chemical Substances	QRA	Quantitative risk assessment
ELINCS	European list of notified chemical substances	QSAR	Quantitative structure activity relationship
EN	Norme Européen (European standard maintained by CEN)	R43	Designates "sensitizing to the skin"
ESCD	European Society of Contact Dermatitis	RAI	Relative alkylation index
ESSCA	European Surveillance System on Contact Allergies	ROAT	Repeated open application test
EU	European Union	REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
FM	Fragrance mix	SAF	Safety assessment factor
GPMT	Guinea pig maximization test	SAR	Structure activity relationship
GSH	Glutathione	SCCP	Scientific Committee on Consumer Products
HBV	Hepatitis B virus	TIMES-SS	Tissue metabolism simulator for skin sensitization
h-CLAT	Human cell activation test	TOPKAT	Toxicity prediction komputer-assisted technology
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde	TTC	Threshold of toxicological concern
HIV	Human immunodeficiency virus	TRGS	Technische Regel Gefahrstoffe (Technical regulations for hazardous substances)
HR IPT	Human repeated insult patch test	TRUE-test	Thin-layer Rapid Use Epicutaneous Test
IFRA	International Fragrance Association	UK	United Kingdom
INCI	International Nomenclature of Cosmetic Ingredients	WoE	Weight of evidence

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Executive summary

Contact sensitization is caused by a group of reactive chemicals referred to as allergens, mostly man-made, that are able to permanently change a subgroup of immune cells so that they will proliferate and target the skin compartment upon allergen re-exposure. Reactive chemicals and metals are present in the domestic and the occupational environment. Natural occurring contact sensitizing chemicals or substances are known but represent a very limited clinical problem. The *induction* of allergen specific memory T-cells is a frequent happening since up to 20% of European adults are contact sensitized. The allergen specific T-cells will in all foreseeable future recognise the chemical in question and react with an inflammatory response upon re-exposure, defined as *elicitation* and clinically expressed as allergic contact dermatitis.

Individuals who are contact sensitized can be identified by a diagnostic test referred to as the patch test. The patch test mimics biological re-exposure since an individual is exposed to small amounts of chemicals applied in small metallic chambers directly on the skin, performed under internationally standardized conditions. The most common contact sensitizing chemicals include metals such as nickel and chromium, preservatives, fragrances and hair dye chemicals. Approximately 25 chemicals are currently included in the European baseline patch test series used by dermatologists; a test series that include chemicals where the frequency of positive test reactions exceed 1%. In studies of random samples from the general population, 10-15% of children and 15–25% of adults have a positive patch test reaction to one or more of these chemicals and are therefore contact allergic. A total of 4 000 chemicals are known to have a contact sensitizing potential and approximately 100 are regularly encountered with positive patch test reactions in dermatology practice.

Allergic contact dermatitis appears primarily at areas of skin contact. It is therefore not surprising that dermatitis is mainly located on the hands and face, skin areas that are in contact with e.g. cosmetics, and jewellery. The clinical picture may include redness, oedema, scaling, fissures and in the acute phase vesicles, bullae and eventually oozing. Secondary infection might be present as well. Figure 1-4 illustrate the variability in the morphology of the disease. Patients with dermatitis need to undergo diagnostic evaluation and treatment. If a single causative allergen can be identified and future contact avoided, dermatitis will typically disappear within 2–3 months. However, hand dermatitis tends to have a more chronic course with 50% of patients having persistent or intermittent symptoms.

Contact sensitization and related skin diseases (allergic contact dermatitis at site of exposure and hand dermatitis) may severely affect an individual's quality of life and working capabilities. It is difficult to quantify national expenses associated with contact sensitization but they are high. The contact sensitization problem has mainly been caused by human engineered chemicals introduced into consumer and occupational products over the last 100 years. Knowledge obtained from observing contact sensitization epidemics over the 20th century provides the basis for future prevention of contact sensitization and related disease. In general, genetic predisposition plays a very little role for contact sensitization as the condition is mainly caused by environmental exposure. The decisive factors include the inherent sensitizing potential of a chemical, skin exposure concentration and the number of exposures.

The present report describes and discusses available methods to identify the potency of contact sensitizing chemicals and to understand the dose-response effects in humans and the effect of regulation of some of these important chemicals. The report is divided into 3 parts.

Part 1 describes methods that are available to determine whether a chemical holds a contact sensitizing potential. Some methods have been in use for more than 50 years, others still undergo final standardisation. *In vitro* tests include binding assays between chemicals and standard receptor molecules based on benchmarking with chemicals known not to be sensitizing. *In silico* tests represent computer identifications of chemical structures known to be present in contact sensitizing chemicals. A new group of methods, based on cultured human cells, might to some extent replace traditional animal testing based on mice or guinea

pigs as the latter after 2013 will be outsourced due to the REACH agreement. The most standardised method used today, to establish the sensitizing potency of chemicals, is the local lymph node assay (LLNA). Historically the Human Repeated Insult Patch Test (HRIPT), a test conducted in healthy controls, has been widely used, particularly in the US. As the end-point is prevention of human disease, human data, if available, have a particular importance and impact for the final decisions. Human data include clinical, epidemiological and experimental dose-response patch test data. The latter may be used to determine threshold concentrations and no effect levels in contact sensitized individuals. New chemicals developed by industry are screened for their contact sensitizing potential using the described methods. Most potential contact sensitizing chemicals developed in more recent years are probably never marketed because of these tests. This belief is enforced by the fact that most chemicals that result in clinical disease have been in use for decades (preservatives and fragrances) or even a century (hair dyes). There are however examples of risk assessment failures (e.g. methyl dibromo glutaronitrile). Part 1 of the document ends with a general evaluation and comparison of the different available methodologies.

Part 2 describes common contact sensitizers that have caused health problems at large scale and initiatives launched by the EU and industry to prevent and reduce morbidity. Also, the scientific background for these regulations is presented.

Part 3 offers documentation for the possible effects of the initiatives in terms of possible decreases of morbidity. For availability and overview, part 2 and 3 are discussed as one continuous document in the executive summary.

The largely successful EU Nickel and Chromium Directives were developed based on human dose-response data collected in patients who were primarily sensitized following exposure to respectively, nickel releasing consumer products or to wet cement containing hexavalent chromium. The frequency of nickel sensitization and hand dermatitis caused by nickel exposure has afterwards decreased significantly among young EU citizens in several member states. In other parts of the world e.g. Asia and the US, where nickel and chromium exposure has not yet been regulated, increasing frequencies of nickel allergy in children and chromium allergy in workers have been observed during the same period. In Denmark, a small country with approximately 1% of the EU population, it has been estimated that regulatory intervention on nickel exposure has reduced national expenses to health care and sick leave with 2 billion Euros over a 20 year period. It is perceived that the industry has not suffered any significant loss due to these regulations. The methods for control are currently evaluated and intended modifications might further increase the effects of the Nickel Directive. The Chromium Directive has been dramatically effective. Before chromium regulation, 10% of building workers, who came in contact with wet cement, suffered from moderate to severe hand eczema due to chromium allergy. This significant occupational skin disease is still frequent worldwide, but it is now nearly eradicated in the EU countries. The total decrease in health care costs has not been calculated for this entity. The Chromium Directive only adds minor extra costs to cement production. Allergic chromium dermatitis caused by consumer leather products, particularly shoes, is still an important clinical problem. Technologies, e.g. enzymatic ones, should replace chromium leather tanning in the future.

Regulation of preservatives, which comes into skin contact, is difficult. Preservatives are needed to maintain durability and quality of both consumer products (e.g. shampoos, lotions, etc) and industrial products (e.g. paints, cooling oils, etc). All known effective preservatives are moderate to potent skin sensitizers. Every time a new preservative is permitted (most recently methylisothiazolinone) the total burden of contact sensitization from this product category is increasing for EU citizens as the total number of allergic individuals increase. *In vitro* and animal methods as well as the Quantitative Risk Assessment methods all underestimate the risk of contact sensitizing in humans. The main explanation for the risk assessment failures is probably the underestimation of real life exposure situations with prolonged and repeated exposure. Another explanation for the persisting problem relates to the demand by industries to use high concentration use levels, which is considered unnecessary for standard products. An important lesson from the “preservative history” is that if any of the methods gives a signal of potential contact sensitization of a new chemical under evaluation, great caution should be taken. When a few human cases have been observed

following occupational allergen exposure, our experience from past contact sensitization epidemics have taught us that significant problems can be expected if the same chemical is permitted in mass-marketed consumer products. The regulation of formaldehyde exposure is a failure. The permitted use concentration of 2000 ppm is known to induce contact sensitization although such as high concentration of 2000 ppm is usually not required to preserve standard consumer products. According to the Cosmetic Directive, a concentration exceeding 500 ppm formaldehyde should be declared on the label. It is however well established that most formaldehyde sensitized individuals react to substantial lower concentrations preserved with formaldehyde releasing preservatives and that 200 ppm might be a better concentration limitation.

Following 2 recent EU funded programmes; current understanding of contact sensitization to fragrance chemicals has increased significantly. All the presented methods, both *in vitro*, animal and human ones are able to identify well-known contact sensitizing fragrance allergens. Exposure assessment based on modern chemical analysis combined with human dose-response elicitation studies and clinical/epidemiological patch test studies has clearly illustrated that the former use concentrations of isoeugenol and HICC have been too high in e.g. fine perfumes and deodorants. A very significant decrease in the use concentration recommended by industry is regarded to be a consequence of the EU sponsored fragrance research programme. A decrease of the frequency of isoeugenol sensitization has been observed in several member states. Despite these limited signs of progress, fragrance contact sensitization remains very common in the general population and among dermatitis patients. New diagnostic methods continue to identify hitherto unrecognized sensitizing fragrance chemicals making the total fragrance burden for EU citizens unchanged.

Hair dyes represent a special category of consumer products. para-Phenylenediamine (PPD), the main chemical in most hair dyes, was invented in the 1880's and has since the early 19th century been used together with related chemicals and oxidisers for permanent hair dyeing and fur dyeing. All tests that are available to evaluate whether chemicals are contact sensitizers have classified PPD as an extreme potent sensitizer. Based on the accumulation of scientific data, PPD based hair dyes are not suitable for human exposure. From recent population surveys, it is known that 80–90% of women and 30–40% of men dye their hair at some point in life, many starting in their teens. Furthermore, it has been estimated that 5% develop adverse skin reactions and that some individuals experience very severe reactions resulting in hospitalization and assisted respiration for days. However, the great majority of individuals tolerate exposure to hair dyes. Recent research performed in animals suggests that individuals who tolerate hair dyes develop immunological tolerance which potentially might increase the risk of cancer and autoimmune diseases. This is an area that will be further explored in the near future. PPD containing hair dyes has for these reasons been forbidden temporarily in a number of current EU member states (before entry into the EU). For years, there has been an acceptance of the sensitizing capacity of PPD due to a “social need” for dyeing grey hair in middle-aged and elderly citizens. However, during the last 20–30 years, the use of hair dye products has exploded, not due to the “social need” in elderly, but due to fashion changes in teenagers and young people. There is no need for more data to underscore the sensitizing capability of hair dye chemicals. All studies demonstrate that PPD is an extreme contact sensitizer. Thus, the hair dye problem can only be addressed, either by the introduction of use limitations and/or by demands for technical developments. Because of the extreme contact sensitizing capabilities and the new research data showing severe immune toxicity in animals, this particularly problem needs to be studied in humans.

Effects of regulatory interventions on contact allergy to chemicals and metals with in the European Union

METALS	Substance	Regulating body (EC authority / industry association)	Type of regulation	Limit	Product category	Year of introduction	Scientific background	Estimated preventive effect on sensitization/elicitation
	Nickel	EU Communities Directive 94/27/EC	a) Limitation of release b) limitation of content	a) 0.5 µg Ni/cm ² /week b) 0.05%	a) Products in prolonged contact with the skin (e.g. jewellery, buttons) b) Piercing posts	1994 (full force 2001)	Dose-response patch testing	Likely
	Nickel	Commission Directive 2004/96/EC	Limitation of release	0.2 µg Ni/cm ² /week	Piercing posts	2004 (put into force 2005)	Not Clear	Likely
	Chromium	European Parliament and Council Directive 2003/53/EC	Limitation of content	2 ppm Cr(IV) in cement	Cement	2005	Dose-response patch testing & Exposure Stay-on	Likely
COSMETICS								
	Formaldehyde	Council Directive 76/768/EEC	a) labelling b) limitation of content	a) ≥ 500 ppm b) 2000 ppm	Cosmetics	1976	Based on other toxicology parameters than sensitization	Probably insufficient
	MCI / MI (3:1)	Council Directive 76/768/EEC (Directive 89/174)	Limitation of content	15 ppm	Cosmetics leave-on and rinse off	1989	Animal testing	Probably insufficient
	Methyl/dibromoglutaronitrile	Directive 2003/83/EC	Limitation of content	Ban	Cosmetics leave-on	2003 (put into force 2005)	Animal testing	likely
	Methyl/dibromoglutaronitrile	Directive 2007/17/EC	Limitation of content	Ban	Cosmetics leave-on and rinse off	2007 (put into force 2008)	Human testing	likely

	HICC	Directive 2003/15/EC	Labelling a) leave-on b) rinse off	a) ≥ 10 ppm b) 100 ppm	Cosmetics a) leave-on b) rinse off	2003 (put in to force 2005)	Not clear	unlikely
	HICC	IFRA Guidelines 44rd Amendment	Limitation of content	200 – 2000 ppm	Cosmetics a) leave-on b) rinse off	2009	Not clear	likely
	Isoeugenol	Directive 2003/15/EC	labelling	a) ≥ 10 ppm b) 100ppm	Cosmetics a) leave-on b) rinse off	2003 (put in to force 2005)	Not clear	unlikely
	Isoeugenol	IFRA Guidelines 43rd Amendment	Limitation of content	100 – 200 ppm	Cosmetics a) leave-on b) rinse off	2008	Not clear	unknown
	p-Phenylenediamine	Regulation 1223/2009 of the European Parliament and Council	a) Limitation of content b) Limitation of use	a) 6% as free base b) Warning:” can cause allergic reactions” and “ wear suitable gloves”	Hair dyes	2009	Not clear	insufficient

Part I

Aim

To critically review currently available methods, or methods under development (*in vivo*, *in vitro*, *in silico*, etc.) used in the evaluation of skin sensitization potential and their applicability in the derivation of quantitative “safety thresholds”.

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1. **Introduction**

Skin allergies affect 20% to 25% of the general European population. Depending on exposure to allergens in either the domestic or occupational environment, a person will be at risk of developing the skin disease allergic contact dermatitis typically on the hands and face. There are examples that show that skin allergies and allergic contact dermatitis can be prevented. For this reason a systematic evidence-based review on the methodologies and approaches to assess the inherent skin sensitization potential (skin allergies) of chemicals is needed to translate the knowledge to general preventive strategies, if possible. Such preventive strategies will be of benefit to European populations.

2. **In vivo assays targeting the sensitization phase**

2.1. **Animal tests**

Since early 1940, predictive assessment of the chemical inherent contact allergenic potential has been performed using animals. The animal of choice from the beginning has been the guinea pig (1–3). Over the last decade increasing interest has fallen on using the mouse as the species of choice. From the beginning being thought of as a first line of preliminary test, the mouse test local lymph node assay (LLNA) has come to be a stand-alone assay (4, 5).

2.1.1 **The Beuhler test**

The Beuhler test for sensitization was introduced in 1965 (3). It implies a 3 times occluded induction phase under occlusion for 6 h. The animals pass through a rest period and are subsequently patch tested on their flanks. The resulting erythema and oedema is assessed by ocular reading by an experienced technician.

2.1.2 **The guinea pig maximization test (GPMT)**

The GPMT was introduced in 1969 (2). The test method has an induction procedure using both intradermal injections with Freund's complete adjuvant as well as an occluded exposure for 48 h. The result of the induction is assessed using ordinary patch test technique on the flanks of the animals. The resulting erythema and oedema is assessed by ocular reading by an experienced technician. In 1985, the introduction of a split injection technique together with the use of logistic statistical assessment gave room for a dose response assessment of induction (6).

2.1.3 **The mouse ear swelling test (MEST)**

The MEST method relies on epidermal exposure and the use of intradermal injected Freund's complete adjuvant for induction (7). The epidermal exposure is performed 3 times on the stripped skin of the abdomen. One variation of the test implies exposure under occlusion. Assessment of sensitization is performed by exposing the dorsal sides of the ears to test compound or vehicle control. Sensitization results in more ear swelling in the ear exposed to the test chemical when compared to the ear exposed to the vehicle. The assessment gives an objective numerical measurement that can be analysed statistically. To increase sensitivity for weaker sensitizers, feeding the animals vitamin A was introduced (8).

2.1.4 **The local lymph node assay (LLNA)**

The LLNA is a method relying on assessing the allergenic potential of chemicals using the induction phase only. Mice are exposed to test materials on the back of the ears. Exposure is done once a day during 3 consecutive days. The mice are divided into 4 dose groups including 1 vehicle control. The resulting proliferation is assessed by injecting ³H-thymidine in the tail vein on day 5. At 5 h after injection,

the mice are sacrificed and the lymph nodes draining the ears are harvested and the lymphocytes extracted and processed to release the DNA. Proliferation is relative to the incorporated thymidine and is assessed by scintillation counting. A stimulation index is calculated, and a concentration giving an index equal to or above 3 is considered positive as a sensitizer (9). There are also alternatives to the radio isotope LLNA, namely the integrated model for the differentiation of chemical induced allergic and irritant skin reactions (10).

Conclusion

The GPMT method is, with the slight modification of using several challenge patches, an acceptable method for analysing thresholds at elicitation. This technique can also be used to assess cross reactivity at challenge. The introduction of split injections gave a possibility for dose response assessment during induction. The drawback of the method is the use of adjuvant as this leads to unspecific stimulation of the immune system.

The LLNA method is well suited for assessment of thresholds at induction. The method has also a big advantage in being fast and less labour intensive than the guinea pig methods. There is, however, some concerns about suitability of the use of olive oil as a first choice vehicle, as it itself induces some proliferation in the lymph nodes. The concern of ingestion of test material is also present since the exposure is open.

All animal tests have a good ability to predict potency of contact allergens in man for classification and labelling purposes (11).

2.2 Human Tests

2.2.1 The human repeated insult patch test (HRIPT)

To make an adequate risk assessment or safety evaluation, it can be argued that a test has to be performed in naïve human subjects. For this purpose, there has been a number of sensitization tests designed. The most used is the HRIPT, originating from a test described by Draize in 1944 (1, 12). The test includes subjects that are exposed to the chemicals 9 x 24 or 48 h during a 3 weeks period. Following a 2 weeks rest period, challenge is performed on an exposed site and an unexposed site. The challenge is performed using a 24 or 48 h patch test and the resulting reaction is graded for clinical signs. The test can be performed in a variety of manners with varying exposure time and occluding methods. The test is performed on a comparative large group of subjects, typically around 100. Although the use of human subjects for risk assessment is considered unethical and not recommended it has been widely employed with claimed good result (13, 14).

2.2.2 Clinical and epidemiological data

Contact allergy, comprising allergic contact dermatitis as the clinical disease and underlying delayed-type sensitization as the latent condition, is an inflammatory skin disease triggered by chemicals of usually low molecular weight. Manifest contact dermatitis may give rise to diagnostic patch testing by a dermatologist using common contact allergens suspected to have caused sensitization. If the individual is sensitized to an allergen, the patch test will *elicit* an eczematous reaction in the patch tested area, which is, according to certain morphological criteria, interpreted as an allergic reaction. Thus, the chemical was

shown to be an allergen through its capacity for *elicitation*. In a second step, the exposure containing the chemical is sought (e.g. a cosmetic product). If the individual is shown to be exposed to the allergen, it is concluded that *induction* must have taken place through that very exposure. This topic will be further covered in chapter 3.2.4.

In this section on induction/sensitization, this perspective is reversed. The primary question would be: Does a defined exposure, characterized by certain chemicals, bear the risk of sensitization? As there is no ethically acceptable tool to demonstrate induction in a patient directly (not to mention the rarely used laboratory methods, where allergen specific T-lymphocytes are shown (15, 16)), the method to prove sensitization is an indirect one, namely through elicitation (dermatitis after re-exposure to the chemical via patch testing or a use test where the products containing the allergen are applied to the skin).

The question: “Does a defined exposure to a certain chemical bear the risk of sensitization” is put and answered in different ways:

- a) Work-up of a single case with dermatitis suspected to be allergic
- b) Clinical-epidemiological studies
- c) Traditional epidemiological studies in the general population or specific (occupational) subgroups.

a) Single cases

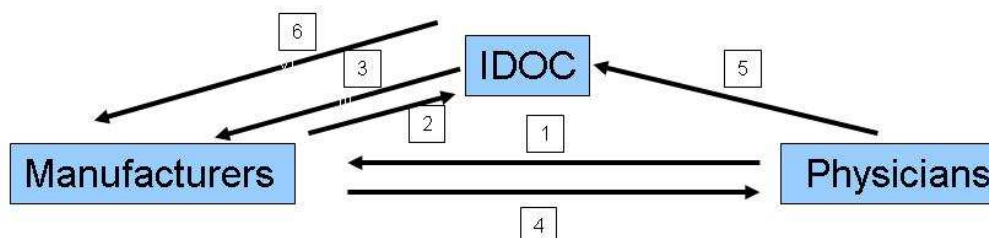
A cosmetic may have caused contact dermatitis. Thus, “the exposure may bear the risk of sensitization”. The causal allergen is identified through patch testing of the single constituents of the cosmetic (“break down testing”). This way numerous case reports have identified new allergens, with a proven path from exposure to sensitization.

In Germany, this pragmatic approach is used in a systematic way by the IDOCsystem (Information and Documentation Centre for Contact Allergy) (17). After a case of intolerance reaction to a cosmetic has been notified to a cosmetic company, IDOC gives advice how to prepare the single components, which are then tested by the local physician. Test results are reported to the company and to the IDOC (Fig. 1).

Up to 2009, 1650 inquiries from 990 dermatologists to 70 producers were registered. A feedback was given in about 50%. The inquiries concerned more than 1450 different products with more than 2100 substances or raw material mixtures. As well, new allergens have been identified, which are not yet part of the commercial patch test series.

Figure 1. The single steps of work up of an intolerance reaction by the IDOC system in Germany.

Information- and Documentation Office on Contact allergy



1. The physician asking for single ingredients
2. manufacturer asking IDOC for suitable test procedures
3. IDOC gives advice how to prepare the patch test preparation of ingredients
4. Manufacturer sends preparation to physician
5. Physician reports results to IDOC
6. ...which are then submitted with comments to the manufacturer

A similar procedure is used by the German KAB system (“Kontaktallergie Berufsstoffe”) for the working up of occupational cases of dermatitis. There, the break down of suspected single components of material brought in by a worker is organized systematically and the results from all over Germany are documented. IDOC and KAB are situated at the IVDK centre in Göttingen.

b) Clinical-epidemiological studies

Clinical-epidemiological studies are performed in larger groups of patients suspected of being sensitized to a contact allergen. The question “does a defined exposure to a certain chemical bear the risk of sensitization” was, for instance, applied to exposure to metalworking fluids, which were shown to cause allergic contact dermatitis. Metal working fluids contain hundreds of different chemicals. One of our colleagues (a chemist) scrutinized the chemical structures of a list of chemicals for structural alerts and suspected diglycolamine as a potential allergen, although predictive animal tests were negative. The substance was therefore included in a specific test series. Patch testing of a larger group of metal workers confirmed a non-negligible sensitization potential of this chemical (18).

There is even more evidence for sensitizing properties of the chemical, if a chemical is shown to be an allergen in a subpopulation exposed to certain products, e.g. hair-cosmetics in hairdressers, or water-based paints, and subsequent removal or reduction of the concentration of the substance lead to a decrease or disappearance of contact sensitization (as was shown for glyceryl monothioglycolate and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) (19, 20)).

c) Traditional epidemiological studies in the general population or specific (occupational) subgroups

This is an approach rarely used for several reasons, amongst them the economic feasibility. One example is a study on sensitization to hair-dyes in Thailand (21). One group of volunteers (n=548) dyed their hair for a period of 6 months, and another group (n=516) was instructed not to use hair dyes. At the end of this period, the participants were patch tested with p-phenylenediamine (PPD), and it was shown that the sensitization rate in the study group was substantially higher (1.3%) than in controls (0.4%). Thus, the sensitizing potential of PPD was confirmed.

3. In vivo assays targeting the elicitation phase

3.1. Animal tests

3.1.1 Modified version of the local lymph node assay

The LLNA was initially described using 3 dose groups and a vehicle control group resulting in a dose response where a 3 times increase in proliferation of the lymphocytes above the vehicle control is considered a positive contact allergen (9). To meet the increasing demands on reduction of animal assays a refinement of the assay has recently been introduced. Instead of 3 dose groups the introduction of a single dose group has been validated and proposed as a routine screening test for assessment of contact allergenic potential in unknown chemicals (22). A further reduction of this method with the use of only 2 animals in the vehicle control group has resulted in some loss of sensitivity and uncertainty of identification of weak sensitizers (23).

3.2. Human tests

3.2.1. Elicitation tests in subjects sensitized experimentally

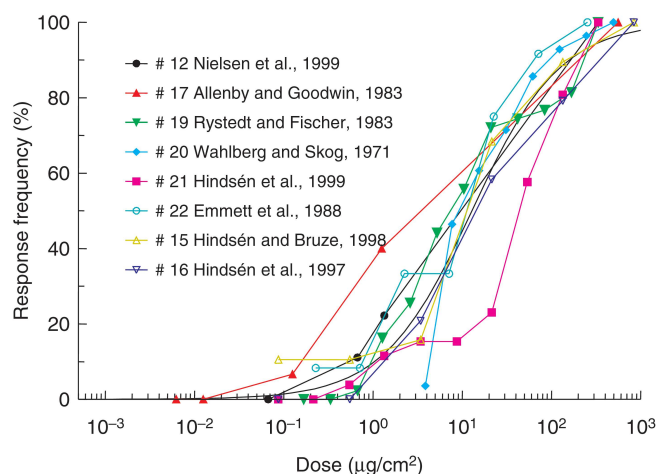
The threshold level of contact allergy and allergic contact dermatitis to various allergens encountered in our environment, e.g. nickel and chromium, was investigated in the first half of the 20th century; however, such investigations were rapidly abandoned due to ethical considerations (24, 25). Few elicitation data are available from subjects who have been experimentally sensitized to allergens used in consumer products, e.g. fragrances (26). It is not reasonable to compare elicitation thresholds in patients and experimentally sensitized subjects as the biological mechanisms and the reactivity threshold are likely to differ markedly.

3.2.2. Dose-response patch tests in dermatitis patients

To gather information about the dose-response (elicitation) relationship of an allergen, serial dilution patch testing with the substance under investigation can be performed in sensitized patients. This is useful when one aims to determine the optimal allergen patch test concentration for clinical evaluation or when one wishes to identify threshold levels that can be used in risk assessment and prevention of contact allergy and allergic contact dermatitis (see repeated open application test (ROAT)) (27). The serial dilution patch test is performed on the upper back similar to the traditional patch test. However, the allergen under investigation is diluted in different concentrations ($x > 3$). The dilution steps depend on the allergen but usually steps of 2, 3 or 10 are applied, covering a span of factor 100-10,000 (27). Threshold levels are determined as the minimal elicitation concentration (MEC) or as the maximum no effect level (NOEL). When the investigator reads and interprets the serial dilution patch test, it is advisable to slightly differ from the traditional criteria defined for

patch test reading as papules typically develop initially without erythema or infiltration. Thus, Fischer et al. suggested that the threshold concentration can be defined as the weakest concentration giving a visible reaction (minimum score 1 defined as few papules with no erythema) on day 3 or 4 in a continuous line of patch test reactions starting from the highest test concentration. In general, the elicitation response in humans is complex. Thus, no uniform elicitation threshold can be determined that applies to all subjects as great inter- and intra-individual variability exists; e.g. investigators have found a 250-fold variation in the elicitation threshold in nickel allergic patients (28). Despite these biological fluctuations and limitations probably caused by recent preceding allergen exposure, the serial dilution patch test is of great value as there is only very little variation in the outcome of dose-response response testing when data from different studies are compiled and combined (29, 30) (Fig. 2). Furthermore, there seem to be little variation in the dose of different allergens that is needed to elicit allergic contact dermatitis in 10% of a sensitized population (ED10) (31).

Figure 2. Dose-response curves from 8 different studies on nickel allergy gave very similar results when analyzed in a logistic regression model (30).



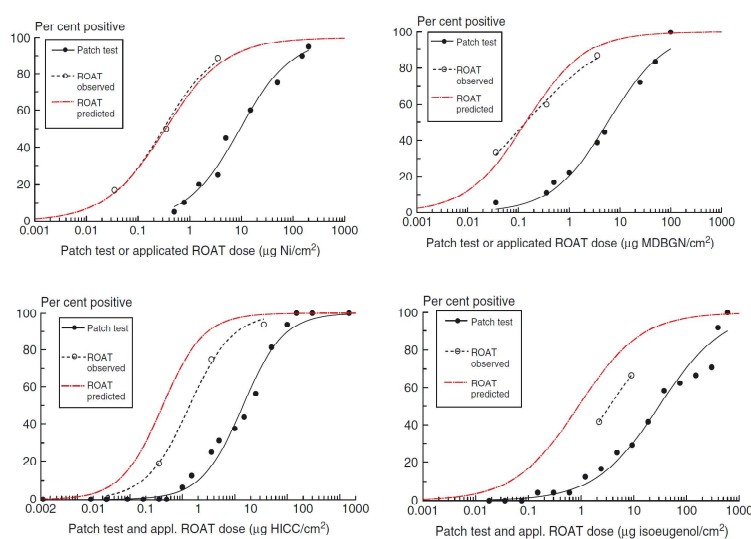
3.2.3. The repeated open application test in dermatitis patients

The repeated open application test (ROAT), also referred to as the use test, provocative usage test, or open patch test is as its name states an open exposure test that intends to mimic real-life exposure situations (32, 27). A finished product, e.g. a skin lotion or a well-defined vehicle containing the allergen under investigation is applied once or twice per day for a 1-3 week period at a 5x5 cm² marked skin area in sensitized individuals, typically on the forearm close to the antecubital fossa. Several test sites (x>3) may be challenged at the same time using different test concentrations, which is typically done by reducing the area size to e.g. 3x3 cm². It should be noted that the size of the test area is generally not crucial except when low concentrations are applied (33). Application in the antecubital fossa should be avoided as the degree of natural occlusion may vary from person to person. Studies have shown that reactivity is stronger when application is performed at other test sites than the forearm, e.g. on the neck or face (34). Application of the test agent or allergen containing solution is either performed by rubbing the lotion at the test site or by using a micropipette followed by spread with a glass rod or the tip of the micropipette. This step is followed by air drying of the test site for a period of 10 minutes. The investigator should consider leaving a control test site without application of the allergen but only the vehicle and also consider performing the

investigation in a blinded or double-blinded manner. The marked challenge site is inspected visually and by palpation according to a reading schedule that may vary between investigators, e.g. at day 2, 3, 4, 7, 14 and 21. However, test subjects should be seen following initial signs of an allergic dermatitis reaction to verify this. A positive response usually develops after 2-4 days of application. Prior to visible signs of dermatitis, test subjects may experience itching. The first sign of an allergic contact dermatitis reaction typically includes follicular papular eruptions due to accumulation and absorption of the allergen through the follicles and sweat duct orifices. However, erythema with or without infiltration may also be an early manifestation. Thus, clinical manifestations are different from those defining positive allergic reactions when performing the patch test as positive allergic ROAT test reactions are weaker and represents earlier stages of allergic contact dermatitis. A semi-quantitative reading scale for the ROAT has been proposed which requires assessment of the degree of area involvement, erythema, infiltration, vesicles and overall clinical impression (35). The ROAT is typically terminated before allergic contact dermatitis reactions corresponding to 2+ or a 3+ reaction in the patch test method are observed. If a ROAT is performed with a formulated product, the observed reaction may be due to the allergen under suspicion, another allergen not under suspicion or due to irritancy.

The ROAT represents a standardized way to mimic real-life exposure to allergens. However, the experimental design of the ROAT has its inherent weaknesses as 1 or 2 applications per day may deviate from real-life exposure. Furthermore, real-life dose per application varies considerable between individuals and probably also within the same individual. Nevertheless, the ROAT undoubtedly serves as an important experimental tool when one wishes to estimate the elicitation threshold of an allergen. As opposed to the patch test, the ROAT is an open test in which the allergen may evaporate or become rinsed or rubbed off during normal activities making it more a realistic exposure test system. Furthermore, most studies include a control group and assessments are done, not only by comparing the reaction site, the vehicle control site and the non treated area but also by comparing different test subjects. Recently, an equation was developed to predict the response to an allergen in the ROAT based on dose-response data obtained from serial dilution patch testing (36). Based on nickel and methyldibromoglutaronitrile (MDBGN), the dose per area per application required to elicit a reaction in the ROAT was lower than the dose per area required to elicit a reaction in the patch test (Fig. 3). Perhaps, the accumulation of allergen in the skin may determine the threshold level of elicitation rather than the dose per application (37). For volatile compounds (e.g. fragrances), the outcome of the ROAT was influenced by evaporation. The proposed equation still needs validation but may be a promising future tool able to define safe-levels of allergens in consumer products. Other important ROAT studies have recently been performed (38).

Figure 3. Comparison of predicted (red line) and observed (broken black line) dose-response relations for the ROAT (36). The predicted dose-response relationship was calculated from dilution patch test results obtained for the respective allergens (upper line, left to right: nickel and MDBGN); lower line, left to right: hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) and isoeugenol). Note that for volatile compounds, the observed and predicted ROAT results are discordant.



3.2.4. Clinical and epidemiological data

As explained in section 2.2.2., elicitation of an allergic reaction following dermal exposure to the chemical (or a mixture), proves that the chemical is an allergen. To diagnose sensitization, elicitation is normally provoked by single allergens through routine patch testing. The results of patch testing a single patient is very rarely published, except in case reports, in contrast to results gained by testing larger groups of patients. These studies can be regarded as examples of clinical-epidemiology.

Large networks of clinical epidemiology of contact allergy include the ‘Information Network of Departments of Dermatology’ (IVDK) (39) and the ‘European Surveillance System on Contact Allergy’ (ESSCA) (40). In both systems, many dermatology departments register on a local PC the results of patch testing together with data from the patient’s history, clinical data, and data on suspected exposure. These data are transferred in regular intervals to a centre (Göttingen and Erlangen, respectively), where further data analyses are done using appropriate statistical tools. Thus, large numbers of reactions to allergens (elicitation) are documented. The IVDK receives data on more than 12.000 patients per year. About half of them have at least one positive reaction to an allergen.

The consequence for the identification of a chemical as an allergen is straightforward. If there are thousands of reactions to a chemical (and not only one

or two in the world literature), there is unequivocal evidence that the chemical is indeed an allergen (Table 1). And even in chemicals considered as “rare allergens”, with possibly 1 case per year per department, the number accumulated in networks is eventually quite impressive (Table 2).

Perspectives

The figures in Table 1 and Table 2 may illustrate the absolute risk of being sensitized to an allergen. However, it is important to consider the relative risk, in this context risk related to exposure. In rare situations only, exposure can be quantified with sufficient validity and detail, as in the case of topical drugs (47) (Table 4). Although the frequency of sensitization is higher for gentamycin in the clinical population and also in the general population, according to the CE-DUR model (48), this is put into perspective through the lower prescription rate of kanamycin, resulting in an actually higher risk to be sensitized to kanamycin.

Table 1. Frequency of allergic (+ to +++) reactions to different chemicals (selection of frequent allergens), IVDK 2000-2009, 96.224 patients tested from 56 departments.

Substance	Number of positives
Nickel (II)-sulfate 6*H ₂ O	12923
Balsam of Peru	7513
Fragrance Mix	7279
Potassium dichromate (chromium)	4547
Colophony	3886
Wool wax alcohols	2830
Fragrance-Mix II	2243
Propolis	2129
Thiuram Mix	2065
PPD (Free Base)(CI 76060)	1991
MCI/MI	1983
Lyrar	1814
Methyldibromo glutaronitrile + 2-Phenoxyethanol	1739

Table 2. Frequency of allergic (+ to +++) reactions to different chemicals (selection of allergens considered to be rare), IVDK 2000-2009, 96.224 patients tested from 56 departments.

Substance	Number of positives
<i>“Rare” allergens</i>	
Iodopropinylbutylcarbamate	588
Zinc-diethyldithiocarbamate	561
Diazolidinyl urea (Germall II)	407
Quaternium 15 ¹⁾	395
1,2-Benzisothiazolin-3-one, Sodium	365
Imidazolidinyl urea (Germall 115)	353
Sorbic acid	348
Sesquiterpenelactone Mix	307
Ylang-ylang (I + II) Oil	287
DMDM hydantoin ²⁾	268
Triclosan	175
Benzylalcohol	165
Dexpanthenol	137
<i>N,N'</i> -Diphenyl-p-phenylenediamine (DPPD)	97
Clioquinol (Iodochlorhydroxyquin)	96
Polidocanol	76

¹⁾ *N*-(3-Chloroallyl)hexaminium chloride, Dowicil 200[®]

²⁾ 5,5-Dimethyl 1,3-dimethylolhydantoin

Furthermore, as suspected exposures are documented, the association between allergen and exposure/occupation can be studied, resulting for each allergen in a list of factors increasing (or decreasing) risk of sensitization (41–45) (Table 3).

Table 3. Results of a poisson regression analysis concerning occupation as risk factor for contact allergy to 2 allergens (chromium and epoxy resin) (46).

Chromium		
Occupation / Occupational group	%	PR (95% CI)
Construction labourer	1.46	3.79 (3.18 - 4.51)
Metal finisher (e.g., electroplater)	0.14	3.07 (1.82 - 4.84)
Metal furnace operator, etc	0.19	2.03 (1.18 - 3.24)
Miner	0.18	2.02 (1.13 - 3.32)
Sheet metal worker	0.13	1.79 (0.85 - 3.26)
Office worker	13.01	1.00 (Reference)
Epoxy resin		
Occupational major group	%	PR (95% CI)
Construction and mining industry	1.63	4.08 (2.81 - 6.00)
Painter, Carpenter, Potter	1.42	3.76 (2.52 - 5.63)
Chemical industry workers	1.59	2.70 (1.73 - 4.20)
Metal worker	5.16	1.43 (0.99 - 2.09)
Technicians	2.99	1.30 (0.83 - 2.01)
Service occupations NEC	5.02	1.00 (Reference)

Table 4. The relative incidence of sensitization to 2 topical antibiotics.

Allergen	Frequency of sensitization in patients (% of patients tested) (IVDK data)	Frequency of sensitization in general population (# of cases) (according to CE-DUR)	Prescriptions (Mio DDD p.a.). (Data form WIdO Research Inst. Bonn)	Relative incidence (cases/100.000 DDD p.a.) (95% CI)
Kanamycin sulphate	2.9	1,336	15.4	8.7 (3.7-13.7)
Gentamycin sulphate	3.5	2,077	43.3	4.8 (2.5-7.1)

Final comments

Clinical data, helping to identify a chemical as an allergen, become even more pertinent in a situation where pre-clinical data (animal tests) are missing. However, there should be agreement on the grades of evidence required for designation (e.g. the number of cases observed, possibly put into perspective by the number of individuals exposed or by doses unrealistically high). The final decision will probably always be based on an expert judgement (49–51).

4. Animal and human welfare

Testing for prediction of allergenic potential or safety assessment of chemicals on animals or human subjects is increasingly associated with ethical considerations. There is always a consideration of health benefit contra costs in suffering of animals or research subjects. Knowledge about sensitization properties of chemicals usually leads to marketing of safe products for the public or industrial chemicals/products. It can also be the scientific base for classification and labelling of products.

Regarding animal testing, particular concern has been given to the use of the mineral oil based Freund's complete adjuvant in the scientific community as well as among the public. This adjuvant contains mineral oil as well as muramyl dipeptide and is associated with a range of side effects when given to experimental animals. The most severe adverse effect is considered to be formation of granulomas and necrosis following injection (52), a trait it shares with some other adjuvants (53). For this reason, test methods without the use of adjuvant may arguable be preferred due to ethical reasons i. e. Beuhler test or LLNA.

Regarding cosmetic products, a ban on animal testing has been put forward through amendments to the EU Cosmetics Directive (76/768/EEC) (54). For this reason the Scientific Committee on Consumer Products (SCCP) has adopted the reduced LLNA (rLLNA) for hazard identification and LLNA as a "reduce and refinement" risk assessment test method since there at the moment is no replacement method available (55).

The Helsinki declaration of the World Medical Association clearly regulates the participation of human subjects into research studies (56). One among those is the compulsory review of research studies by Independent Ethics Committees prior to start. Since predictive and safety testing of products and chemicals may not necessarily be considered research, it can be argued that it not should be performed on human subjects. One major reason is that if a subject is sensitized to a product or chemical commonly used in the general environment, it can create significant problems for this subject subsequently. This opinion is strongly advocated in the guidance documents for the implementation of

the EU Registration, Evaluation, Authorisation and Restriction of Chemicals programme (REACH) (57, 58).

5. In vitro assays targeting the sensitization phase

Current assessment of skin sensitization relies on animal tests. The future ban in the EU, in 2013, on in vivo testing of the skin sensitization potential for cosmetic ingredients (59), together with the EU REACH programme (60), call for the urgent development of in vitro and in silico methods for the prediction of the sensitizing potential of chemicals.

5.1. In silico tests

In silico methods in the field of skin sensitization are mainly (quantitative) structure-activity relationships models [(Q)SARs] and expert systems. Principles for validating (Q)SARs models for their use in regulatory assessment of chemical safety exist from 2004 (61, 62). Any (Q)SAR model should be associated with the following information: a defined endpoint, an unambiguous algorithm, a defined domain of applicability, appropriate data on robustness and predictivity, and a mechanism of interpretation if possible. Another issue is their application in the context of endpoint specific Integrated Testing Strategies (ITSs).

5.1.1. Structure-activity relationships (SARs)

SARs aim to identify relationships between chemical structure, or structural-related properties, and biological activity of studied compounds. In the case of skin sensitization, the identification of electrophilic features in chemicals is the basis of studies where qualitative associations between structure and sensitization response are investigated. The earliest SAR study correlating chemical reactivity and skin sensitization was reported on halogenated nitrobenzene derivatives and their allergenic response in guinea pigs (63). It was proposed for the first time the formation of a hapten-protein complex as a prerequisite for the development of skin sensitization. The modern concept of SAR in contact allergy was pioneered by Dupuis and Benezra (64). These authors showed that structural requirements for haptens are highly specific. Many authors started then to define structural relationships based on an evaluation of a large data set of guinea pig and LLNA sensitization data (65). Other efforts included the identification of structural alerts and of common reaction mechanisms following the grouping of chemicals into potency categories (66, 67). Today, derived structural alerts for sensitizers are continuously encoded into the Derek for Windows (DfW) expert system (see later). Even if SARs give only qualitative information, the structural alerts are useful for identifying potential sensitizers as a first stage in an ITS.

5.1.2. Quantitative structure-activity relationships (QSARs)

A complementary approach is to search for empirical quantitative SARs (QSARs) by application of statistical methods to sets of biological data and structural descriptors. QSARs models can be “global”, derived empirically using statistical methods, but also “local”, specific to a chemical class or reaction mechanism. Some global QSARs have been characterized in accordance with the OECD validation principles and show to be of limited use for regulatory purposes (68). The main criticism is that, instead of exploring the chemical behaviour of compounds, they intend to predict just a yes/no-sensitizing outcome using descriptors hard to interpret physically. Predictive rates are apparently high, but there is a lack of mechanistic

basis and of rationalisation of the underlying skin sensitization mechanism of molecules. The majority of local models derive from the Relative Alkylation Index (RAI) approach (69). The RAI model quantifies the degree of carrier alkylation, as a function of hydrophobicity and reactivity, with sensitization potential. It has successfully been applied to evaluate chemicals such as sultones, aldehydes, sulfonates, and butyrolactones. Its main limitation is that it only estimates allergenic capacity with good results for series of compounds of the same chemical class. Thus, it was thought of limited predictive coverage. This view changed with the introduction of a classification of compounds into reaction mechanistic applicability domains, to rationalize their behaviour on the basis of reaction chemistry (70, 71). These new guidelines are being used for reaction mechanism classification in the context of QSARs and in quantitative mechanistic models (QMM) (72). The QMM approach, integrating mechanistic principles for skin sensitization, has the potential to create robust models that could be useful as part of an ITS in a regulatory context.

5.1.3. Expert systems

Expert systems are available for the prediction of skin sensitization (68). DfW is a knowledge-based expert system that contains about 360 alerts covering a wide range of toxicological endpoints. Version 9.0.0 contains 64 alerts for skin sensitization (73). Each alert describes a structural feature, in general having the potential for electrophilic binding to skin proteins, associated with the occurrence of skin sensitization. The Toxicity Prediction Komputer-Assisted Technology (TOPKAT), with modules aiming to discriminate sensitizers/non-sensitizers and weak/moderate *versus* strong sensitizers, and the Computer Automated Structure Evolution program (CASE), identifying structural fragments associated with sensitization independently of the action mechanism, rely on a statistical/empirical approach. The Tissue Metabolism Simulator for Skin Sensitization (TIMES-SS) is a hybrid between knowledge-based and statistical expert systems. The program constructs QSARs for skin sensitization potential by considering also skin metabolism and the potential interaction of reactive metabolites with skin proteins. Some of these expert systems (DfW, TOPKAT) have been validated according to the OECD principles for QSARs validation. However, further improvements are still necessary, results for non-sensitizers prediction being unsuccessful. More refined models need to be developed including skin penetration, chemical reactivity and metabolism. Recently, the OECD has started the development of a (Q)SAR Application Toolbox intended to filling gaps in toxicity data needed for assessing the hazard of chemicals. It incorporates information and tools from various sources into a logical workflow based in grouping chemicals into chemicals categories. The aims are to identify relevant structural characteristics, potential mechanisms of action and use of existing experimental data to fill the data gap(s) (74).

5.2. In chemico tests

In chemico tests are based on the already mentioned relation observed between the ability of a sensitizer to react with proteins to make covalent adducts and its sensitizing potential. To the opposite of *in silico* methods based on calculation, *in chemico* methods are based on experimentally measured parameters (75). Despite the fact that some of this information has been the base for *in silico* developments, it is only recently that alternative methods based on this approach have been developed. Different model nucleophiles have been proposed ranging from small molecules to proteins (75).

5.2.1. The GSH reactivity database

Schultz and co-workers have examined the value of using a non-enzymatic glutathione (GSH) reactivity assay as a potential non-animal approach to skin sensitization testing (76). The assay is a simple and rapid spectrophotometric-based concentration-response assay for non-enzymatic chemical reactivity, following the incubation of GSH with a chemical. Results are expressed as the 2-hour RC50 GSH value reported in mM units. Current RC50 GSH values are available for over 200 compounds, which represent various mechanistic domains. It is important to note that this method provides a means to obtain rate constants data for potential use in *in silico* models.

5.2.2. The Direct Peptide Reactivity Assay (DPRA)

This assay, currently under pre-validation at the European Centre for Validation of Alternative Methods (ECVAM) is based on the observed reactivity of test molecules with 2 nucleophile-containing synthetic peptides, by measuring peptide depletion (77). 82 Chemicals were evaluated for their ability to react with the 2 peptides containing cysteine and lysine. The chemical represented in the dataset comprised weak (n = 15), moderate (n = 19), strong and extreme sensitizers (n = 18), as well as non-sensitizers (n = 30) as based on potency categorisation criteria that have been developed by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). The peptide reactivity data were compared with the existing LLNA data, by recursive partitioning methodology to build a classification tree that allowed a ranking of reactivity as minimal, low, moderate and high (78). In general, non-allergens and weak allergens demonstrated minimal to low peptide reactivity, whereas moderate to extremely potent allergens displayed moderate to high peptide reactivity. The cysteine and lysine peptides represent softer to harder model nucleophiles, which should help in detecting skin sensitizers, which have different reaction mechanisms. To evaluate the approach for hazard identification purposes, Cooper statistical analysis was used. Classifying minimal reactivity as non-sensitizers and low, moderate and high reactivity as sensitizers, it was determined that the model based on cysteine and lysine gave a prediction accuracy of 89%. Recently, an interlaboratory study was carried out to evaluate the peptide reactivity of 15 chemicals, showing a similar peptide depletion values. The data were analysed which showed that each participating laboratories classified reactivity similarly for more than 90% of the chemicals evaluated. Natsch et al. (79) have evaluated a similar approach based on alternative test peptides derived from protein sequences. Overall, all the different heptapeptides evaluated (n = 7) gave similar results and ranking of the compounds although the Cor 1 peptide (Cys420 of Human Coronin 1C) was slightly more reactive than the other peptides.

The main limitation of the DPRA is that pro- and pre-haptens, molecules needing a metabolic/chemical activation to become reactive, are not detected in this assay. To overcome this limitation, a peptide-based assay including a peroxidase/hydrogen peroxide activation of pro- and pre-haptens has been proposed and is currently under development and evaluation (80).

5.3. Cellular tests

These methods are mainly based on the use of isolated cells or cell-lines. To that respect, examination of the phenotypic changes induced by chemicals on various antigen presenting cells (APC) has been one of the main approaches (81). As APC are difficult to isolate from the skin, methods have been developed to generate dendritic cells from human peripheral

blood mononuclear cells (PBMC) or CD34+ hematopoietic progenitor cells (HPC) cultured in the presence of specific cytokines. It is then possible to follow expression of CD86/CD54 (82), IL-1b release (83) or internalization of MHC class II molecules (84) in the presence of chemicals. However, generation of DC like cells takes several days and requires complicated procedures. Moreover, problems including availability of human blood and donor-to-donor variability are limiting factors. Therefore, cell-lines with some characteristics of DC have gained much attention and two assays, based on such cell-lines are currently under pre-validation at ECVAM after transferability tests.

5.3.1. The human cell activation test (h-CLAT)

This assay is based on the use of THP-1 (human monocytic leukemia cell line). Typically, cells in culture are exposed to increasing doses of the test molecules. After 24 h of incubation, cells are reacted with anti-CD86 and/or anti-CD54 labelled antibodies prior to cytometry analysis (85). These authors have been able to show that expression of CD86 and CD54 were good predictive markers for the discrimination between sensitizers and non-sensitizers. For CD86 expression, allergens augmented significantly the relative fluorescence intensity (RFI) compared to non-sensitizers and a threshold of 150 gave a prediction accuracy of 89% (n = 9). On the same panel of molecules measure of the CD54 up-regulation gave also a prediction accuracy of 89% based on a threshold of 200 (86). In another study 21 sensitizers and 8 non-sensitizers were evaluated in the h-CLAT based on EC150 (CD86) and EC200 (CD54) values. The prediction accuracy (sensitizers/non-sensitizers) was found to be of 93.1% (87).

5.3.2. The myeloid U937 skin sensitization test (MUSST)

This assay is very similar to the h-CLAT but based on the use of U937 (histiocytic lymphoma cell line). Typically, cells in culture are exposed to increasing doses of the test molecules. After 48 h of incubation, cells are reacted with anti-CD86 labelled antibodies prior to cytometry analysis (88). For CD86 expression, allergens augmented significantly the relative fluorescence intensity (RFI) compared to non-sensitizers and a threshold of 120 gave a prediction (sensitizer/non-sensitizer) accuracy over 90% (n = 50). The potential of this test was further confirmed (89) and the MUSST is currently in pre-validation at the ECVAM.

5.3.3. Others

The Sens-it-iv project, financially supported by a grant from the European Commission (LSHB-CT-2005-018681), is focusing on the development of “in vitro” alternatives to animal tests currently used for the risk assessment of potential skin or lung sensitizers. New approaches can then be expected in the next years. Up-dated information can be found on the Sens-it-iv web site: <http://www.sens-it-iv.eu/>.

Table 5. Overview of methods

Overview of currently available methods or methods under development (in vivo, in vitro, in silico, etc.) used in the evaluation of skin sensitization potential and their applicability in the derivation of quantitative “safety thresholds”.

Test Method	Transparency of test method	Reproducibility	Number of doses ≥ 3	Relevance of dose metric	Relevance of endpoint for human disease	Correlation to human data	Controls included
2. Sensitization							
2.1 Animal Tests							
The Buehler test	Good	Good	Good	Good	Good	Fair	Good
The GPMT	Good	Good	Good	Good	Good	Good	Good
MEST	Fair	Fair	Good	Good	Good	Fair	Good
LLNA	Good	Good	Good	Good	Good	Good	Good
2.2 Human Tests							
HR IPT	Poor	Unknown	Fair	Good	Unknown	-	-
Single cases	Good	Good	Poor	Good	Good	-	Fair
Clinical epidemiological studies	Good	Good	Poor	Fair	Good	-	Good
Traditional epidemiological studies	Good	Good	-	Poor	Good	-	Good
3. Elicitation							
3.1 Animal tests							
rLLNA	Good	Good	Good	Good	Good	Good	Good
3.2 Human tests							
Elicitation test in subjects sensitized experimentally	Fair	Unknown	Good	Good	Poor	-	-
Dose-response patch test in dermatitis patients	Good	Fair	Good	Good	Good	-	Good
The ROAT in dermatitis patients	Good	Good	Good	Good	Good	-	Good
Clinical and epidemiological data	Good	Good	Poor	Poor	Good	-	Good
4. In vitro assays							
4.1 In silico							
SARs	Fair	Good	-	-	-	Unknown	-
QSARs	Good	Good	-	-	-	Unknown	-
Expert systems	Fair	Good	-	-	-	Unknown	-
4.2 In chemico							
GSH reactivity	Good	Good	Good	Good	-	Fair	-
DPRA	Good	Good	-	Good	-	Fair	-
4.3 Cellular tests							
h-CLAT	Good	Good	Good	Good	-	Fair	Good
MUSST	Good	Good	Good	Good	-	Fair	Good

Good
Fair
Poor

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Part II:

Aim

To identify specific cases, classes or specific use situations of chemicals for which “safety thresholds” or “safety limits” were set (in regulations, standards, in scientific research/clinical work, etc.) and critically review the scientific and methodological parameters used to set those limits.

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6. Regulations

6.1 Chromium

The EU Chromium Directive (2003/53/EC) came into force in January 2005 and applies to cement and products containing cement marketed in EU member states (Table 1) (1). It dictates that the level of soluble hexavalent chromium (VI) shall be limited to a maximum of 2 ppm by mass of cement when water is added. In most cement, the soluble chromium (VI) levels will be controlled by reducing agents added to the cement at the grinding stage. One should be aware that the length of time over which the reducing agents will be effective is limited. Typically, a manufacturer declares a two-month shelf life although this can be increased by using other reduction technologies than ferrous sulfate. The EU Chromium Directive only applies to cement that is handled manually; hence cement that is used in entirely automatic processes does not need to fulfil the requirements. The EU Chromium Directive was introduced long after the introduction of similar regulations in Scandinavian countries (2,3).

The content of chromium VI in cement depends on the chromium content, mostly trivalent chromium (III), of the raw material but also on the kiln lining and on chromium steel abrasion during the grinding process. Oxidation of chromium III to chromium VI occurs during cement processing in the kiln at temperatures between 1400–1500 °C.

Reduction of chromium VI in cement is typically obtained by adding 0.35% (w/w) ferrous sulfate. Burckhardt et al. were the first to show that ferrous sulfate had the capacity to reduce chromium (IV) to chromium (III) (4), whereas Fregert et al. found no water-soluble chromium in cement to which ferrous sulfate had been added (5).

The scientific basis for the 2 ppm chromium content limit was derived from chromium exposure studies and the necessary data were mainly collected prior to regulation (for details, please see 8.3)

Table 1. The EU Chromium Directive (1)

I	Cement and cement-containing preparations may not be used or placed on the market, if they contain, when hydrated, more than 0.0002% soluble chromium VI of the total dry weight of the cement.
II	If reducing agents are used, then without prejudice to the application of other Community provisions on the classification, packaging and labelling of dangerous substances and preparations, the packaging of cement or cement-containing preparations shall be legibly and indelibly marked with information on the packing date, as well as on the storage conditions and the storage period appropriate to maintaining the activity of the reducing agent and to keeping the content of soluble chromium VI below the limit indicated in paragraph 1.
III	By way of derogation, paragraphs 1 and 2 shall not apply to the placing on the market for, and use in, controlled closed and totally automated processes in which cement and cement-containing preparations are handled solely by machines and in which there is no possibility of contact with the skin.

6.2 Nickel

The EU Nickel Directive (94/27/EC) covers metallic consumer items that are intended to come into direct and prolonged skin contact (6). The Directive was passed in 1994 but did not come into full force until 2001 (Table 2). Part I was modified in 2004 which resulted in a nickel release limit rather than a nickel content limit of items inserted into pierced parts of the body (7). Thus, part I dictates that piercing posts and post assemblies may not release more than 0.2 μg nickel/ cm^2 /week; part II dictates that consumer items intended to come into direct and prolonged skin contact may not release more than 0.5 μg nickel/ cm^2 /week; part III dictates that coated consumer items should withstand nickel release exceeding 0.5 μg nickel/ cm^2 /week for at least 2 years of normal use. The EU Nickel Directive was mainly based on the Danish and Swedish nickel regulations from respectively, 1990 and 1991 (8, 9). The Danish nickel regulation contained part II of the EU Nickel Directive whereas the Swedish regulation contained the original part I of the Nickel Directive (Table 2).

The scientific basis for the EU Nickel Directive was mainly obtained during the 1980's when the nickel epidemic accelerated due to the popularity of ear piercing, jewelry and blue jeans (3). The original part I restricted the nickel content of post assemblies used in piercing holes to 0.05% nickel. Fischer et al. analyzed discs made of white gold containing various concentrations of nickel (i.e. 2.5-15% and 75-80%) and showed that nickel allergic dermatitis patients with a high sensitivity reacted to discs with low nickel content upon patch testing (10). The 0.05% content limit was a conservative limit aimed at the protection of nickel allergic patients. In 2004, an amendment to the EU Nickel Directive changed part I. The scientific background for this change is not clear. However, prior to the amendment, Ingber et al. performed a small study on AISI316L stainless-steel ear piercing post assemblies with a nickel content between 10% and 14% (11). Ear Piercing Manufacturers of

Europe Ltd (UK), Spalding, UK, supported the study financially. Nickel release from piercing post assemblies was between 0.11 and 0.21 $\mu\text{g}/\text{cm}^2/\text{week}$ for 10 unused posts.

Part II of the EU Nickel Directive was based on the Danish nickel regulation. A Danish study aimed to identify a general threshold limit below which nickel allergic subjects could safely wear metallic items that contained nickel (12). 267 nickel allergic patients were patch tested with 15 metal alloys of known composition (nickel content ranged from 0-100%). The discs were also analyzed in synthetic sweat for 1 week and with energy dispersive x-ray to confirm their composition. Discs that released more than 1 μg nickel/ cm^2/week gave strong patch test reactions whereas discs that released less than 0.5 μg nickel/ cm^2/week showed weak reactivity. However, one disc, Inconel 600 (77% nickel, 8% iron, 15% chromium), gave dermatitis reactions in a large proportion of patients but released less than 0.5 μg nickel/ cm^2/week when analyzed in synthetic sweat. This finding could be explained by the formation of chromium oxides on the surface during corrosion testing. The authors concluded that the nickel allergy problem could be minimized by the use of alloys that released less than 0.5 μg nickel/ cm^2/week . When the DMG test was applied on the discs used for patch testing, it mostly gave positive reactions in alloys that released more than 0.5 μg nickel/ cm^2/week (13). Based on this finding, the DMG test became a rapid and inexpensive screening method to discriminate between safe and unsafe nickel alloys and it was hence used as the reference test method in the Danish nickel regulation.

The scientific basis for Part III of the EU Nickel Directive has not been published in the medical literature.

The decreasing trends of nickel allergy following nickel regulation suggest an effect of this major public health intervention.

Table 2. The EU Nickel Directive (6, 7) and reference methods (14-16).

		CEN standard
Part 1	<p><i>Original requirement</i> (before 2005): Nickel was prohibited in post assemblies which were inserted into pierced ears and other pierced parts of the human body during during epithelialization of the wound, unless they were homogenous and the nickel concentration was below 0.05%.</p> <p><i>New requirement</i> (from 2005): Nickel release from all items inserted into pierced parts of the body (not only during epithelialization after piercing) should be less than 0.2 µg/cm²/week.</p>	<p>EN1810 (Flame Atomic Absorption Spectrometry. Nickel content is expressed as mass of nickel to total mass.)</p> <p>EN 1811 (Items under investigation are placed in artificial sweat for 1 week and the concentration of dissolved nickel in the solution is determined by atomic absorption spectrometry (or other methodology) and expressed in µg/cm²/week.)</p>
Part 2	Nickel may not be used in products intended to come into direct and prolonged contact with the skin such as earrings, necklaces, bracelets, chains, anklets, finger rings, spectacle frames, wrist-watch cases, watch straps, zippers, buttons and mobile phones if nickel release from the parts coming into direct and prolonged contact with the skin is greater than 0.5 µg/cm ² /week	EN 1811
Part 3	Nickel is prohibited in products such as those listed under point 2 if they have a coating and if they do not fulfil the requirement under point 2 for a period of at least 2 years of normal use of the product	<p>EN 12472 (Method for simulation of wear and corrosion for the detection of nickel release from coated items. The item under investigation is exposed to a corrosive atmosphere and then placed in a container together with abrasive chips, water and a wetting agent. The container is rotated to smooth the surface and abrade the coating. Finally, the item is subjected to the EN 1811)</p>

6.3

Cosmetics Directive (76/768/EEC)

According to the EU Cosmetics Directive from 1976, cosmetic products are defined as any substance or preparation intended to be placed in contact with various external parts of the human body or with the teeth and 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 (17). Cosmetic products may contain different preservatives of which the use of some is regulated within the EU (18), i.e. formaldehyde, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (MCI/MI) and MDBGN.

6.3.1 Formaldehyde

Formaldehyde is a strong sensitizer that may be used for preservation as such or under the chemical form of formaldehyde releasers. Formaldehyde is allowed in cosmetic products in the EU in a use concentration of 0.2% (2000 ppm) but should be declared when used in higher concentrations than 0.05% (500 ppm). Formaldehyde is prohibited in aerosol dispensers but allowed in nail varnish up to 5%. The scientific background for the concentration limits on free formaldehyde in cosmetic products seems to be based on the general toxicological properties of formaldehyde and not to be related to its skin sensitizing properties (19). However, in the 1986 report by the Scientific Committee on Cosmetology, it was noted that formaldehyde sensitizing properties may appear at use-levels of 0.1% (19). This could possibly be related to the study by Marzulli and Maibach who showed that 0 of 45 healthy volunteers were sensitized to 0.1% formaldehyde whereas 4 of 89 subjects were sensitized to 1% formaldehyde (20). In a recent comprehensive review, de Groot et al. state that formaldehyde levels that exceed 200 ppm are not safe for formaldehyde allergic patients (21). Taken together, the EU restriction on formaldehyde in cosmetic products (2000 ppm) does not correspond well to the concentrations that may elicit dermatitis in formaldehyde allergic patients (for details, please see below). The persistence of formaldehyde allergy in European dermatitis patients reflects this (22).

6.3.2 Isothiazolinones

Kathon[®] biocide was developed by Rohm and Haas (Philadelphia, USA) in the 1960's and is a broad-spectrum antimicrobial agent in an aqueous solution containing a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (MCI/MI) in an approximate ratio of 3:1. MCI/MI has been widely used in occupational, household and cosmetic products since the late 1970's and early 1980's (3). When introduced, the use concentration of MCI/MI in cosmetic products sold within the EU was restricted to 30 ppm in both leave on and rinse-off cosmetic products. However, most manufacturers used concentrations that were below this limit, also because cases of MCI/MI allergy and dermatitis were reported. Today, MCI/MI is only allowed in a use concentration of 15 ppm in rinse-off and leave on cosmetic products sold within the EU despite the Cosmetics, Toiletries and Fragrance Association recommends a use concentration of no more than 7.5 ppm in cosmetic leave on products (3). In a recent Scientific Committee on Consumer Safety report on MCI/MI in cosmetic products, it was found that no adequate data is given to support safe use at a maximum concentration of 15 ppm in leave on cosmetic products (23). The EU restriction on MCI/MI use in cosmetic products (15 ppm) does not correspond well to the observed elicitation concentrations that may

elicit dermatitis in MCI/MI allergic patients, especially for leave on products (for details, please see below). The persistence of MCI/MI allergy in European dermatitis patients reflects this (22).

6.3.3 **p-Phenylenediamine (PPD)**

p-Phenylenediamine (PPD) is an extreme sensitizer (24). The maximum authorized concentration of PPD in finished cosmetic products sold within the EU area was for long 6% calculated as free base (3% when added to the oxidizing solution required to develop the colour). A study from 2005 investigated the concentrations of more than 2000 PPD containing commercial products marketed worldwide (25). 3 groups of shades were defined based on the results: a) light shades (typical range: 0.02-0.39% PPD in colorant base before mixing with the developer), b) medium shades (0.14-1.34% PPD) and c) dark shades (0.74- 2.0% PPD). In 2002 and again in 2006, reports of the Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers and the Scientific Committee on Consumer Products concluded that insufficient information was submitted to allow an adequate risk assessment (26, 27). The insufficiency was mainly related to the carcinogenic and genotoxic properties of PPD. However, the concentration limit of PPD in hair dye products was recently changed to a maximum of 2% PPD, calculated as free base. This concentration limit seems not to further restrict the use of PPD when one appreciates the concentrations typically used in hair dyes (25). Furthermore, products containing PPD should be labelled with warnings about allergic reactions and warnings against the use of PPD for the purpose of dyeing eyelashes and eyebrows. The scientific background for the concentration limits on free PPD in cosmetic products is based on the general toxicological properties of PPD and seems not to reflect its skin sensitizing properties. Studies have shown that allergic subjects react to PPD concentrations well below the limit and that de novo sensitization occurs in healthy individuals when repeatedly exposed to hair dyes (for details, please see below). The persistence of PPD allergy in European dermatitis patients reflects this (28-30).

6.3.4 **Methyldibromo glutaronitrile**

MDBGN is synonymous with 1,2-dibromo-2,4-dicyanobutane. MDBGN was initially introduced during the mid 1980's as Euxyl K400® (Schülke & Mayr, Hamburg, Germany), a combination of MDBGN and phenoxyethanol in a ratio of 1:4. Its popularity grew rapidly as Euxyl K400® efficiently prevented the growth of micro organisms. It was used in occupational products such as paint and cleaning agents but also in moisturizers, shampoos, soaps, sunscreen lotions, hair-care products and make-up products (3). In 1986, the EU scientific Committee on Cosmetology authorized the use of MDBGN at a maximum concentration of 0.1% in both leave on and rinse-off cosmetic products (18). However, the use concentration was not allowed to exceed 0.025% in sunscreen products. In 2003, MDBGN was banned in stay-on products (31) and in 2005, MDBGN was recommended not to be used in rinse-off products (32). Taken together, the MDBGN story shows that the gradual increasing scientific evidence on the sensitizing properties of MDBGN resulted in step-wise increasing restriction and finally in total ban (33).

6.3.5. **Fragrances: isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)**

According to the Regulation No 1223/2009, the presence of isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) must be indicated in the list

of ingredients, when their concentrations exceed 0.001% in leave on cosmetic products and 0.01% in rinse-off products. However, no maximum use concentration was defined (34).

6.3.5.1 Isoeugenol

Because of the sensitization potential of isoeugenol (see below), the International Fragrance Research Association (IFRA) has, from 1980 to 2008, repeatedly revised the recommended concentrations, reducing it from 0.2% to 0.02% in 1998, and finally, in the 43rd amendment in 2008, 0.01% and 0.02%, depending on the product type (35). The use concentrations were determined using repeatedly quantitative risk assessment (QRA) (see 8.1), essentially based on the results of the local lymph node assay (LLNA) and the human repeat insult patch test (HRIPT). Future epidemiological monitoring will show, whether sensitization rates decline significantly, which could cautiously indicate, that the QRA approach might be considered useful.

6.3.5.2. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Syn.: hydroxy-methylpentyl-cyclohexenecarboxaldehyde (HMPCC), Lyrall[®] IFRA recommended concentration levels of 1.5% in finished cosmetic products (leave on as well as rinse-off) in 2003 (35). Because of the sensitization potential of HICC (see below), in the 43rd amendment of IFRA standards, published in July 2008, lower concentration limits for HICC in various kinds of products were recommended, e.g. 1.5% in hydroalcohols for unshaved skin, 1% in hand creams, 0.6% in hydroalcohols for shaved skin, and 0.15% in deodorants (35). In the 44th amendment in 2009, a further (essential) reduction of concentration down to 0.02% and 0.2% depending on the product type was recommended (35).

The use concentrations were determined using repeatedly QRA (see above), essentially based on the results of the LLNA and the HRIPT.

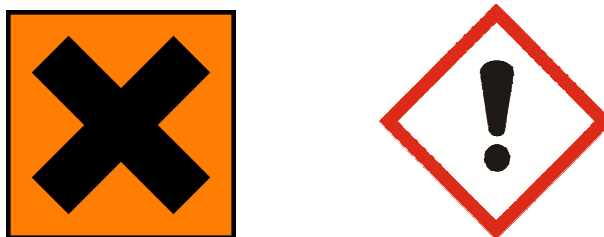
Epidemiological monitoring will show if in the future sensitization rates decline significantly, which could cautiously indicate that the QRA approach might be considered useful.

6.4. Dangerous Preparations Directive (67/548/EEC)

To ascertain a high level of protection of human health and environment, the regulations for classification and labelling were introduced in 1967 with subsequent amendments and followed by the latest Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures in response to changes introduced by the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (36). These documents identify chemicals that are hazardous to the health and environment. One group that is identified consists of sensitizers. The document specifies a generalized concentration level of 1% in certain products, above which a written warning phrase should be printed on the products container, together with a hazard pictogram for easy identification of the hazard involved when handling the product. Among these is the risk phrase R43 for skin sensitization together with the black Andreas cross on an orange coloured square (Figure 1). This is, through the implementation of GHS, being altered to the Hazard Statement H317: 'May cause an allergic skin reaction' together with the Signal Word 'Warning' and the Hazard Pictogram of an exclamation mark on a red framed white diamond (Figure 1). The Hazard Statement and

Pictogram system is an easy way to signal to users the possible hazard when using a product and recommendation to apply proper techniques to prevent cutaneous exposure. Based on potency assessments of certain chemicals in use in society it has been proposed that there should be an addition of at least 2 new limits for classification and labelling to increase health protection of the public (37, 38).

Figure 1. Left pictogram: R43 for skin sensitization, the black Andreas cross on an orange coloured square. Right pictogram: Hazard Statement H317.



6.4.1. List of R 43 labelled substances with concentration limits

In the Regulation 1272/2008 and Directive 67/548/EEC several sensitizing chemicals are given special concern and have lower limits for classification and labelling. These are potent allergens or chemicals with large use in society. These chemicals are summarized in table 3.

Table 3. List of R 43 labelled substances with concentration limits

Name	CAS	EINECS	Lowest limit labelling with R43 or H317
ammonium dichromate	7789-09-05	232-143-1	0.2 %
1,2-benzisothiazol-3(2H)-one	2634-33-5	220-120-9	0.05 %
1,2-benzisothiazol-3-one			
4,4'-methylenedicyclohexyl diisocyanate	5124-30-1	225-863-2	0.5 %
diethylene glycol diacrylate	4074-88-8	223-791-6	0.2 %
oxydiethylene diacrylate			
2,3-epoxypropyl acrylate	106-90-1	203-440-3	0.2 %
formaldehyde	50-00-0	200-001-8	0.2 %
glutaral	111-30-8	203-856-5	0.5 %
glutaraldehyde			
2,2',2''-(hexahydro-1,3,5-triazine-1,3,5-triyl)triethanol	4719-04-04	225-208-0	0.1 %
hexamethylene diisocyanate	822-06-0	212-485-8	0.5 %
2-hydroxyethyl acrylate	818-61-1	212-454-9	0.2 %
2-hydroxy-1-methylethyl acrylate	2918-23-2	220-852-9	0.2 %
2-hydroxypropyl acrylate	999-61-1	213-663-8	0.2 %
acrylic acid, monoester with propane-1,2-diol	25584-83-2	247-118-0	0.2 %
3-isocyanatomethyl-3,5,5-trimethylcyclohexyl isocyanate	4098-71-9	223-861-6	0.5 %
isophorone di-isocyanate			
N-isopropyl-N'-phenyl-p-phenylenediamine	101-72-4	202-969-7	0.1 %
potassium dichromate	7778-50-9	231-906-6	0.2 %
potassium chromate	7789-00-6	232-140-5	0.5 %
2-chloroacetamide	79-07-2	201-174-2	0.1 %
Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1)	55965-84-9		0.0015 %
chromyl dichloride	14977-61-8	239-056-8	0.5 %
methacrylonitrile	126-98-7	204-817-5	0.2 %
2-octyl-2H-isothiazol-3-one	26530-20-1	247-761-7	0.05 %
sodium dichromate, dihydrate	7789-12-0		
sodium dichromate	10588-01-9	234-190-3	0.2 %
sodium chromate	7775-11-3	231-889-5	0.2 %
2,2,4-trimethylhexamethylene-1,6-di-isocyanate	16938-22-0	241-001-8	0.5 %
2,4,4-trimethylhexamethylene-1,6-di-isocyanate	15646-96-5	239-714-4	0.5 %

Source ESIS database (39).

6.5. **General Product Safety Directive (2001/95/EC) of dimethyl fumarate**

In the fall of 2006, an epidemic outbreak of severe allergic contact dermatitis following contact with furniture produced in the Peoples Republic of China emerged in several countries in Europe. Intense clinical and analytical work established that it was caused by dimethyl fumarate. Dimethyl fumarate is generally used as a systemic anti psoriasis drug (40, 41). It is also a well known contact allergen together with other fumaric acid esters (42, 43). In the case of “furniture dermatitis” it was used as a volatile anti fungus agent concealed in the finished products such as furniture, textiles and shoes (44-50).

Following the outbreak, the EU Commission assisted by the General Product Safety Directive 2001/95/EC decided to regulate the use of dimethyl fumarate within the EU. The decision banned the use of dimethyl fumarate as a biocide (51). The implementation of this regulation is still so recent that there has not been any investigations on the effect on the number of cases as it has with other recently regulated chemicals e.g. MDBGN.

6.6. **Toys**

Contact allergies in children are frequent. The most important allergens are nickel and fragrances (a comprehensive list of studies in children is offered in (52)).

Although nickel and fragrances are regulated by substance specific directives (EU) or recommendations (IFRA), national and international authorities in safety and health felt it necessary to implement a directive on the safety of toys (52, 53) which addresses, among many other issues, the question of allergenic risks associated with contact to toys. Annex II of Directive 2009/48/EC “Particular safety requirements” contains a list of substances (p. 24-26 substances No 1 55), which are prohibited in toys. The list covers the most important fragrance allergens, such as oak moss, treemoss, isoeugenol, cinnamic aldehyde.

Critical comments

Substances no 41 to 55 (and the 11 to be labelled, see below) shall be allowed in olfactory games, cosmetic kits and gustative games, provided certain conditions are met (see point 12 p. 27). This is highly surprising, as substance no 54 and 55 are oakmoss and tree moss extracts, the most potent sensitizers known.

In addition, 11 substances (“allergenic fragrances”) contained in another list are to be labelled if added at a concentration exceeding 100 mg/kg in the toy. It could be commented that some are probably weak sensitizers (e.g. benzyl benzoate) and others (e.g. farnesol) are clearly more important sensitizers than some of the prohibited ones.

In point 13 of Annex II (p. 27), migration limits of metals from toys are defined, among others for chromium (III) and chromium (VI) salt and nickel. The migration limits from a) dry, brittle, powder-like or pliable toy material, b) from liquid or sticky toy material and c) from scraped-off toy material (all in mg/kg) are for chromium (VI): 0.02, 0.005, and 0.2, respectively, and for nickel 75, 18.8 and 930. It should be mentioned that the limit 930 mg/kg (=930 ppm) is exceeding the limit of 500 ppm (0.05%) of the EU nickel regulation from 1994. Furthermore: A “migration limit” should contain time (e.g. week and exposure area (e.g. cm²), and not an absolute quantity.

7. **Standards**

Standardisation is a method for international cooperation within EU or the global community to increase mutuality for products and trade goods and hence increase and make trade easier. However, it can also be a tool to increase safety and reduce exposure to harmful products, thus a general increase in health and welfare.

- 7.1. The European Committee for Standardization (CEN), which consists of 31 national standardisation institutes, is responsible for the development of standards within the EU. Standardisation is done either on individual national proposals for items of standardisation or it can be mandated by the European Commission.

7.1.1. **Standards on release of nickel and wear of objects in relation to Nickel Directive. EN 1811 and EN 12472**

In relation to the Nickel Directive two standards have been developed. The first (EN 1811) gives specifications for assessment of release of nickel from objects in contact with skin and the second (EN 12472) for simulation of wear and corrosion of objects. The EN 1811 specifies details for the necessary solutions and reference objects for determination of the release of nickel from commercial objects. It states procedures for preparation of extraction solution, i.e. synthetic sweat, and exposure to this solution for possible release and subsequent analytical procedure. The analytical procedure is specified as a spectrometric method. The limit for acceptance is a release equal to or less than $0.5\mu\text{g nickel/cm}^2/\text{week}$ (54). The EN 12472 specifies details for methodology of simulated wear and corrosive environments. It specifies type of container for corrosion exposure and dimensions and form for the required container in which the wear exposure is performed. It also specifies the composition of the corrosive solution and abrasive paste to be used. Suspension of the test objects and length of abrasive exposure is specified (55).

7.1.2. **Standards on protective and medical gloves. EN 374 and EN 455**

Protection against exposure to chemicals and products can be provided in several ways. In the local workplace replacement of dangerous products, technical actions, work organization, and as a last resort, the application of personal protective equipment can be employed. On higher levels structural measures such as legislation against the use of certain chemicals or products is often very effective.

The choice of protective equipment such as protective gloves against chemical exposure or medical gloves against microorganisms needs detailed knowledge about the nature of the exposure and length of work. Protective gloves against chemicals may be impermeable to some chemicals but not to others. To assess this, a series of standards have been developed. The standard gives guide to terminology and performance criteria of gloves and how to test for penetration. It tests for holes in the glove membrane using a water or air leakage test. The third test is for resistance against permeation of chemicals through the membrane. This is a diffusion test where a glove membrane is placed in between two halves of a test cell. The membrane is exposed to a test chemical or product and the resulting diffusion through the membrane is measured. (56 – 58). The break through time will be the basis for a classification of the protective material against the tested chemicals in a 6-grade scale.

The introduction of the standards has generated a higher scientific level for the testing of and selection of protective gloves. However, there is not sufficient

scientific evidence that the use of better protective equipment reduces the incidence of contact allergy to specific allergens. Though there has been an interest to test protective gloves for a number of chemicals, allergenic as well as toxic, ones used in various occupational environments. The earliest tests were done in a non-standardised manner and thus gave variable non-comparable results (59 – 63). After the first efforts to standardise permeation testing in the US during the 1980's the results were more consistent and testing has gone from a method development stage to a routine technique performed by the manufacturer (64 – 66). However, a few reports are still produced in the scientific domain although all are not performed by standardised methods (67 – 74).

Testing by manufacturers has in turn led to greater awareness of the protective effect of gloves and a better knowledge base for proper selection of personal protective equipment (75).

Medical gloves are at the moment subject to a European standard divided into 4 parts. These parts specify the details for tests for quality assurance and biological safety. The first part tests for freedom of holes using a water leakage test and specifies a quality assurance for leak proof medical gloves. The second test describes dimensions of sizes for surgical and examination gloves and how to perform and the acceptable limits for a break force test, ensuring the gloves are intact during normal work procedures. The third part is a description of a method for extraction and analysis of latex proteins in medical gloves. It also states the maximum levels of starch the gloves can hold to be considered powder free. This is to ascertain exposure of medical personnel only to very low doses of allergenic latex proteins on the skin and on mucous membranes. Thirdly it states that production chemicals shall be "As Low as Reasonably Practicable". The fourth part of the standard describes a method for and analysis of results for accelerated ageing to ensuring the right storage time is given for each product (76 – 79).

The increased awareness of blood borne infections such Human Immune Deficiency Virus (HIV) and Hepatitis B (HBV) in the early 1980's increased the use of medical gloves in hospital glove users but also forced large groups of paramedical staff to use gloves. Since natural rubber latex is comparatively cheap, large amounts of gloves were made from this raw material. This led to an increasing number of reports about local or serious systemic adverse reactions caused by latex gloves of this material (80 – 86). Intense scientific work led to the identification of several of the proteins allergens responsible for the local and systemic reactions (87 – 89).

Previously, all medical gloves were powdered with talcum or cornstarch. This led to formation of internal granulomas and peritonitis in patients after surgery and also facilitated the spread of latex allergens to personnel. In the current standard it is stated that medical gloves should be non-powdered for safety reasons.

The introduction of the standard CEN EN 455 has led to a dramatic increase in quality of medical gloves. This has together with local arrangements and changes in glove use reduced the number of new recruited natural rubber latex allergic persons in the staff of the health services (90 – 94). The introduction of the standard has also resulted in a decline in frequency of detected allergic patients for certain production chemicals in the healthcare services but not so obvious in other occupations where protective gloves are not subject to this regulation. However, interchange procedures

at manufacturing gloves may just make this to a shift to other allergenic chemicals (95 – 98).

7.1.3. **ISO standards**

In 2006 the technical report CEN/TR 15278:2006 and the technical specification CEN/TS 15279:2006 were adopted by the CEN organisation following the work of the CEN/TC 137/WG 6 NEN Dermal Exposure working group (99, 100). These documents have their origin in the ‘Conceptual model for skin exposure’ and several methodological development articles and specify terminology and available methods for assessment of skin exposure (101 – 104). This has since been followed by the development of an international ISO technical report which at the moment is in its finishing stage by the technical committee ISO/TC 146/SC 2/WG 8 (105). Since this document is, as the CEN documents, a general specification of available methods, it is planned that it should be followed by documents specifying detailed methods for particularly interesting chemicals giving the possibility to standardise assessments for skin exposure to several contact allergens.

7. 2. **National standards**

7.2.1. **MAK commission**

The official name of the ‘MAK Commission’ is: “Commission for the Investigation of Health Hazards of Chemical Compounds in the Work area” of the Deutsche Forschungsgemeinschaft (DFG). MAK is the acronym for “Maximale Arbeitsplatz-Konzentration” (maximum workplace concentration). The most important practical results of the Commission’s work are scientific recommendations for the establishment of MAK values, for the classification of carcinogenic substances, for the evaluation of embryotoxic and/or foetotoxic effects and of germ cell mutagens, as well as the investigation and evaluation of analytical methods for controlling exposure.

In addition, sensitizing effects of substances encountered at the workplace are evaluated. A subgroup of the commission, the ‘working group skin and allergy’, analyses and evaluates all available published and unpublished data with an impact on the characterization of a chemical as an allergen, resulting in a recommendation to the Commission. The Commission finally decides, if a substance is designated as “sensitizing for the airways ” and as “sensitizing for the skin” with the symbols “Sa” and “Sh”, respectively, in the *List of MAK and BAT Values* (106). The reasons for designation of a given substance with “Sa” or “Sh” are published in a separate document (107).

A similar (but not identical) designation procedure is taken by the EU, which requires that substances and preparations are classified according to the EU criteria (108). If a substance or preparation fulfills these criteria, then it must be designated as a contact sensitizer and assigned the symbol “Xi” (“irritant”) and the risk phrase R43 (may cause sensitization by skin contact) and as a respiratory sensitizer with the symbol “Xn” (harmful) and the risk phrase R42 (may cause sensitization by inhalation). Assignment of the risk phrase R42 does not necessarily require the evidence that the mechanism of action is immunological.

Besides some differences in the evaluation criteria between R-phrases and MAK-designations (109), the reasons for assigning an R-phrase are normally not published.

In order to make the procedure of classification of substances in the *List of MAK and BAT Values* a) more rational, b) more consistent, c) more comprehensible and also d) more transparent for outsiders, criteria for the designation of a substance with “Sh” and “Sa” have been elaborated, published and discussed (110 – 112). The working group considered it necessary to differentiate between

a) the qualities of the evidence for allergenicity of a substance (resulting in graded levels of evidence sufficient or not sufficient for designation) and

b) the algorithm used to decide whether or not a substance is designated as an allergen.

In a first step, the inherent allergenic properties of the substance are examined (“hazard”). In the second step, additional information is considered, namely the amount (duration) of exposure, the range of exposure concentrations, the allergenic potency, co-factors, or potential susceptibility factors. Depending on available information, case-to-case deviation from the rules is possible in this second step when justified (110).

The objective of the designation of substances in the *List of MAK and BAT Values* with “Sa” and “Sh” is the prevention of sensitization and subsequent allergic disease (in particular bronchial asthma and allergic contact dermatitis). Even if a certain substance is not designated with an R-phrase (109), only on the basis of a MAK-designation concrete preventive measures can be arranged for by the inclusion of appropriate recommendations in the “Technische Regeln für Gefahrstoffe“ (TRGS, technical regulations for hazardous substances) which are published at regular intervals in the *Bundesarbeitsblatt*.

While the working group “skin and allergy” will provide categorical ((yes/no) classifications only resulting in designation or not), concrete measures required by the TRGS may comprise substitution of a substance or the reduction of concentration, but also may require compliance with certain thresholds established.

Thus, the whole process is a 2-step procedure: a) designation of a substance with “Sh” or “Sa” in the *List of MAK and BAT Values* (the result of a scientific evaluation) and b) establishment of a TRGS with subsequent concrete measures of prevention.

8. Scientific models

8.1. Quantitative risk assessment (QRA)

Efforts have been conducted recently by the industry in order to assess dermal sensitization risk for fragrance ingredients used in cosmetic products. The International Fragrance Association (IFRA), together with the Research Institute for Fragrance Materials (RIFM), have developed the Dermal Sensitization QRA model, an exposure-based methodological approach to assess the sensitization risk and identify safe concentration limits for fragrance substances (113). The QRA approach deals with the induction phase only. It is constructed

on 3 elements: predicted no-effect levels of sensitization under experimental conditions, safety factors and exposure assessment. The first step of the QRA model is the determination of a No Expected Sensitization Induction Level (NESIL), based on weight of evidence (WoE) built with relevant available data from animal assays, essentially the LLNA, and confirmatory human assays as the HRIPT conducted with an exposure level identified as a no effect level (NOEL). In case animal data is used (LLNA), the use of default values of expected no-effect levels NOELs has been suggested based on the potency of the substance (114). The NESIL is expressed as dose per unit area (i.e. $\mu\text{g}/\text{cm}^2$), as there is evident empirical support for the dose per unit skin area being the crucial determinant of induction and not the total dose (115). In a second step, the NESIL is divided by a set of safety factors (SAFs), aiming to extrapolate from experimental to real life exposure scenarios, to give an Acceptable Exposure Level (AEL). SAFs are based on inter-individual variability (value of 10), vehicle/product matrix effects and use considerations (values ranging from 1 to 10). SAFs values can thus range from 10 to 1000, depending on the differences between the experimental situation and the specific use situation of a cosmetic product. Finally, a Consumer Exposure Level (CEL) to the fragrance ingredient is calculated (expressed as dose/unit area/day) taking account of the frequency and duration of use, practices and amount of cosmetic product used per application/use. At the end, to establish the acceptability of consumer exposure to a fragrance ingredient in a given product, the ratio AEL/CEL is determined. The percent concentration of the fragrance ingredient in a product is acceptable if $\text{AEL} \geq \text{CEL}$. In order to implement the QRA, cosmetic products have been classified into several different categories (116).

IFRA submitted the QRA model to the Scientific Committee on Consumer Products (SCCP), an independent committee providing the European Commission with scientific advice, together with 3 specific cases assessed by the QRA approach: citral, farnesol and phenylacetaldehyde (117). The SCCP was requested to critically review the QRA methodology. In its opinion, the SCCP established several conclusions. First of all, the QRA does not consider the protection of consumers already sensitized to fragrance ingredients. Epidemiological/experimental data on sensitization/elicitation reactions in consumers are not integrated in the QRA model. It is also unclear if and how the model covers the significant part of the population that suffers from skin disease without previous sensitization to fragrance substances. On another hand, the QRA model is based on data from experimental sensitization tests in humans such as the HRIPT, which takes precedence over all other data from other predictive human tests. The SCCP considered that the validity of this test, sensitivity and reliability is sparse outside the industry. The HRIPT is not part of any official test guidelines. Also, no clear guidance exists in the performance of a HRIPT for the safe choice of test concentrations. Furthermore, the predictive sensitization testing in humans was considered unethical to perform. In parallel, even though criteria are given for the SAF assignment, it is still a pragmatic approach and scientific consensus in determining safety factors for skin sensitization is yet to be achieved. For example, a factor of 10 is always assigned for inter-individual variability, which is in accordance with general principles of toxicology. However, in the field of contact dermatitis the inter-individual variability could be higher than 10. Several studies on nickel contact allergy suggest for example that individual susceptibility to nickel sensitization and elicitation is extremely variable (118 – 120). Also, predictive sensitization tests in healthy volunteers have shown a difference of a factor of 8 in susceptibility when DNCB was tested (115). Another example, a matrix SAF of 3 is assigned to very different products such as aerosol antiperspirants, hand wash detergents and baby creams, covering very different matrixes. Concerning the exposure assessment (CEL), the SCCP considered that the fact that several sources are used to

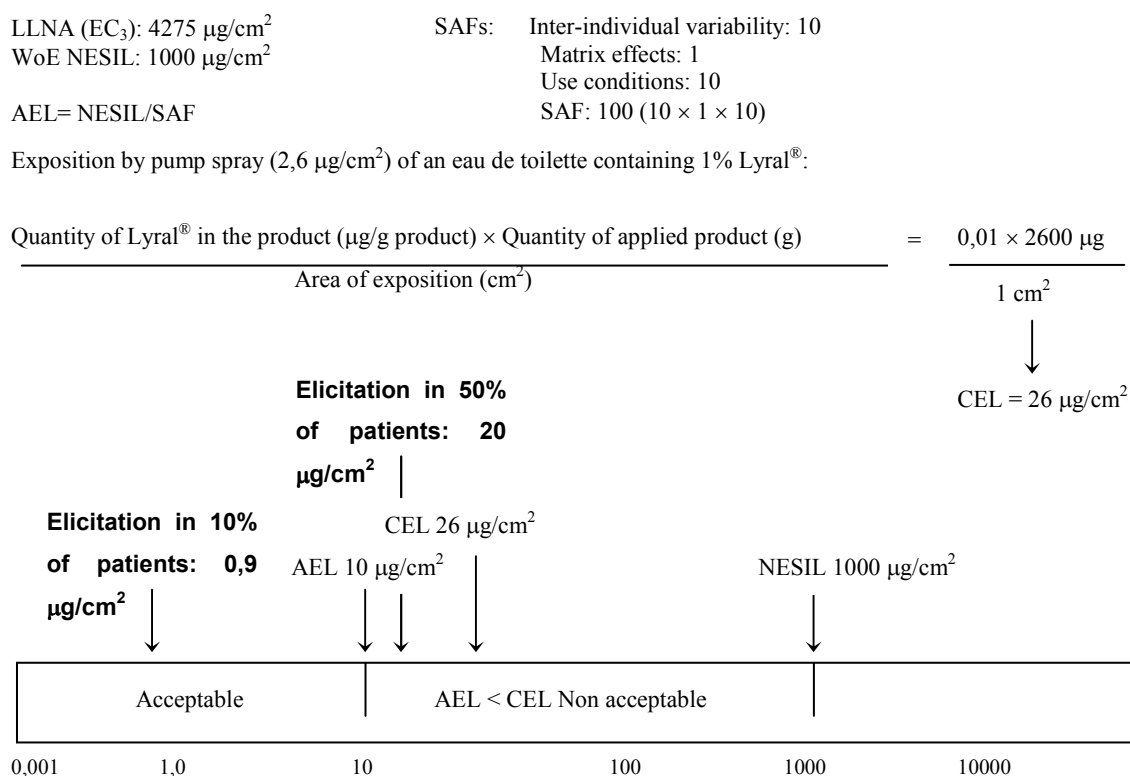
establish the CEL gives significant differences in the estimates. Also, the model operates with multiple product categories but does not consider risk from aggregated exposures (use of several products containing the substance in question), neither from occupational exposure. Moreover, the allergen load of structurally similar substances is not considered. Finally, there is no data to support the proposed AEL scientifically as safe for the consumer. The QRA is a theoretical model and no validation has been done. Under these conclusions, the SCCP stated that models like the QRA need strong refinement and validation, and that an independent post-marketing surveillance system is essential. Aggregated exposures must be included, validation must be performed employing a broad range of different chemicals, data from substantial clinical investigations are needed and scientific consensus must be obtained concerning the choice of SAFs. As an example, Table 4 shows data on the application of the QRA model to the use of citral in two different categories of consumer products (116). Limits based on the QRA will be 0.05% citral in deodorants and 0.6% in hydroalcoholic products for unshaved skin. In liquid soaps 7% citral can be used, 8.2% in shampoos and 100% in baby diapers and hand dish washing (explained by a low estimated exposure). The limits calculated for these wash-off products are changed into maximum pragmatic concentrations of 5% for shampoos, and 2.5% for baby diapers and hand dish washing (116). The SCCP considers that the maximum pragmatic level is identical with the usual concentration of fragrance in a product, which is a blend of fragrance ingredients in the final product. This would mean that citral, as an individual ingredient, cannot exceed the usual concentration of the whole fragrance formula in that product type. The SCCP considered thus not to endorse the proposed QRA approach for setting safe levels of exposure to citral. Similar remarks have been stated for cinnamic aldehyde and isoeugenol (117).

Table 4. Application of the QRA to citral

Citral	Deodorant	Hydroalcoholic product for unshaved skin
LLNA (EC ₃): 1414 µg/cm ²		
NOEL-HRIPT (induction): 1400 µg/cm ²		
LOEL-HRIPT (induction): 3876 µg/cm ²		
WoE NESIL	1400 µg/cm ²	1400 µg/cm ²
Sensitization Assessment Factor (SAF)	300 (10 × 3 × 10)	100 (10 × 3 × 3)
Acceptable Exposure Level (AEL)	4.7 µg/cm ²	14 µg/cm ²
Consumer Exposure Level (CEL)	9.1 mg/cm ² /day	2.2 mg/cm ² /day
AEL/CEL	0.0005	0.0064
Concentration of citral in the product giving AEL≥CEL	≤ 0.05%	≤ 0.64%

Another example of the QRA limits is given by the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde HICC(121), known as Lyr[®]. The risk assessment model for induction of contact allergy, give a sensitization reference dose of 10 µg/cm² in a fine fragrance (Fig. 2).

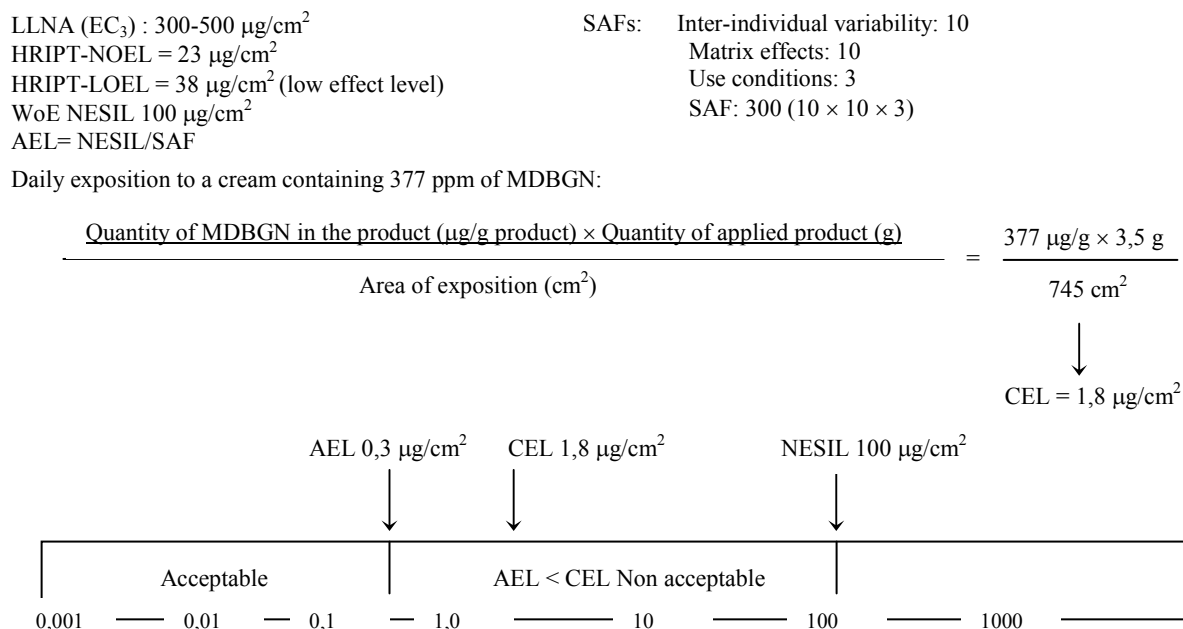
Figure 2. QRA for the use of hydroxyisohexyl-3-cyclohexene carboxaldehyde in an eau de toilette



The dose-response relationship of HICC contact allergy was evaluated with doses relevant for normal exposure in cosmetic products. On patch testing, 10% of the patients studied reacted to around $1 \mu\text{g}/\text{cm}^2$, i. e. a fivefold lower dose than a usual deodorant exposure (the content of 0.1% HICC in a deodorant corresponds to an exposure of $5 \mu\text{g}/\text{cm}^2/\text{application}$) (122), and 50% of the patients elicited at $20 \mu\text{g}/\text{cm}^2$. In order to mimic real-life exposure situations, repeated open application testing (ROAT) was conducted (123). The aim was to identify the sufficiently low concentration of the fragrance compound not causing an allergic reaction in patients with proven sensitization. The results of the study showed that concentrations tolerated by 90% and by 50% of the patients were $< 88.2 \text{ ppm}$ and $< 1791 \text{ ppm}$ in the case of a cream, and $< 270 \text{ ppm}$ and $< 3420 \text{ ppm}$ in the case of a perfume. In other words, 10% thresholds for no response were $1.2 \mu\text{g}/\text{cm}^2$ for the perfume, and $4.9 \mu\text{g}/\text{cm}^2$ for the cream. 50% thresholds were $15.2 \mu\text{g}/\text{cm}^2$ and $99.5 \mu\text{g}/\text{cm}^2$ respectively. The relationship between patch test preparation and ROAT thresholds has been recently studied for HICC (124). Authors of the study concluded that the ROAT threshold in dose per area per application is lower than the patch test threshold, but also that the accumulated ROAT threshold is higher than the patch test threshold.

To conclude, another example showing the limits of the QRA can be given for the use of MDBGN (125) as bactericide in a hand moisturizing cream (Fig. 3). Today, the use of MDBGN has been banned in all cosmetics, including soaps and shampoos. This ban took effect from 22 June 2008. Consequently, from 22 June 2008 it has been prohibited to sell cosmetic products within the EU that contain this bactericide. These products cover, for example, make-up, moisturizing creams, cleansing creams, sun protection creams and personal-hygiene products.

Figure 3. QRA for the use of methyldibromo glutaronitrile (MDBGN) in a hand moisturizing cream



8.2 Thresholds of toxicological concern

Recently it has been proposed to apply a Threshold of Toxicological Concern (TTC) approach to allergic contact dermatitis (126). To the opposite of the QRA model, which is based on a classical toxicological approach (NOAEL/LOAEL modulated by uncertainty factors), the Dermal Sensitization Threshold (DST) is based on a statistical evaluation of the sensitization risk. The TTC approach, which assumes that a human exposure threshold below which there is no appreciable risk to health can be determined, has mainly been used to assess the risk associated with low level of chemicals (additives, impurities, contaminants etc.).

The aim of this approach is to avoid animal testing, arguing that the cost of chemical evaluation should be weighed against the potential risk of sensitization assumed to be low in the case of molecules used at a low concentration in products leading to a low dermal exposure such as rinse-off products.

The DST has been developed based on the European List of Notified Chemical Substances – ELINCS – (127) and published data of LLNA results. From these sources, it was assumed, on the one hand, a 20% incidence of sensitizers in the world of chemicals, and on the other hand, a potency (128) distribution for skin sensitizers ($n = 167$) of 7.7, 12.4, 40.8, 39.1% for extreme, strong, moderate and weak sensitizers, respectively. It should be mentioned that chemicals listed in the LLNA paper probably do not reflect the world of chemicals as some of them have selectively been chosen for the development of the test.

For each chemical a NESIL ($\mu\text{g}/\text{cm}^2$) was calculated according to the mouse/human conversion factor proposed by Basketter et al. (129) from which Acceptable Exposure Limits (AELs) were derived for two categories of products (shampoo and deodorant) according the QRA principles (see point 8.1). Distributions of AELs for the list of sensitizing chemicals (shampoo and deodorant) were plotted on a negative log scale, showing a gamma distribution. Based on this distribution, probabilities that an untested chemical would exceed the AELs at a given dose can be established for either shampoo or deodorant. Assuming 20% of chemicals to be sensitizers, dermal sensitization threshold values of 1.64 and 0.55 $\mu\text{g}/\text{cm}^2$ for shampoo and deodorants respectively, would give a 95% probability of not exceeding the AEL. This means that, with these figures, there is a 5% probability that the sensitization risk for an untested chemical would exceed $1/10^6$ (130). As mentioned by Safford (126) a wider discussion of what is considered to be an acceptable risk is needed.

When comparing AEL values for individual chemicals, based on previously calculated dermal sensitization threshold values (1.64 and 0.55 $\mu\text{g}/\text{cm}^2$ for shampoo and deodorants, respectively) and actual Consumer Exposure Level (CEL), it was found that for 34 over 167 chemicals $\text{CEL} > \text{AEL}$ (Table 5).

Table 5. Examples of major sensitizers for which CEL > AEL

Chemicals	EC ₃ µg/cm ²	AEL/CEL
5-Chloro-2-methyl-4-isothiazolin-3-one	2.25	0.004
Glutaraldehyde	25.0	0.06
1,4-Phenylenediamine	40.0	0.11
Formaldehyde	152.5	0.5
Methyldromo glutaronitrile	225.0	0.8

Obviously, this approach, which has been extensively used for trace chemicals, is not yet fully accurate for cosmetic or household ingredients, which are often used in much higher concentrations. As it can be seen on the few examples listed above, such a DST will set up a threshold of toxicological concern far above the actual one. Several factors can be responsible for such a poor assessment. As already mentioned, while the ELINCS database is composed of chemicals tested for their skin sensitization properties without any selection, the Master Table published by Gerberick et al. is mainly composed of chemicals selected for their already known sensitizing properties (131). It can be also pointed out that the data set used to establish the distribution of sensitization potency is relatively small compared to the ones used for carcinogenicity. It is therefore suggested by the author that this approach could be limited to set a DST for chemicals that are negative in chemical reactivity tests.

Table 6. Examples of major sensitizers for which CEL>AEL, after application of quantitative safety thresholds and QRA

Case example: Dermal sensitization threshold	Applicability in derivation of quantitative safety threshold according to part 1	Safety thresholds used: no effect or low effect	defaults used	modelling of data	safety factors	conclusion
5-Chloro-2-methyl-4-isothiazolin-3-one	YES	No effect	YES	YES	QRA	CEL > AEL
1,4-Phenylenediamine	YES	No effect	YES	YES	QRA	CEL > AEL
Formaldehyde	YES	No effect	YES	YES	QRA	CEL > AEL
MDBGN	YES	No effect	YES	YES	QRA	CEL > AEL
Case example QRA						
MDBGN ¹	YES	No effect	YES	NO	QRA	CEL > AEL
Citral ²	YES	No effect	NO	NO	QRA	CEL > AEL
Hydroxyisohexyl-3-cyclohexene carboxaldehyde ³	YES	-	YES	NO	QRA	CEL > AEL

¹ Figure 3: exposure to a hand moisturizing cream

² Table 4: exposure to deodorant and hydroalcoholic product for unshaved skin

³ Figure 2: exposure to an eau de toilette. The ratio AEL/CEL may depend on the category of the cosmetic product considered

8.3 Scientific data on experimental dose-response models

Toxicologists, dermatologists and researchers have repeatedly performed experimental sensitization and elicitation studies for prevalent contact allergens that have resulted in contact allergy epidemics. Below is a presentation of studies that have addressed the sensitizing potential of important allergens including chromium, nickel, formaldehyde, PPD, isothiazolinones and MDBGN, as well as their elicitation thresholds.

8.3.1 Chromium:

Sensitization

Kligman et al. showed that repeated application of 2% potassium dichromate sensitized all 23 subjects in a human maximization test (24).

Dose-response

Allenby and Goodwin showed that 1 of 14 chromium allergic patients reacted to about 1 ppm chromium under occlusion (132). Basketter et al. showed that on normal skin, the patch test threshold was 10 ppm when 17 chromium allergic patients were patch tested. In the presence of an irritant, sodium lauryl sulphate, the threshold level was 1 ppm in 2 of 17 patients (133). Irritants are of relevance as wet cement has a high pH of 12.5, which may

alter the *stratum corneum* of the skin resulting in inflammation and in later stages penetration of chromium ions. Utilizing a log-probit analysis of 9 patch test studies that evaluated allergic contact dermatitis elicitation thresholds for hexavalent chromium at different pH levels, Stern et al. estimated a threshold for elicitation of allergic reactions to be 15 ppm of hexavalent chromium for 10% of the population and 7.6 ppm for 5% of the sensitized population (134). A more recent review on chromium allergy gathered published dose-response patch test studies (135). Exposure to occluded patch test concentrations of 7-45 ppm hexavalent chromium elicited allergic contact dermatitis in 10% of chromium allergic patients. The collection of repeated open exposure studies showed that either exposure to 5 ppm hexavalent chromium in the presence of 1% sodium lauryl sulfate (SLS) or exposure to 10 ppm hexavalent chromium alone elicited allergic contact dermatitis in chromium allergic patients. The eliciting capacity of trivalent chromium has not been systematically investigated but, compared to hexavalent chromium, much higher concentrations are needed to elicit allergic chromium dermatitis (135).

Exposure stay-on

Based upon a review by Shelnutt et al., a chromium immersion study including 26 chromium allergic subjects performed by Fowler et al. incorrectly concluded that exposure to 25 ppm hexavalent chromium in the environment does not pose an allergic contact dermatitis hazard to chromium sensitized persons (136, 137). Shelnutt et al. dissected the study and concluded that at least 10 of 26 (38%) of individuals exposed to 25 ppm hexavalent chromium developed dermatitis consistent with allergic contact dermatitis (136). Basketter et al. performed repeated open application testing (ROAT) with aqueous solutions of potassium dichromate containing 1% SLS and potassium dichromate in the concentration range 5–50 ppm (133). The respective solutions were applied to the antecubital fossa twice daily for 1 week and 20% had allergic reactions to 5 ppm. Nielsen et al. exposed the fingers of 3 chromium allergic patients to chromate concentrations of, respectively, 10 and 100 mg chromium/l during week 1 and 2 by immersion of one finger into a chromium containing solution 10 minutes per day (138). They showed that all 3 patients had a flare of hand eczema.

8.3.2 Nickel:

Sensitization

Kligman et al. showed that repeated application of 10% nickel sulfate sensitized 12 of 25 subjects in a human maximization test (24). Räsänen et al. observed 9 women with no prior symptoms suggestive of nickel dermatitis following ear-piercing (120): 6 developed itching, swelling or discharge on the earlobes, which was sufficient to terminate the use of earrings. They all displayed positive nickel patch test reactions and had their earrings analyzed for nickel release. Higher nickel concentrations were found in plasma (in $\mu\text{g}/\text{cm}^2/\text{week}$: 0.15, 0.17, 2.03, 10.06, 22.44, and 104.59) than in distilled water (in $\mu\text{g}/\text{cm}^2/\text{week}$: range 0.02–0.79). These data suggested that there is wide variation in susceptibility to nickel sensitization. Larsen and Brandrup found that nickel release was 100-1000 times higher from buttons suspected of inducing primary nickel allergy when compared to buttons eliciting nickel dermatitis in already nickel allergic patients (139).

Dose-response

Fischer et al. have extensively reviewed nickel dose-response studies (140). The authors identified 8 occluded nickel dose-response studies. Statistical analysis showed that 5% of a sensitized population reacted to $0.44 \mu\text{g}$ nickel/ cm^2 and that 10% reacted to $1.04 \mu\text{g}$ nickel/ cm^2 . In another study with a single open application, 7.8% of sensitized subjects

responded to a dose 6 times higher than the dose to which 10% reacted in occluded exposure. Concomitant nickel and irritant exposure resulted in a diverging outcome, although the literature shows evidence of an augmented response when combining exposure to an allergen and an irritant. The thresholds of penetrating exposure were found to be lower than the thresholds of single occluded exposure (140).

Exposure stay-on

One study performed the ROAT using nickel sulfate in nickel allergic patients and controls (141). Thus, 20 nickel-allergic patients underwent patch testing with a dilution series of 19 concentrations at the same time as ROAT with a dilution series of 3 concentrations, with duration of up to 21 days. The predicted dose that would elicit allergic contact dermatitis in 10% of nickel allergic individuals was calculated to be 0.78 µg nickel/cm² in the patch test. The threshold for the ROAT (in µg nickel/cm² per application) was significantly lower than the threshold for the patch test, while the dose-response for the accumulated ROAT dose at 1 week, 2 weeks and 3 weeks was very similar to the patch test dose-response. The same group has also shown that for the elicitation of allergic nickel dermatitis, the size of the exposed area and therefore the total amount of applied nickel, influenced the elicitation reaction at some concentrations, even though the same dose per unit area is applied (142).

8.3.3 Formaldehyde:

Sensitization

Kligman et al. showed that repeated application of 5% formaldehyde sensitized 18 of 25 subjects in a human maximization test (24). Marzulli and Maibach showed that 0 of 45 healthy volunteers were sensitized to 0.1% formaldehyde whereas 4 of 89 subjects were sensitized to 1% formaldehyde (20). Lee et al. investigated the sensitizing capacity of formaldehyde in guinea pigs and showed a linear relationship between formaldehyde dose and the proportion of animals that reacted (143). Other animal studies have also shown that formaldehyde is a strong sensitizer (144).

Dose-response in elicitation

De Groot et al. performed serial dilution patch testing in formaldehyde allergic patients and showed that 8 of 35 patients reacted to 100 ppm, the lowest concentration in the dilution series (145). Fischer et al. showed that 5 of 22 formaldehyde allergic patients reacted to 630 ppm formaldehyde when serial dilution patch tested (146); the lowest concentration tested was 0.015% (150 ppm) to which only one patient reacted. Flyvholm et al. investigated the elicitation threshold concentration for formaldehyde in formaldehyde allergic patients (147). They performed occluded and open serial dilution patch testing (25-10.000 ppm) in 20 formaldehyde allergic patients and in 20 healthy volunteers. 1 patient reacted to 250 ppm formaldehyde in the occluded dilution patch test series (the next concentration was 50 ppm), 2 patients reacted to 500 ppm and 3 patients reacted to 1000 ppm. Retesting in the subject that reacted to 250 ppm (1 year later) revealed no positive response to 50, 100 or 250 ppm formaldehyde. No positive reactions were observed in the open patch test series. Isaksson et al. performed dilution patch testing with formaldehyde in 7 formaldehyde allergic patients and showed that 3 of 7 reacted to 0.125% (1250 ppm) formaldehyde and 1 reacted to 0.0312% (312 ppm) formaldehyde (148).

Exposure stay-on

Horsfall pioneered the investigation on formaldehyde exposure in 1934 (149). He showed that a formaldehyde allergic patient reacted to an intradermal injection with 1:8 x 10⁶ dilution of a 35% formalin solution whereas controls did not respond to a 1:10³ dilution.

Marzulli and Maibach showed that respectively, 2, 4 and 5 of 10 formaldehyde allergic volunteers reacted to 0.6%, 0.4% and 0.5% formaldehyde in a semi-use test (20). Later, Jordan et al. performed a double blind controlled study on 16 formaldehyde allergic patients (150). First, 4 patch tests (0, 30, 60, 100 ppm) were repeatedly applied for 1 week in 9 patients. The closed patch test method revealed positive reactions to 30 ppm formaldehyde in 4 patients. Then, 2 creams containing 0.1% formaldehyde were repeatedly applied in a similar manner. Results corresponded with those observed in the closed patch test series for 60 and 100 ppm formaldehyde. Finally, a repeated axillary spray test was performed with 28.86 ppm formaldehyde in one axilla twice a day for 2 weeks and with a control vehicle in the other axilla. Among 13 patients, 2 developed dermatitis to 30 ppm formaldehyde. The authors concluded that most formaldehyde allergic subjects might tolerate formaldehyde concentrations below 30 ppm (150). Flyvholm et al. briefly discussed unpublished data by Maibach and Franz who found that 80 ppm formaldehyde in an antiperspirant gave “no response” in a provocative use test study (151). Flyvholm et al. performed the ROAT (300 ppm formaldehyde) for 1 week in 20 formaldehyde allergic patients and in 20 healthy controls and found mild allergic reactions in 5 of 20 patients (147).

Exposure studies using formaldehyde releasers have also been performed in formaldehyde allergic patients. Isaksson et al. exposed 7 formaldehyde allergic patients with dermatitis and 17 controls to respectively, a corticosteroid cream containing 175 ppm free formaldehyde released from imidazolidinyl urea and a corticosteroid cream without formaldehyde in a double-blind fashion (152). They showed that dermatitis in formaldehyde allergic subjects healed significantly less than in controls. De Groot et al. performed a ROAT in 12 formaldehyde allergic patients with 1% DMDM hydantoin (145). 4 patients reacted and were then exposed to 0.25% DMDM hydantoin (containing approximately 200 ppm free formaldehyde) in a ROAT and 1 reacted with mild dermatitis. Zachariae et al. performed an experimental ROAT study in 30 formaldehyde allergic patients (153). They were exposed to a cream preserved with diazolidinyl urea (0.05%, 0.15%, 0.3% and 0.6% containing respectively, 130, 370, 730 and 1500 ppm free formaldehyde). Exposure was initially performed for 2 weeks in the antecubital fossa and in case of negative outcome; exposure was instead performed on the neck and face. 2 patients reacted to 0.15% diazolidinyl urea (370 ppm free formaldehyde) and 7 reacted to 0.3% (730 ppm free formaldehyde). Of interest, none reacted following exposure in the antecubital fossa but only following exposure on the neck or face.

There is a convincing accumulation of scientific data to support that the current concentration limit of free formaldehyde in cosmetic products is insufficient and may result in allergic contact dermatitis in sensitized subjects as well as de novo sensitization.

8.3.4 **p-Phenylenediamine (PPD):**

Sensitization

Kligman et al. showed that repeated application of 10% PPD sensitized all 24 subjects in a human maximization test (24). Marzulli and Maibach performed a PPD sensitization study in healthy volunteers and showed 7.2% of 97 healthy volunteers were sensitized to 0.01% PPD, 11.2% to 0.1% PPD and 52% to 1% (10.000 ppm) PPD (20). Basketter et al. performed the HRIPT with 1% PPD in 98 healthy subjects without PPD allergy and showed that 3 reacted when later exposed to 1% PPD (154, 155). In further studies, the authors exposed healthy Thais without PPD allergy to respectively, hair colorant containing 0.5% PPD (n=1107) (group 1), permanent hair dye containing 1.5% PPD (n=548) (group 2) and no hair colouring product (n=516) (group 3). Subjects in group 1 used hair dye products 5 min/day the first 4

days and then once per week thereafter (still 5 min/exposure). Subjects in group 2 used permanent hair dye (approximately 30-40 min of exposure) once per month (total 6 exposures). Group 3 was unexposed but they could theoretically have been exposed to hair dyes due to non-compliance. At the end of the 6-month period, 1% PPD patch testing was performed. It revealed that the prevalence of PPD allergy was 7.2% in group 1, 1.3% in group 2 and 0.4% in group 3. Approximately 1/3 was also positive to an open patch test with 1% PPD. These data indicated that repeated short-time exposure to hair dyes with a low concentration of PPD increased the risk of PPD sensitization more than prolonged exposure to a higher concentration of PPD but with a longer time interval (154). In further studies, White et al. showed that when 23 PPD allergic patients were exposed to 0.3% and 0.03% PPD for 5 min at the same site every day for up to 8 days and additionally were exposed to single PPD exposures at different sites from 5 to 40 min, 7 patients reacted to the cumulative exposure to 0.3% PPD whereas no one reacted to 0.03% PPD (neither following repeated or single exposure) (156). The authors found a positive correlation between grade of PPD reactivity prior to study start and the strength of reactivity following exposure. They showed that PPD accumulates in the tape stripped rat skin and that intermittent exposure to lower concentrations of PPD may be equivalent to higher concentrations in their animal model (156). Investigators have shown that a PPD concentration of 0.1-0.25% results in induction when performing the LLNA (157 – 159). Finally, a recent Danish study using the LLNA showed that PPD containing hair dye, but not an oxidizer alone, resulted in profound skin inflammation and systemic release of interleukin-6 (160). This study underscored that PPD can be a potent and rapid immune activator. However, in further studies, the authors propose that tolerance induction after repeated hair dyeing may explain why the majority of individuals tolerate hair dyeing with PPD containing hair products without developing allergic reactions (161).

Dose-response

Krasteva et al. elicited allergic contact dermatitis in 100% of 30 PPD allergic patients that were open patch tested on the retroauricular area with 1.8% PPD (162). Later, the same group of investigators performed similar open patch testing in 34 PPD allergic patients with 0.1% PPD and found that 27 reacted (25). Søsted et al. performed occluded serial dilution patch testing in 15 PPD allergic patients on the back, outer aspects of the arms and behind the ears (163). They showed that 1 patient reacted to 50 ppm on the back, arm and behind the ear and that 2 patients reacted to 100 ppm on the back and outer aspects of the arm and 3 patients to 100 ppm behind the ears. The threshold value that elicited allergic contact dermatitis in 10% (ED₁₀) of the patients was 38 ppm on the back, 56 ppm on the upper arm and 75 ppm on retroauricular region.

McFadden et al. performed patch testing in 16 PPD allergic patients to investigate the elicitation response over time (164). 7 patients were patch tested with 1% PPD for 15 min, 30 min and for 120 min. The remaining 9 patients were patch tested with 1%, 0.3%, 0.1% and 0.01% (100 ppm) PPD for 15 min, 30 min and for 120 min each. With exposure for 120 min, 11 of 16 subjects reacted to 1% PPD and 2 of 9 reacted to 0.01%. With exposure of 15 min, 6 of 16 reacted to 1% PPD and 0 of 9 reacted to 0.01% PPD. The study concluded that prolonged exposure and high exposure concentrations increase the risk of elicitation. Jowsey et al. showed that when PPD allergic subjects were patch tested with a permanent hair dye product containing 0.5% PPD for respectively, 30 min, 1 h and 24 h, positive reactions were only observed after 30 min in subjects who had 2+ or 3+ patch test reactions to 1 PPD prior to the study (165). Xie et al. showed that 0.1% PPD resulted in dermatitis in 6 of 6 mice when performing the LLNA whereas 0.01% PPD resulted in dermatitis in 5 of 6 animals (158).

Exposure rinse-off

Hextall et al. performed a ROAT in 18 PPD allergic patients. Application of 0.2 mL of 1% PPD in petrolatum was performed daily to the antecubital fossa for up to 8 days; each application was rubbed onto the skin for 1 min, then left for a further 4 min; excess material was then wiped away (166). After 8 days, 39% of the patients had reacted to the ROAT. It was concluded that the number of applications matters when evaluating the risk of elicitation.

There is a convincing accumulation of scientific data to support that the current concentration limit of PPD in hair dyes is too high and may result in allergic contact dermatitis in sensitized subjects as well as de novo sensitization.

8.3.5 Isothiazolinones:**Sensitization**

According to a personal communication between Chan and Beuthe in 1982, no delayed type hypersensitivity reactions could be observed in guinea pigs at induction and elicitation concentrations reaching 1500 ppm (application 1/week for 3 weeks) (167). Furthermore, a personal communication with Parson revealed that when MI alone was applied in guinea pigs, a dose of 16000 ppm (16%) resulted in dermatitis reactions whereas 1600 ppm (1.6%) gave no response (167). Chan et al. investigated the relationship between allergic contact dermatitis and different induction/elicitation concentrations. They used a modified Buehler's occluded epicutaneous patch technique in outbred Hartley guinea pigs. Groups of guinea pigs received 9 induction doses of Kathon®, 3 times a week, at concentrations ranging from 25-2000 ppm. They were then challenged with the biocide at concentrations ranging from 20-2000 ppm and the application sites were scored for erythema 24 and 48 h after the challenge. They showed that the incidence of delayed contact dermatitis in induced guinea pigs was dependent on both the induction and challenge concentrations. Thus, 20 of 20 animals reacted to 2000 ppm MCI/MI when previously sensitized to a dose of 2000 ppm. One animal that was sensitized to 100 ppm also reacted to 100 ppm, the lowest challenge dose that gave a reaction. However, one animal that was sensitized to 2000 ppm MCI/MI reacted to 25 ppm upon challenge (167). Hausen et al. used a modified Freund's complete adjuvant (FCA) method to determine the sensitizing potency of Kathon® CG (168). He showed that the mean response was 1.57 when compared to 0.27 for MDBGN. Botham et al. showed that the sensitizing potential of MCI/MI was significantly stronger than for 2 other common isothiazolinones by using the LLNA (169). The authors stated that lowest dose of MCI/MI that gave proliferation was equivalent with 100 ppm. Their findings were confirmed in a later combined LLNA and HRIPT study (170).

Dose-response

Weaver et al. performed dose-response patch testing in MCI/MI sensitized volunteers and showed that 1 of 9 subjects reacted to 25 ppm MCI/MI whereas no one reacted to concentrations down to 1 ppm (171). Björkner et al. found that 2 of 34 MCI/MI allergic patients reacted to 10 ppm MCI/MI upon patch testing (172).

Exposure stay-on

Björkner et al. performed use testing in the antecubital fossa with 15 ppm MCI/MI and showed that 54% of 13 MCI/MI sensitized patients developed contact dermatitis (172). Cardin et al. performed the HRIPT in 1540 volunteers (173). Induction was made with MCI/MI 3 times weekly for a 3-week period on the upper arm. 2 weeks later, challenge and

2 episodes of re-challenge (second re-challenge was done with 100 ppm MCI/MI) were made in duplicate on both upper arms. No sensitization was induced at 5, 6 or 10 ppm in 1121 subjects or at 15 ppm in 200 subjects. 1 of 84 subjects reacted to 12.5 ppm MCI/MI. Schwartz et al. performed double blind studies in healthy volunteers in which subjects were exposed daily to either 15 ppm MCI/MI in a lotion or to a placebo lotion over a 13-week period (174). No evidence of dermatitis was found over the test period. Following exposure, subjects were evaluated for de novo MCI/MI allergy by means of 0.1% MCI/MI patch testing but no positive reactions were found.

Hjort and Roed performed use testing in 11 MCI/MI sensitized patients (175). They were instructed to apply a skin lotion with 8.6 ppm MCI/MI daily in one elbow flexure and a similar lotion but without MCI/MI in the other elbow flexure for 1 week. No positive reactions were identified. Meneghini et al. performed a similar use test in 20 MCI/MI allergic patients although the MCI/MI content was 15 ppm in the lotions (176). They showed that 8 of 20 patients developed allergic contact dermatitis to the MCI/MI containing lotion. Frosch and Schulze-Dirks performed the ROAT in 7 MCI/MI allergic patients and showed that 4 (57%) reacted to 15 ppm MCI/MI preserved lotion and that 3 (42%) reacted to 9 ppm MCI/MI preserved lotion (177). Hannuksela et al. showed that 5 of 10 MCI/MI allergic patients reacted to 7 ppm MCI/MI in a ROAT (178). Marks et al. performed a double-blind provocative study in which 10 MCI/MI allergic patients were exposed to a skin lotion containing either 15 ppm MCI/MI or a combination of parabens and DMDM hydantoin (179). 4 patients did not react to any of the lotions, 5 reacted to the MCI/MI lotion and 1 reacted to the control lotion. Furthermore, a European multi-centre study including 101 MCI/MI allergic patients was designed in a double-blind placebo manner to investigate the reactivity to 15 ppm MCI/MI in a lotion vs. a lotion without MCI/MI (180). Patients were instructed to apply the lotions twice daily for 1 week and 31% reacted to the MCI/MI containing lotion, 3% to the placebo lotion and 5% to both lotions. Finally, Zachariae et al., performed a double blind, placebo controlled dose-response ROAT study in 25 MCI/MI allergic patients and 10 healthy controls (181). As opposed to previous studies, the investigators expressed exposure in dose/unit area rather than as weight/volume (% or ppm). They showed that 7 of 25 patients reacted to 0.025 $\mu\text{g}/\text{cm}^2$ (2 ppm) MCI/MI and that no lower elicitation threshold could be established. The accumulation of patients based data show that all exposure to MCI/MI containing leave on products may pose a risk of eliciting dermatitis in sensitized subjects.

Exposure rinse-off

Few rinse-off exposure studies and case reports have been published. Weaver et al. performed a 3-6 week provocative use test with different rinse-off cosmetic products containing 4-6 ppm MCI/MI. No dermatitis reactions were found in 18 healthy volunteers (171). Bruze et al. reported MCI/MI dermatitis on the hands and forearms following the use of a cleansing cream containing 10 ppm MCI/MI in a worker that had used the cleansing cream up to 5 times a day for 2 years. A controlled use test with the MCI/MI-containing cleansing cream elicited skin reactions (182). Another study revealed that when 4 volunteers were experimentally sensitized to a shampoo preserved with 25 ppm MCI/MI, all developed a reaction to the product (183). Frosch et al. performed a randomized multicentre, double-blind, 2 period cross-over study (184). 27 MCI/MI sensitized subjects were exposed to two different shampoos containing respectively, 15 ppm MCI/MI and imidazolidinyl urea. They found that most subjects tolerated the shampoos without developing dermatitis. Also, they showed that no difference could be identified in the frequency of dermatitis following use of MCI/MI or imidazolidinyl urea preserved shampoo. The accumulation of data shows that if

MCI/MI is used in rinse-off products in a concentration < 7.5 ppm it is considered safe for the vast majority of subjects.

8.3.6 **Methyldibromo glutaronitrile:**

Sensitization

In 1983, Mathias reported allergic contact dermatitis to MDBGN in a maintenance worker who handled paste glues (185). According to the manufacturer, toxicology data indicated that MDBGN was a moderate skin irritant and that a modified Draize test in albino rabbits produced a mean score of 4.46 (185). Furthermore, a HRIPT utilizing 3% (3000 ppm) MDBGN in corn oil failed to sensitize any of 52 volunteers following 12 daily applications over a 3-week period. Bruze et al. used the guinea pig maximization test (GPMT) to evaluate the sensitizing potential of MDBGN and concluded that despite MDBGN did not sensitize the animals, one could not rule out that MDBGN had a sensitizing capacity (186). Hausen challenged the Swedish study as he claimed that the modified Freund's complete adjuvant (FCA) method was more sensitive than the GPMT (187). Hausen showed that Euxyl K400® possessed a distinct but weak sensitizing potency. Thus, half of the guinea pigs were sensitized to 3% Euxyl K400® and 7 of 10 animals were sensitized to 0.3% MDBGN. Wahlkvist et al. reflected on the increasing prevalence of MDBGN allergy despite previous investigations had shown that MDBGN was a weak allergen (188). They studied the allergenicity of MDBGN and Euxyl K400® by using 3 different animal models for predictive testing: the LLNA in mice, the GPMT and the cumulative contact enhancement test (CCET) using a dose-response protocol in guinea pigs. They found a few positive reactions to 1% MDBGN in the GPMT but no statistically significant results. However, the CCET and the LLNA showed that MDBGN had an allergenic potential. The authors concluded that investigators should use a variety of predictive test models for the investigation of contact allergens and that MDBGN allergy typically require multiple topical applications (188).

Exposure stay-on

Tosti et al. performed a double-sided provocative use-test in 11 Euxyl K400® allergic patients (189). Subjects applied a lotion containing 0.1% Euxyl K400® and a similar lotion but without Euxyl K400® in the antecubital fossa twice daily. They showed that 5 patients reacted with dermatitis after 5 days in the fossa challenged with Euxyl K400® containing lotion. In 2002, the Scientific Committee on Cosmetic Products and Non-food Products (SCCP) of the European Commission recommended that the use of MDBGN in leave on products should be prohibited and that the use of MDBGN should be restricted to rinse-off products. In September 2003, the EU Commission adopted Commission Directive 2003/83/EC and banned MDBGN in cosmetic leave on products marketed within the EU (31). This was a response to the increasing prevalence of MDBGN allergy in European member states (190). Gruvberger et al. showed that 18 of 51 patients with doubtful or positive patch test reactions to MDBGN reacted to a ROAT using 0.03% MDBGN (191). In 2005, Schnuch et al., attempted to define a maximum non-eliciting concentration of MDBGN in leave on products in 39 patients (192). They performed a ROAT with 3 concentrations of MDBGN and phenoxyethanol (50, 100 and 250 ppm). However, 33% reacted to 50 ppm (0.005%) and they concluded that no safe-limit could be defined. Finally, Kynemund-Pedersen et al. performed a ROAT study in 18 volunteers with MDBGN allergy and in 10 healthy controls (193). They confirmed that 50 ppm MDBGN elicited allergic contact dermatitis in sensitized subjects.

Exposure rinse-off

The MDBGN epidemic did not level off and clinical cases with dermatitis following exposure to MDBGN in rinse-off products were continuously reported (125). Jensen et al. set out to further investigate the risk of MDBGN exposure from rinse-off products by performing a double-blind randomized ROAT study using two coded liquid soaps with and without 0.1% MDBGN in 19 MDBGN allergic individuals and 9 controls (194). Soaps were used twice a day for up to 34 days on the lower arms and 37% of MDBGN allergic subjects developed allergic contact dermatitis. In 2005, the SCCP recommended that MDBGN should be prohibited in all rinse-off cosmetic products (32). The industry, later suggested that the use concentration in rinse-off products should be lowered from 0.1% to 0.02% based on a small study (195). Tosti et al. claimed that MDBGN in rinse-off products rarely produced allergic contact dermatitis and therefore challenged 12 MDBGN allergic patients with shampoo containing 0.02% MDBGN 3 times per week for a total of 9-13 weeks (195). None developed allergic contact dermatitis and the authors concluded that 0.02% was a safe use-concentration in rinse-off products. To strengthen their argument, they calculated that cutaneous exposure to 0.02% MDBGN in a rinse-off product was 7500 times lower than the concentration used for MDBGN patch testing. The SCCP concluded that no safe-level had been demonstrated so far. Recently, Heratizadeh et al. set out to identify a maximum non-eliciting concentration for MDBGN containing rinse-off products in MDBGN sensitized patients (196). 37 patients performed a ROAT by using soap twice daily for up to 4 weeks. Initially the use concentration was 50 ppm but if no reaction occurred patients were instructed to use soap with first 200 ppm MDBGN and later 400 ppm MDBGN (if no reaction was observed to 200 ppm MDBGN). They showed that 1 patient reacted to 50 ppm, 3 to 200 ppm and 1 to 400 ppm. However, up to the highest concentration of 400ppm, 32/37 (86.5%) did not react. Therefore the authors concluded that a use concentration of 50 ppm would be safe for most sensitized subjects, and new sensitization through this concentration would be highly improbable.

8.4 Contact allergy epidemics and unacceptable exposures

Contact allergy epidemics observed over the 20th century were recently debated (3). It appeared that these epidemics had several common features (Table 6) (3). Thus, allergic contact dermatitis to a given chemical is firstly described among workers and later among consumers. Once the epidemic is established, essentially substantiated through many cases observed in multicentre studies and surveillance networks, it tends to be long-lasting as allergens persist in consumer products for decades (3). Thus, when consumer cases are reported in the medical literature, one should suspect that many subjects in the general population are already sensitized and that morbidity may increase unless something radical is done. The control of contact allergy epidemics has traditionally been achieved through communication between toxicologists, dermatologists, and administrators. Generally, public and industrial interference is negligible and rarely affect the course of an epidemic. Finally, European governments have traditionally been more motivated to regulate contact allergy epidemics than governments on other continents (3).

A categorization of contact allergy epidemics was also recently suggested (197) (Table 7). When more than 1/20 subjects in the general population are sensitized, an epidemic should be categorized as an “outbreak”. Thus, nickel may be regarded as an allergen that has caused (and still is causing) an outbreak (197). The suggested categories may serve as useful tool to detect and monitor future contact allergy epidemics (Table 7).

Table 6. Characteristics of contact allergy epidemics (3)

Initial cases of allergic contact dermatitis are detected among workers
Once consumer cases are reported and substantiated through many cases observed in multicentre studies and surveillance networks, the epidemic is already accelerating
The control of an epidemic is time consuming and requires that toxicologists, dermatologists and politicians communicate with each other
Industrial and public interference is usually scarce
Regulations seem mainly to be a European measure to fight epidemics

A discussion on acceptable exposure to contact allergens is closely tied to a discussion on acceptable risk of contact sensitization. Before an attempt is made to define acceptable risk of sensitization, it is important to emphasize the difference between sensitization and elicitation. Thus, sensitization is an asymptomatic condition defined by immunological alertness in an individual following repeated or prolonged skin contact with a given allergen. Re-exposure to the allergen in sufficient concentrations will in most cases result in allergic contact dermatitis, the elicitation phase defined by dermatitis reactions. Thus, a person can be contact allergic (defined by a positive patch test reaction to the allergen) without knowing it and without ever having experienced any symptoms such as redness, itch or dermatitis. In line with this, dermatologists sometimes find it difficult to establish relevance of positive patch test reactions. This can be due to 1) patient recall bias, 2) cross-reactivity to an allergen that has not been included in the test battery, 3) or simply that the patient has been sensitized but never experienced dermatitis following exposure. Thus, as such, it can be argued that the prevalence of contact sensitization is of minor relevance when discussing public health whereas the prevalence of allergic contact dermatitis is of much more relevance. However, this is a very dangerous and misleading interpretation. There are several arguments. First, general population studies have repeatedly shown that contact allergy is strongly associated with self-reported allergic contact dermatitis and hand eczema for allergens such as nickel and fragrances (198, 199). Thus, despite some scientists have raised concern about false positive reactions in patch test studies from the general population, the proportion is suspected to be very small. Second, the strength of patch test reactivity is linearly associated with self-reported allergic contact dermatitis, emphasizing that contact sensitization leads to allergic contact dermatitis. Third, the threshold level of contact sensitization is higher than the threshold level for elicitation of allergic contact dermatitis. This means that once a proportion of the general population is contact allergic, this subgroup is at special risk of developing allergic contact dermatitis as well as hand eczema following re-exposure. Fourth, some allergens are very potent and result in concomitant sensitization and elicitation, e.g.

PPD in temporary henna tattoos. Taken together, it is wise to keep the number of contact allergic subjects low since this is the best way to prevent disease and related health care costs. Also, it is very difficult to measure the prevalence of allergic contact dermatitis, as it may be confused with other conditions.

There are most certainly many reports about the level of acceptable exposure and thereby the acceptable risk of sensitization in the general population. In an ideal world, most might agree that no more than 1/1 000 000 subjects should be contact sensitized by an ingredient. However, in the real world it may be difficult to reach consensus about a limit as contact allergy constitutes a different type of hazard than e.g. cancer and since politics is often based on individual reports. Thus, determination of the acceptable level of exposure, sensitization and elicitation is definitely political, and regulators based on hard evidence provided by researchers, dermatologists and epidemiologists should hence make decisions. In general, it should be remembered that contact allergy is preventable, and furthermore that this condition causes morbidity and high costs to society. Different measures have been used over the years to reduce the contact allergy problem (Table 8). Despite prohibition of a contact allergen is the only way to totally remove the contact allergy problem, it may not be warranted in many cases. Based on exposure studies performed in contact allergic patients with dermatitis, allergen concentrations in consumer products should be limited so that the vast majority of patients are protected from developing dermatitis following exposure. It may be an illusion to protect all subjects so this have only be sought when an allergen have resulted in widespread problems and/or very severe clinical symptoms as seen with dimethyl fumarate and MDBGN.

Table 7. Categorization of contact allergy epidemic (197)

Number of contact allergic 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	-

Table 8. Measures to prevent contact allergy epidemics

Methods:
Early detection of an epidemic of CA through continuous surveillance (eg. ESSCA or IVDK) and ad hoc multicentre studies
Prohibition or voluntary withdrawal from the market
Limitation (of the use concentration or use permitted only in specific types of products)
Information campaigns
Mandatory labelling of consumer products

Conclusion and discussion part II

In vitro and *vivo* methods used to identify the inherent sensitizing potential of chemicals have been described and evaluated. Most methods are well established and have been in use for decades whereas others, particularly *in vitro* methods, are still under development. The process seems to be dynamic and it is foreseen that new cellular methods will be developed and applied in the near future. The way safety evaluations of the contact sensitization potential of new chemicals is addressed by the industry is not known in detail and different strategies are probably used, depending on tradition, experience and available technologies.

Based on pure economic considerations, the first logical steps for the industry would be to use *in vitro* and cellular test methods followed by animal tests and then finally the human repeat insult patch test (HRIPT). Public insight in the number of chemicals that has been evaluated by such risk assessment programmes is very limited but it is likely that many tested chemicals hold a contact sensitizing potential and therefore never enter use in consumer products. It is understandable that the industry will try to keep such information in-house, but on the other hand, valuable scientific data that could be used in the evaluation of the contact sensitizing potency of chemicals, never reach the scientific databases.

Due to these circumstances, it is only possible to describe and evaluate methodologies that have been used to estimate and regulate exposure from contact sensitizing chemicals post-marketing. Thus, our knowledge seems to be restricted to such “historical challenges and failures”, e.g. the metals nickel and chromium, the hair dye chemical p-phenylenediamine (PPD), the preservatives formaldehyde and isothiazolinones, and finally various fragrance chemicals. More recent events such as the preservatives methyldibromo glutaronitrile (MDBGN) and dimethyl fumarate, clearly illustrate a novel alertness in the regulatory system within the EU.

Historically, both nickel and chromium contact sensitization has been prevalent in the general population and among dermatitis patients. Accordingly, these metals have resulted in high frequencies of allergic skin disease affecting quality of life and occupational capabilities in millions of Europeans. Nickel and chromium are known to have moderate to strong sensitizing potentials. The contact sensitization epidemic caused by these metals has remained present for more than 100 years. Sensitization is not caused by natural occurring nickel and chromium compounds but by human industrial activities. Nickel is present in consumer items such as buttons and inexpensive jewellery whereas chromium is present in cement and leather products.

Research leading to their regulations was mainly driven and financed by academia and public institutions. The industry has first at a very late stage participated in the process of developing exposure regulations.

The present EU regulations limiting nickel in consumer products and chromium in cement are both based on clinical research and the outcome of experimental dose-response elicitation studies in already sensitized individuals. The general aim was to identify the non-elicitation concentrations in contact sensitized individuals. These values have afterwards been used as regulatory limits as it is well established that the primary sensitizing dose is generally of higher magnitude than the minimal elicitation dose.

Formaldehyde is a historically important preservative used in a wide variety of consumer and industrial products. Formaldehyde is also a degradation by-product from many chemicals; e.g. the formaldehyde donors are a group of preservatives that release formaldehyde. The regulation of formaldehyde is largely based on historical traditions as well as risk assessment regarding other health hazards than contact sensitization. Thus, the concentration limit has not been set to limit formaldehyde allergy and dermatitis, and in that sense, it is not scientifically based. The permitted use concentration of 2000 ppm in cosmetic products is far beyond the contact sensitizing threshold concentration. The 500 ppm limit for declaration is also much higher than the elicitation concentration established in clinical studies including formaldehyde sensitized individuals. The same limitation applies to the regulation of formaldehyde releasing preservatives (formaldehyde donors such as diazolidinylurea and quarternium-15), both when it comes to exposure concentration of the “mother chemical” and the released formaldehyde. Interestingly, experimental dose response elicitation studies showed that a standard cosmetic stay-on product can be adequately preserved with a formaldehyde releaser in a concentration that is safe in terms of elicitation, and hence also sensitization, in a concentration level well below the actual permitted.

The isothiazolinones, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) preservatives, have been used in cosmetic products since the early 1980's. It was established from animal studies and HRIPT studies that this group of chemicals had strong to extreme contact sensitizing capabilities. Initial dose response studies performed in animals as well as humans showed that low concentrations of these chemicals retained preservation activities but did not induce contact sensitization. Based on these studies, isothiazolinones were permitted in cosmetic products. Epidemics of MCI/MI contact sensitization caused by industrial and cosmetic products were observed rapidly after initial use (months to years). It was obvious that the permitted exposure concentration was too high. Interestingly, it has later been showed that one may obtain sufficient preservation with only 1/5 of the original permitted MCI/MI concentration. Contact sensitization and elicitation remains frequent from isothiazolinones albeit at a lower level. An important mistake in the toxicological and pre-consumer-market evaluation was the translation of animal and human experimental dose response studies (performed in healthy volunteers) to the general use situation. Particularly, the effect of repeated exposure of even very low concentrations of this extreme potent sensitizing chemical was grossly underestimated. With our present knowledge, it is unlikely that the isothiazolinones preservatives would have been permitted in cosmetic stay-on products if introduced today. The use in shampoos seems to be safe in the vast majority of individuals.

Many perfume chemicals have inherent contact sensitization capabilities. Isoeugenol and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) represent respectively, one of the traditional strong sensitizing fragrance chemicals and a more recently introduced perfume chemical with a moderate sensitizing potential. The contact sensitizing capabilities of perfume chemicals in general has been known by the industry for decades based on animal and human exposure studies. For many years, diagnostic methods used to identify fragrance sensitization seemed to lag far behind the sophisticated ability of the industry to mix products containing hundreds of different chemicals, many of these with some degree of sensitizing potency. The introduction of the diagnostic patch test material “Fragrance Mix I and II” as well as chemical analysis of marketed perfumes and dose response elicitation studies in individuals sensitized to fragrance products made it obvious, that perfume sensitization mainly was related to the use of a relatively limited number of

moderate to strong sensitizing perfume chemicals, used in high concentrations. Combined efforts have led to a more effective regulation and lower use concentrations of e.g. isoeugenol and HICC.

PPD was invented as one of the pivotal chemicals for the colour industry in the late 19th century. French chemists developed the permanent hair-dye based on PPD and closely related chemicals. This invention has led to a global hair-dye industry. PPD has for the last century been known as a contact allergen. Animal and human experimental studies have classified PPD as an extreme potent contact sensitizing chemical in the same group as the most contact sensitizing experimental chemicals known. In contrast to the isothiazolinones, which are also extreme contact sensitizers and only permitted in a concentration up to 15 ppm, PPD is permitted in concentrations up to 2%. Furthermore, marketed hair-dyes contain several similar chemicals and an oxidiser is added to produce the wanted colour. Recent animal studies identified the finished hair dye product as perhaps the most extreme contact sensitizing product in contact with the human skin. Recent studies have proposed systemic immunological effects following hair dye exposure and hitherto unknown immunotoxicological capabilities by normal use. To make white hair permanently black, a high PPD concentration is required. Experimental human exposure and human dose response studies have clearly illustrated that a safe response concentration does not exist.

MDBGN has more recently been introduced in cosmetic products based on mainly animal studies illustrating a low contact sensitizing potential. A few years after introduction, contact sensitization epidemics of severe cases of contact sensitization were observed in several countries. Human dose-response elicitation studies made in patients primarily sensitized to MDBGN from cosmetics illustrated that no safe exposure level existed. Animal studies were repeated and showed that the chemical had a moderate to strong contact sensitizing potency. Its use was rapidly banned in cosmetic products. However, recent research indicates that the use in e.g. shampoos seems to be safe in the vast majority of individuals.

A short but violent epidemic of contact sensitization to dimethyl fumarate used to preserve leather furniture, shoes and other items was followed by a temporary prohibition of its use in the EU. It is interesting that this swift action was mainly done based on clinical observations.

In all the presented cases, a multitude of methods have been applied, particularly animal studies, HRIPT, clinical studies and human experimental dose-response elicitation studies. It comes as no surprise that the *in vitro*, *in silico* and cellular methods have not been used as they are mainly designed for pre-market risk assessment. These methods have also been used retrospectively and will generally identify the discussed chemicals as moderate to extreme contact sensitizing. Similarly the quantitative risk assessment (QRA) model has been used retrospectively. Such methods would not on their own have been able to predict the above mentioned problem cases. As described, the QRA methods have general limitations and tend to underestimate the risk of sensitization. Future monitoring of contact sensitization prevalence rates performed by multiple centres is necessary.

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Part III

Aim

For those chemicals identified in point 2 above, collect and critically analyse clinical and statistical evidence on the incidence and morbidity (clinical picture) of skin contact allergies (contact dermatitis) cases in the European Union (EU) before (at least 3 years) and after the limits were set so as to allow an assessment of the possible effect of the limits in the reduction/prevention of the incidence and morbidity of contact dermatitis.

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10. Effect of regulations

10.1 Nickel Directive

In July 2001, the EU Nickel Directive (94/27/EC) came into full force to protect European citizens against nickel allergy and dermatitis. This Directive was included in the Registration, Evaluation, Authorisation and Restriction of Chemical substances registration (REACH) during 2009. To evaluate whether items are in compliance with the EU Nickel Directive, 3 reference methods have been developed, EN 1810, EN 1811 and EN 12472. Prior to the introduction of the EU Nickel Directive, Northern European governments had already begun to regulate consumer nickel exposure; in Denmark, a statutory order was implemented to reduce nickel release from certain items in 1990 (1); in Sweden, ear-piercing with nickel containing piercers or rings was banned in 1991, if the alloy contained more than 0.05% nickel (2), in Germany, certain nickel containing consumer items were required to be labeled “contains nickel and may cause an allergic reaction” after 1991 (2). Overall, the increased focus on nickel exposure and allergy is suspected to affect the composition of consumer items sold in European countries since the early 1990’s. Below, data from different European countries are presented to demonstrate the development of nickel allergy.

Denmark

Since the Danish nickel regulation was introduced in 1990, 10 years before the EU Nickel Directive into force, possible epidemiological changes of nickel allergy and dermatitis following regulation are expected to appear first in Denmark. So far, a decrease of nickel allergy has indeed been observed in young Danish women from the general population (3), in young Danish female dermatitis patients seen in private dermatology practice (4-6) and from a tertiary university clinic (4;7). Furthermore, school girls and women who were ear-pierced after the regulatory intervention in Denmark had a significantly lower prevalence of nickel allergy (and dermatitis) when compared to school girls (8) and women ear-pierced before regulation (9). Finally, the association between hand eczema and nickel allergy in young Danish women and the strength of positive patch test reactions (2+ and 3+) in Danish dermatitis patients have been reduced after regulation (3;10) (Figure 1). Nickel allergy data are gathered in Table 1.

Sweden

Only one Swedish study has compared the prevalence of nickel allergy before and after nickel regulation. Consecutive patch test data from dermatitis patients tested in 9 centres during the periods 1991-1993 (n=3680) and 1999-2001 (n=3790) showed that the prevalence of nickel allergy decreased from 33.8% to 29.4% ($p<0.05$) in women under 40 years of age (Table 1) (11). Also, there is indirect evidence to suggest an effect of the EU Nickel Directive in Sweden. The proportion of dimethylglyoxime (DMG) test positive items, among the broad range of consumer items covered by the EU Nickel Directive, decreased significantly in Stockholm, Sweden from 25% of 725 tested items in 1999, to 8% of 786 items in 2002/2003 and 9% of 659 items in 2010 (12-14). Since the EU Nickel Directive came into full force in 2001, the decrease in Sweden is likely to be explained by an effect of the regulation.

Germany

In Germany, the prevalence of nickel allergy decreased in female dermatitis patients aged under 31 years from 36.7% in 1992 to 25.8% in 2001 ($p<0.0001$) (Table 1 and Fig. 1) (15;16). However, in further follow up of male and female dermatitis patients, no additional decrease of nickel allergy could be demonstrated in any age-groups after year 2000 except

for female dermatitis patients aged 18-30 years where a significant decrease was observed from 26.7% to 20.2% ($p < 0.0002$).

Italy

Two Italian studies have compared the prevalence of nickel allergy before and after nickel regulation. One study found a stable prevalence of nickel allergy in dermatitis patients from Rome although no stratification for gender and age group was provided (17). Thus, the prevalence of nickel allergy was 54.3% in 931 patients tested in 1994 and 53.5% in 867 patients tested in 2005. Another Italian study suggested a decrease of nickel allergy as the prevalence of nickel allergy was significantly higher in female patients aged 26-35 years when compared to female dermatitis patients aged 15-25 years (18).

Other old EU countries

No data has so far been published on the development of nickel allergy before and after nickel regulation in France. By courtesy of Dr. Martine Vigan, Department of Dermatology, Hôpital St. Jacques, Besançon, France, patch test data suggest a decrease from 24.1% in the period 1989-99 ($n=2996$) to 15.8% in the first 6 months of 2009 ($n=76$). By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, apparently no decrease of nickel allergy has been observed among approximately 600-700 annually patch tested dermatitis patients. Thus, the overall prevalence of nickel allergy was 20.3% in 1990 and 18.5% in 2009. However, as for the Italian data presented above, no gender or age stratification was offered. Gender and age stratification is imperative as observed for nickel allergy data from Denmark and Germany (Fig. 1). Remaining old EU countries have not provided nickel allergy prevalence data from before and after nickel regulation. However, for general comparison of nickel allergy prevalence estimates, current data are offered. For the period 2004-07, the prevalence of nickel allergy in 11 British patch test centres was 21% (courtesy Dr. Statham, Singleton Hospital, Swansea, UK). In line with these data, the 2005/2006 clinical patch test data registered in 10 European countries and reported to the European Surveillance System on Contact Allergies (ESSCA), revealed high prevalences of nickel allergy in both Western, Southern, Central and Northeastern Europe being respectively, 20.8%, 24.5%, 19.7% and 22.4% (19).

New EU countries

Few data exist from new EU countries. In Budapest, Hungary, the prevalence of nickel allergy was 18.6-21.2% during the period 2007-2009 (courtesy, Professor Temesvári, Department of Dermatology, Venerology and Dermatoooncology, Semmelweis University Budapest). The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities. (Courtesy, Dr Razvigor Darlenski, Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 31% were nickel allergic. In Poland, the prevalence of nickel allergy decreased from 15.9% in 1995 to 10.0% in 2004 in female dermatitis patients aged under 20 years patch tested in Warsaw (20). However, the prevalence of nickel allergy among adolescents (12-16 years) who were patch tested between 1970-1994 reached 15.3% in girls and 5.5% in boys (21) as compared to 27.8-31.8% in girls and 6.7%-7.7% in boys aged 16-17 years who were patch tested in the years 2007-2009 (22;23).

Conclusion

Over the second half of the 20th century, the nickel allergy epidemic accelerated, as European and North American consumers became increasingly nickel allergic resulting in morbidity, sick leave and increased health care costs (24). The Nordic nickel regulations and

the EU Nickel Directive may be regarded as pioneering consensus approaches aimed at reducing the nickel allergy problem, but not attempts to totally eliminate nickel allergy. The accumulation of clinical and epidemiological general population studies strongly suggest that the regulations on nickel exposure from consumer items have had a *likely effect* on the prevalence of nickel allergy as it has decreased markedly and significantly in young women and female dermatitis patients. It seems that the decrease of nickel allergy has occurred first in Northern European countries and only partly in Southern European countries.

General remarks

Despite the decrease, it is important to emphasize that nickel allergy remains very prevalent as for instance at least 11% of Danish adult women aged 18-35 years are allergic to nickel (3) and the proportion of positive nickel patch test reactions remained stable at 10-20% among young female German dermatitis patients (<18 years) since the beginning of the new millennium (25). Several reasons for the persistence of nickel allergy can be listed (Table 2): 1) Sensitization before nickel regulation. 2) Violation of the EU Nickel Directive (26;27). 3) Exposure to items not covered by the regulation, e.g. mobile phones until recently (28;29). 4) Lack of control and information by responsible authorities. 5) Insufficiency of the EN 1811:1998 reference test method as it allows one to multiply the amount of nickel release determined by chemical analysis by an adjustment factor of 0.1 before its interpretation of compliance with the EU Nickel Directive. This adjustment factor was introduced to compensate for difficulties when calculating complicated area sizes and due to lack of experience. However, this adjustment factor has weakened the EU Nickel Directive markedly as items in fact may release 10 times more nickel than intended by the Directive. Research is currently being done to assess the real difference between accurate area measurement using computer technology and area measurement using traditional methods. It is currently debated whether the 0.1 factor could be removed or replaced by a smaller adjustment factor, as proposed in the EC mandate of 25 June 2007 to CEN for revision of EN 1811:1998; or replaced by a measurement uncertainty interval as in the Draft prEN 1811 of July 2009, currently in the acceptance process of CEN/TC 347. Despite these important issues, the EU Nickel Directive stands as an example of a successful public health intervention since the nickel allergy problem in Asia and North America seems to be uncontrolled (30).

Table 1. Overview of patch test studies that investigated the development of nickel allergy before and after regulation.

Reference	Country	Population	Number	Nickel allergy prevalence before regulation (study year: %)	Nickel allergy prevalence after regulation (study year: %)	Significance level	Conclusion
(8)	Denmark	Public school girls; mean age=12.4 y	305	<1992: 15.8	>1992: 4.3	0.02	A higher risk of ear piercing before but not after 1992 was found. Implementation of nickel-exposure regulation has protected the female population from becoming allergic to nickel
(8)	Denmark	High school girls; mean age=18.8 y	275	<1992: 26.8	>1992: 12.4	0.01	
(9)	Denmark	Adult women (18-69 y) general population	2 117	Ear pierced before regulation: 15.6	Ear pierced after regulation: 6.9	0.004	Regulatory control of nickel exposure may reduce the prevalence of nickel allergy
(3)	Denmark	Adult women (18-35 y) general population	852	1990: 19.8	2006: 11.4	0.02	Regulatory control of nickel exposure may reduce the prevalence of nickel allergy
(4)	Denmark	Dermatitis patients (<18y), DCDG	2 499	1985-86: 24.8	1997-98: 9.2	0.0008	-
(5)	Denmark	Female dermatitis patients (<20 y), private practice	1 026	1986-1989: 22.1	1996-99: 16.7	0.05	The most likely explanation of this decrease in nickel sensitivity are reduced exposure to nickel and increased public awareness of the risk of nickel sensitization
(7)	Denmark	Female dermatitis patients (5-30 years), University clinic	2 397	1985: 27.6	2007: 16.6	0.002	The prevalence of nickel allergy decreased among young female patients with dermatitis after the introduction of the Danish nickel regulation.

(11)	Sweden	Female dermatitis patients (<40 years), Hospital departments	2368	1991-93: 33.8	1999-01: 29.4	0.05	The decrease seen in younger ages between the two test occasions may reflect decreased exposure due to the Nickel Directive
(15;16)	Germany	Female dermatitis patients (<31 years), Hospital departments	2252	1992: 36.7	2001: 25.8	0.0001	The observed decline indicates that measures to limit the release of nickel from costume jewellery helped reduce the prevalence of nickel allergy
(17)	Italy	Dermatitis patients, University clinic	1798	1994: 54.3%	2005: 53.5%	0.9	The frequency of allergy to nickel due to earrings has not decreased after the introduction of the nickel regulation

DCDG = Danish Contact Dermatitis Group

- = not given

Figure 1. Patch test reactivity to nickel among nickel allergic patients seen at the Department of Dermatology, Gentofte Hospital between 1977 and 2009 (10).

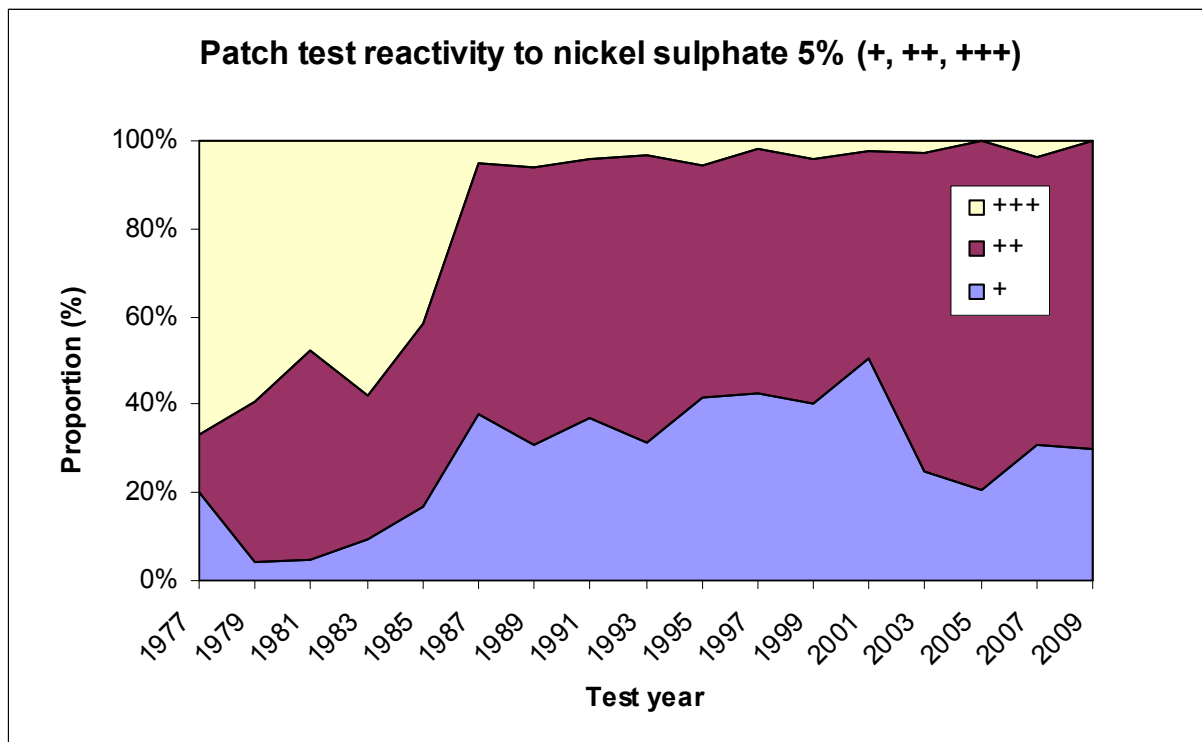


Figure 2. The development of nickel allergy in female dermatitis patients patch tested between 1992 and 2001 in a network of German patch test clinics (15).

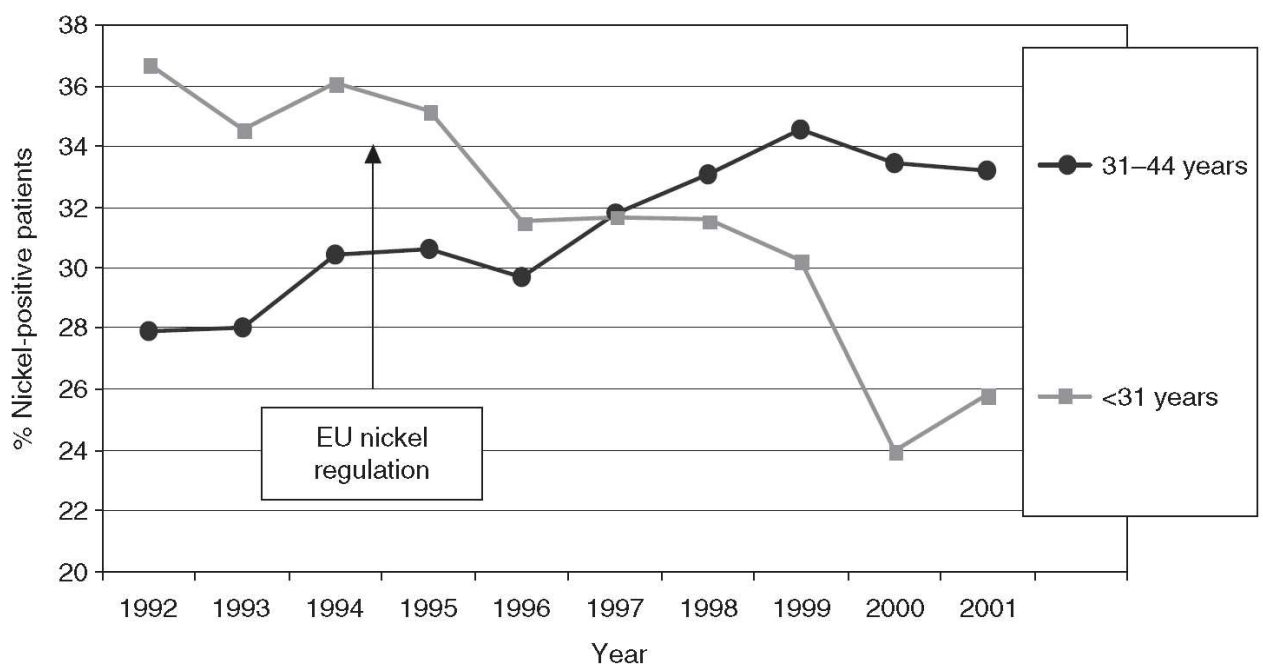


Table 2. Possible explanations for the persistence of nickel allergy and dermatitis following regulatory intervention on nickel exposure.

Causes	Estimated contribution to persistence of nickel allergy and dermatitis (strong/moderate/weak)
<i>Consumer:</i>	
Sensitization before nickel regulation	strong/moderate/weak*
Violation of the EU Nickel Directive	moderate/strong
Lack of control and information by responsible authorities	weak/moderate/strong**
Exposure to items not covered by the regulation	moderate/weak
Exposure to items personally imported from countries outside the EU	weak
Exposure due to defect coatings on consumer items after 2 years use	weak
<i>Occupational:</i>	
Exposure by contact with tools, keys, locks, handles, coins, other equipment, materials, metal working fluids etc.	strong/moderate/weak***
<i>Other:</i>	
“Adjustment” factor of 0.1 in EN 1811:1998, the reference test method for control of compliance with the EU Nickel Directive	strong
Insufficiency of the contents of the EU Nickel Directive	weak
Genetic vulnerability	moderate
Toys	weak
Medical devices with skin contact	weak

* Depending on age group

** Depending on country

*** Depending on occupation

10.2 Chromium Directive

On January 17th 2005, the EU Chromium Directive came into force to protect European construction workers against chromium allergy and dermatitis caused by cement exposure. Since cement is a preparation and not a chemical, cement is not subject to REACH registration. However, the existing chromium restrictions continue to apply to cement and products containing cement marketed in EU member states. Prior to the introduction of the EU Chromium Directive, Northern European governments had already begun to regulate the content of hexavalent chromium in cement. Thus, in 1983 in Denmark, a Working Environment Service Order made the addition of ferrous sulfate to cement compulsory (24). Shortly after, governments in Sweden, Norway, Finland and Germany introduced similar regulations (24;31). Below, data available from European countries are presented to demonstrate the development of chromium allergy before and after regulation.

Denmark

Since the Danish chromium regulation was introduced in 1983, 22 years before the EU Chromium Directive came into force, possible epidemiological changes of chromium allergy and dermatitis following regulation are expected to appear first in Denmark. Avnstorp et al. showed that the prevalence of chromium allergy among cement workers decreased significantly from 10.5% in 1981 to 1.6% in 1987 (p -value=0.002) (32). Furthermore, they found a statistically significant decrease in the number of workers with allergic chromium dermatitis in a cohort of workers exposed to cement with a lower water-soluble chromate concentration (2 ppm) when compared to a cohort of workers exposed to cement with a higher water-soluble chromate concentration (10 ppm) (relative risk of chromium sensitization was 8.3 in subjects exposed to 10 ppm chromium, 5.8 in subjects exposed to 2-10 ppm chromium and 1 in subjects exposed to 2 ppm chromium) (33). Finally, they studied a cohort of workers engaged, or who had been engaged, in the manufacture of prefabricated concrete building components in 1981 and in 1987. They found that workers who had allergic chromium dermatitis in 1981 appeared to show no improvement 6 years after the reduction of chromate in cement whereas improvement of dermatitis was observed in workers with irritant contact dermatitis but no chromium allergy. A significantly larger number of chromium allergic workers required medical services and topical steroid treatment than did those who were not sensitized to chromate (34). Of particular importance, Avnstorp et al. stated that no changes of production methods, besides reducing the content of chromium in cement, had taken place during the study periods (32).

Three studies have investigated the overall prevalence of chromium allergy in Danish dermatitis patients from respectively, a tertiary referral patch test centre and a Danish network of clinics (4;35;36). Between 1989 and 1994, 79 of 4 511 patch tested patients had chromium allergy (36). Relevant chromium exposure was established in 34 patients. In 10 patients, chromium sensitization from cement was considered likely and of these, 7 had been sensitized before 1981, 2 had been sensitized by non-occupational exposure to cement, and only 1 had been sensitized from occupational cement exposure in the 6-year period (36). A clinical patch test study using data from the Danish Contact Dermatitis Group showed a significant decrease of chromium allergy from 3% in 1985–86 to 1.2 % in 1997–98, suggesting an effect of the Danish chromium regulation (4). In subsequent retrospective analysis of chromium patch test data from 16 228 dermatitis patients and medical charts from patients with chromium allergy, it was shown that the prevalence of chromium allergy decreased significantly from 3.6% in 1985 to 1% in 1995 ($P_{\text{trend}} < 0.001$) (Fig. 2). The frequency of clinically relevant cement exposure decreased significantly

from 12.7% in 1989-1994 to 3.0% ($P < 0.01$) in 1995-2007 (35). However, in recent years, the prevalence of chromium allergy has increased significantly, a finding that is explained by exposure to chromium from leather goods such as gloves and shoes (Fig. 2). Finally, one study has investigated the development of patch test reactivity to chromium over a 33-year period (1977-2009) (10). It showed a decrease of 3+ reactivity since 1981, the year from which all cement produced in Denmark contained ferrous sulphate. An increase of 2+ reactivity in recent years may be explained by chromium exposure from leather goods.

During the construction of the combined tunnel and bridge of the Great Belt linking Funen and Sealand in Denmark and of the combined tunnel and bridge over Öresund, the strait between Denmark and Sweden, no cases of cement dermatitis were recorded (31). This is noteworthy as construction workers suffered from chromium dermatitis during the construction of the Channel Tunnel between France and the UK (see below).

Germany

Gailhofer and Ludvan documented a decreasing trend of chromium allergy in 8 247 German dermatitis patients tested between 1975 and 1984, i.e. prior to the regulation of hexavalent chromium in cement (37). Bock et al. investigated the incidence of chromium allergy between 1990 and 1999 among construction workers identified through the occupational skin disease register in Northern Bavaria (38). They showed that the incidence followed a u-shaped pattern and hence concluded that their findings contrasted those from Scandinavian countries. More recent, unpublished, data from the Information Network of Departments of Dermatology (IVDK) network in Germany indicate that the prevalence of chromium allergy remains steadily high. Thus, the prevalence of chromium allergy was 3.8% in 1999 and 3.4% in 2009. The highest prevalence during this 14-year period was observed in 2006-7 (6.3%). No gender difference in the development of chromium allergy was observed. A recent study from Germany investigated data from 1 153 men working in the building trade and who presented with occupational skin symptoms between 1994 and 2008 (39). The authors stratified data according to the outcome of patch testing but also beginning and duration of work. They showed that the prevalence of chromium allergy decreased from 43% in 1994-1996 to 29% in 2006-08 ($p_{\text{trend}} < 0.001$). Adjusted regression analysis showed that patients who began to work in the building trade after 1999 had a significantly lower risk of chromium allergy compared to those who began working before 1994 ($OR = 0.42$; $CI_{95\%} = 0.20-0.90$). The outcome of this study is of particular interest as the authors took into account the duration of occupational exposure.

United Kingdom

The temporal development of chromium allergy in dermatitis patients tested in a London clinic between 1982-3 and 1992-3 was investigated by Olsavszky et al. (40). They showed that the prevalence appeared stable in both genders and that no change occurred in the anatomical distribution of dermatitis or the prevalence of co-sensitivity to cobalt (cobalt allergy was used as an indicator of cement exposure as cobalt and chromium are both found in cement). The prevalence of chromium allergy in 1982-83 and 1992-3 was respectively, 1.6% and 2.0% for women and 4.0% and 4.3% for men. An interesting article investigated morbidity in construction workers who participated in construction of the Channel Tunnel between France and the UK (41). The British drive employed 5 900 underground workers. Between January 1990 and January 1992, 1138 men were seen at a Medical Centre regarding

their skin and 332 were diagnosed as having occupational dermatitis. Patch testing was performed on 86 grouters and revealed chromium allergy in 56 (65%). Recent unpublished data from the period 2004-07 revealed that the mean prevalence of chromium allergy in 11 British patch test centres was 2.8% (courtesy Dr. Statham, Singleton Hospital, Swansea, UK).

Other EU countries

One Swedish study has investigated the development of chromium allergy over time. Consecutive patch test data from dermatitis patients tested in 9 centres during the periods 1991-1993 (n=3680) and 1999-2001 (n=3790) showed that the prevalence of chromium allergy increased in female patients from 2.8% to 5.1% and remained stable in male patients 4.5% (11). Rui et al. recently made a retrospective study on chromium allergy in 14 464 Italian dermatitis patients that were patch tested between 1997 and 2004 (18). The overall prevalence of chromium allergy was 8.7% (7.9% in women and 10.1% in men). Chromium allergy was significantly associated with construction work in both genders and with cleaning work in women. By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, there seem to be an increase of chromium allergy in approximately 600-700 annually patch tested dermatitis patients during the period 1990-2009. The lowest prevalence was 3.3% and the highest 6.6%. However, Professor Goossens notes that she sees many patients with chromium allergy caused by shoe exposure. Few data exist from new EU countries. In Budapest, Hungary, the prevalence of chromium allergy was 3.7-5.0% during the period 2007-2009 (courtesy, Professor Temesvári, Department of Dermatology, Venerology and Dermatooncology, Semmelweis University Budapest). The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities (Courtesy, Dr Razvigor Darlenski, Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 5.4% were chromium allergic. 2005/2006 clinical patch test data registered in 10 European countries and reported to the ESSCA, revealed relatively high prevalences of chromium allergy in both Western, Southern, Central and Northeastern Europe being respectively, 2.4%, 4.5%, 5.9% and 5.3% (19).

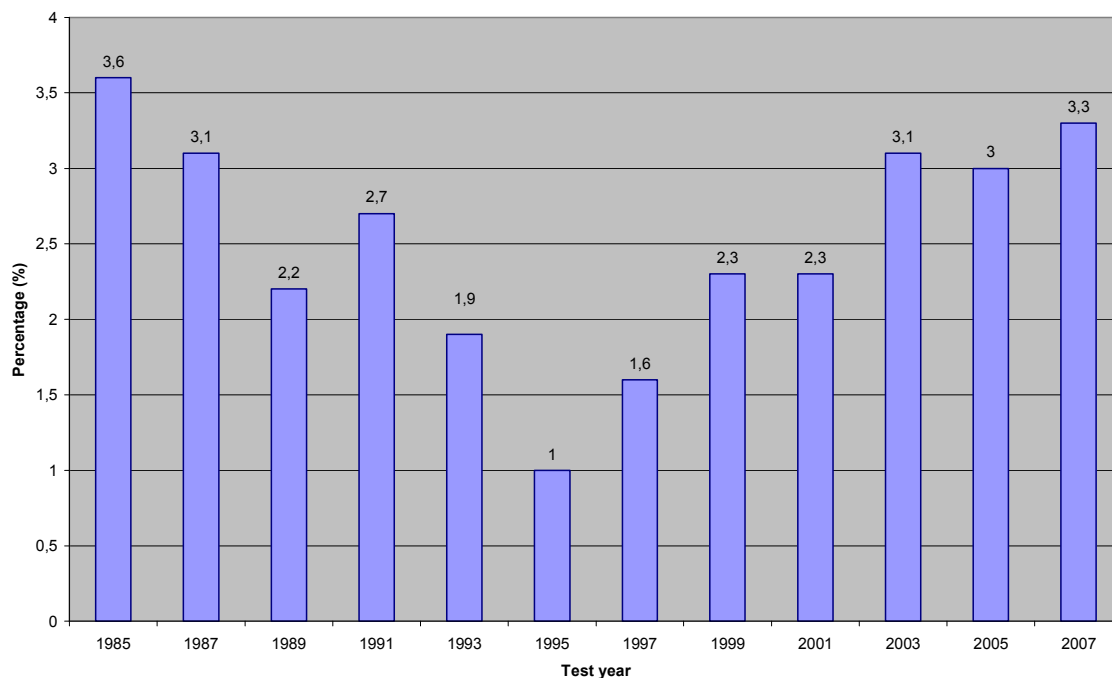
Conclusion

As for nickel allergy, it is interesting to study the development of chromium allergy in Denmark, as changes in its epidemiology are expected to occur first in the nation that pioneered the regulation of chromium exposure from cement. There is no doubt that the epidemiology of chromium allergy has changed in Denmark and elsewhere during the past 30-years due to better education, use of personal protective measures, improved workplace hygiene and decreased contact with construction materials. This has also been documented in the literature (37;42;43). For these reasons, it is unwise to only study temporal prevalence changes but one should also include other factors such as exposure records in patients, duration of occupational exposure in workers under investigation and experiences from large construction projects.

The collection of studies, especially those conducted in construction workers, strongly indicates that the regulation of hexavalent chromium in cement has contributed to the decreasing prevalence of chromium allergy and chromium dermatitis. In Singapore, a change in the manufacturing process of cement giving a lower content of hexavalent chromium was also accompanied by a decline in the prevalence of chromate allergy among construction workers (44). In a follow-up, an increase of chromium allergy was explained by other sources than cement (45). An

indirect evidence of the reduction of chromium exposure following addition of ferrous sulphate came from a Chinese study where workers exposed to cement without the addition of ferrous sulphate had a significantly higher concentration of urinary chromium when compared to workers exposed to cement with the addition of ferrous sulphate (46). The moderate overall decrease of chromium allergy in dermatitis patients undergoing routine testing (and even increase) is explained by increased exposure to chromium from leather items. Taken together, the accumulation of clinical studies, and experience from large scale European construction projects, strongly suggest that the regulations on chromium exposure from cement have had a *likely effect* on the prevalence of chromium allergy and dermatitis as it has decreased markedly and significantly in construction workers.

Figure 3. The development of chromium allergy in dermatitis patients patch tested between 1985 and 2007 in Copenhagen, Denmark (35).



10.3 Cosmetic Directive

10.3.1 Formaldehyde

Formaldehyde is allowed in cosmetic products in the EU in a use concentration of 0.2% (2000 ppm) but should be declared when used in higher concentrations than 0.05% (500 ppm). Formaldehyde is prohibited in aerosol dispensers but allowed in nail varnish up to 5%.

Studies investigating temporal changes of formaldehyde allergy

Studies have investigated the development of formaldehyde allergy over time. A 10-year multicentre analysis on the prevalence of formaldehyde allergy in 16 centres in 11 countries showed a stable but persisting high level of formaldehyde allergy

between 1991 and 2000 (Fig. 4) (47). Of interest, the prevalence of formaldehyde allergy decreased in one Londonian patch test centre at the same time as the prevalence of methyl dibromoglutaronitrile (MDBGN) allergy increased (Fig. 5). A recent Danish patch test study found that the overall prevalence of formaldehyde allergy was 3.1% between 1985 and 2009 and that the prevalence remained stable over time (Fig. 6) (48). Recent patch test data from the IVDK database in Germany also revealed a stable development of formaldehyde allergy between 1996 and 2009 although a small decrease was noted in recent years (Fig. 7). This decrease is not likely to be an effect of the regulation of formaldehyde exposure as this was introduced at least more than 30 years earlier. A recent British study compared the prevalence of preservative allergy in 2000 and 2004-05 and found that the prevalence of formaldehyde allergy remained stable at 2% (49).

Other recent formaldehyde allergy prevalence estimates

2005/2006 clinical patch test data registered in 10 European countries and reported to the ESSCA, revealed relatively high prevalences of formaldehyde allergy in both Western, Southern, Central and Northeastern Europe being respectively, 2.0%, 4.2%, 1.8% and 3.7% (19). Unpublished data from the period 2004-07 revealed that the mean prevalence of formaldehyde allergy in 11 British patch test centres was 1.8% (courtesy, Dr. Statham, Singleton Hospital, Swansea, UK). By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, there seems to be an increase of formaldehyde allergy in approximately 600-700 annually patch tested dermatitis patients during the period 1990-2009. The lowest prevalence was 0.3% and the highest 4.1%. In Budapest, Hungary, the prevalence of formaldehyde allergy was 1.2-4.5% during the period 2007-2009 (courtesy from Professor Temesvári, Department of Dermatology, Venerology and Dermatocarcinology, Semmelweis University Budapest).

The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities (Courtesy, Dr Razvigor Darlenski, Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 2.3% were formaldehyde allergic.

Conclusion

There is a persistently high prevalence of formaldehyde allergy in European dermatitis patients. This may be explained by exposure to formaldehyde as such or to formaldehyde released by formaldehyde donors (e.g. quaternium-15 and diazolidinyl urea) (50). A study from the UK showed that the prevalence of imidazolidinyl urea increased significantly from 0.5% in 2000 to 0.9% in 2004-2005 (49) but such an increase could not be replicated in a recent Danish study although the prevalence was comparable to the one found in the British study (48). Thus, the increase of imidazolidinyl urea allergy in the UK may be a novel trend or perhaps just a result of random fluctuation. The EU restriction on formaldehyde in cosmetic products (2000 ppm) does not correspond well to the concentrations that may sensitize and elicit dermatitis in formaldehyde allergic patients (please refer to part II). It is foreseen that the prevalence of formaldehyde allergy will remain high if formaldehyde exposure from cosmetic products is not further restricted. Taken together, the EU regulation on formaldehyde exposure has not had a *detectable effect* on the prevalence of formaldehyde allergy and morbidity caused by formaldehyde. There might be formaldehyde exposure from a number of occupational products.

Figure 4. Percentage of patch test positive reactions to preservatives averaged from data from 16 European centres in a 10-year period (1991-2000) (47).

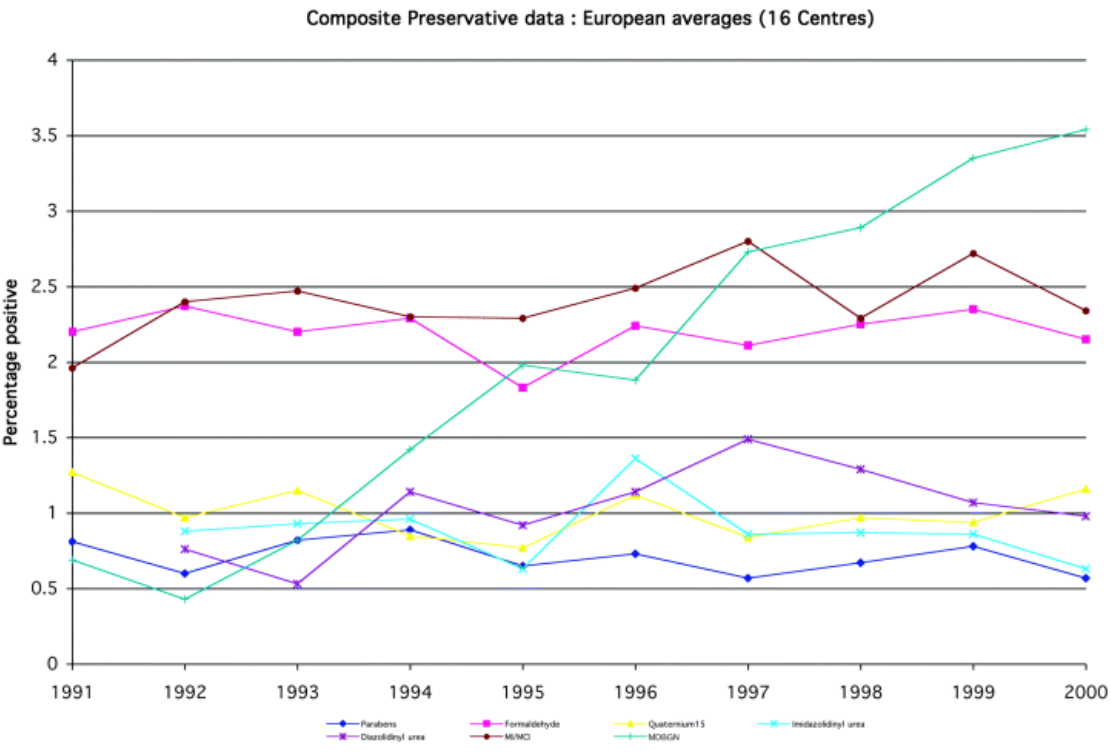


Figure 5. Temporal trends of formaldehyde and methyldibromo glutaronitrile allergy in the UK (1989-2000) (51).

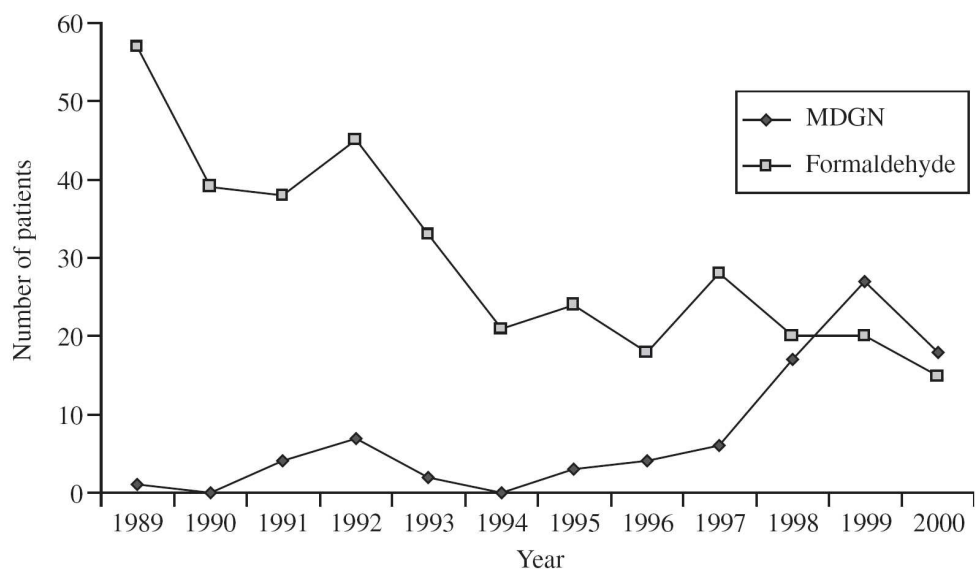


Figure 6. Temporal trends of preservative allergy (%) in Denmark (1985-2008) (48).

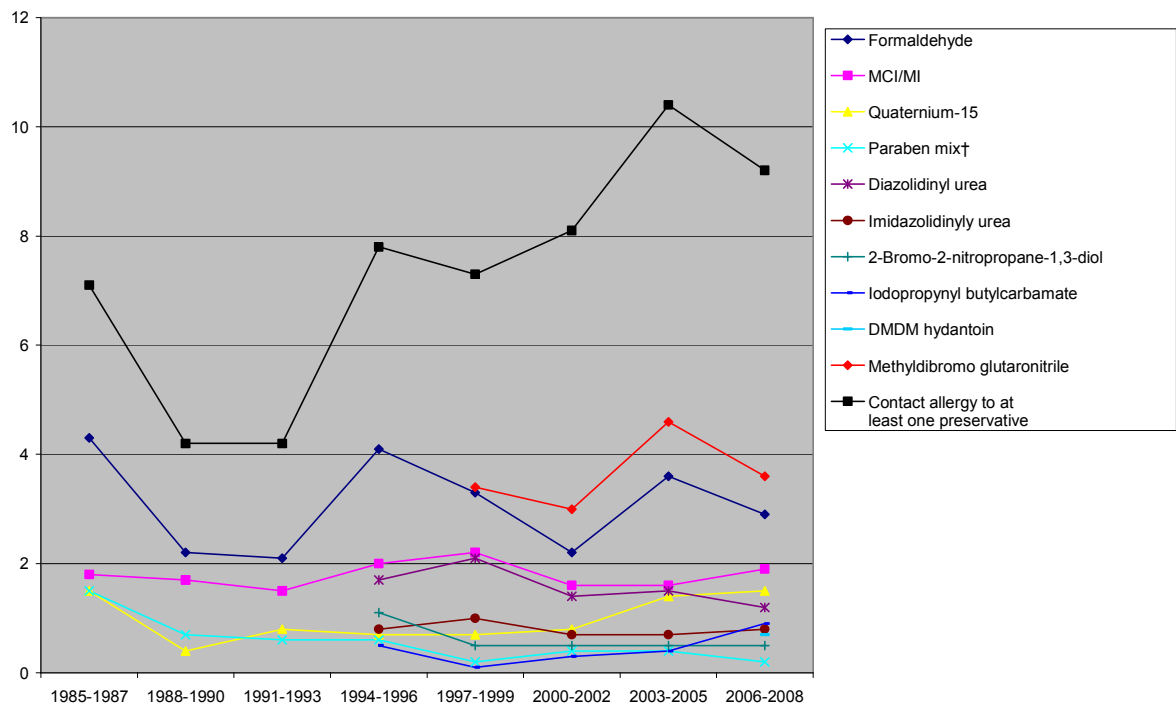
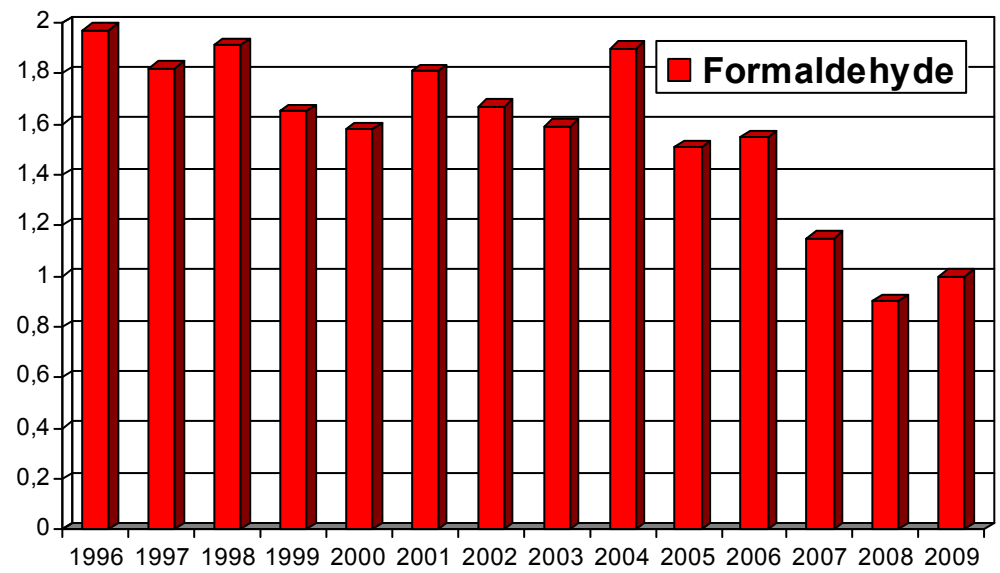


Figure 7. Temporal trends of formaldehyde allergy (%) in Germany (1996-2009) (IVDK 2010; unpublished).



10.3.2

Isothiazolinones

5-Chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3 one (MCI/MI) is allowed in a use concentration of 15 ppm in rinse-off and leave-on cosmetic products sold within the EU region.

Studies investigating temporal changes of isothiazolinone allergy

Studies have investigated the development of MCI/MI allergy over time. A 10-year multicentre analysis of the prevalence of MCI/MI allergy in 16 centres in 11 countries showed a stable but persisting high level of MCI/MI allergy between 1991 and 2000 (Fig. 4) (47). A recent Danish patch test study found that the overall prevalence of MCI/MI allergy was 1.8% between 1985 and 2009 and that the prevalence remained stable over time (Fig. 6) (48). Similar, recent patch test data from the IVDK database in Germany revealed a stable development of MCI/MI allergy between 1996 and 2009 (Fig. 8). A recent British patch test study compared the prevalence of MCI/MI allergy in 2000 and 2004-05 and found that the prevalence of MCI/MI allergy remained stable at about 2% (49).

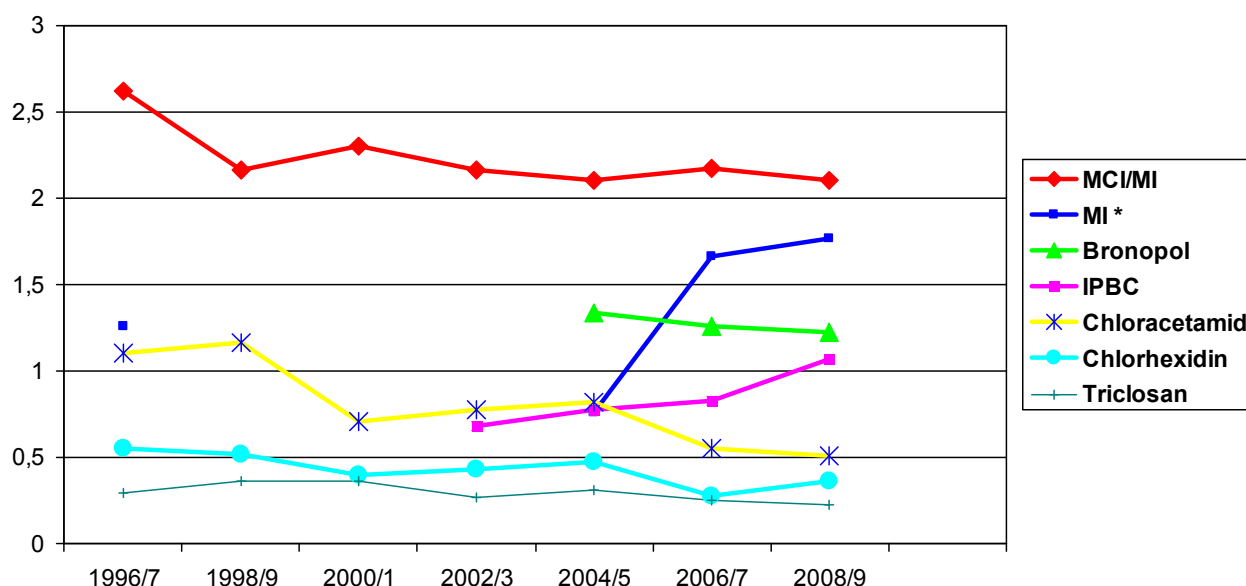
Other recent MCI/MI allergy prevalence estimates

2005/2006 clinical patch test data registered in 10 European countries and reported to the ESSCA, revealed relatively high prevalences of MCI/MI allergy in both Western, Southern, Central and Northeastern Europe being respectively, 2.1%, 4.1%, 2.7% and 2.1% (19). Unpublished data from the period 2004-07 revealed that the mean prevalence of MCI/MI allergy in 11 British patch test centres was 1.9% (courtesy Dr. Statham, Singleton Hospital, Swansea, UK). By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, there seem to be an increase of MCI/MI allergy in approximately 600-700 annually patch tested dermatitis patients during the period 1990-2009. The lowest prevalence was 0.8% and the highest 2.9%. In Budapest, Hungary, the prevalence of MCI/MI allergy was 1.9-3.5% during the period 2007-2009 (courtesy from Professor Temesvári, Department of Dermatology, Venerology and Dermatooncology, Semmelweis University Budapest). The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities. (Courtesy, Dr Razvigor Darlenski, Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 1.0% were MCI/MI allergic.

Conclusion

The EU restriction on MCI/MI use in cosmetic products (15 ppm) does not correspond well to the observed elicitation concentrations that may elicit dermatitis in MCI/MI allergic patients, especially for leave-on products (please see part II). The persistence of MCI/MI allergy in European dermatitis patients reflects this (48). Similar to European patch test data, the prevalence of MCI/MI allergy in North America remained stable at around 2.5% over the past decades (52). It is foreseen that the prevalence of MCI/MI allergy will remain high if MCI/MI exposure from cosmetic *and* industrial products is not further restricted. Taken together, the EU regulation on MCI/MI exposure has not had a *detectable effect* on the prevalence of MCI/MI allergy and morbidity caused by MCI/MI.

Figure 8. Temporal trends of contact allergy to MCI/MI (red line), to MI (dark blue line) and to other preservatives (%) in Germany (1996-2009 (IVDK 2010, unpublished)).



10.3.3 p-Phenylenediamine

The maximum authorized concentration of p-phenylenediamine (PPD) in finished cosmetic products sold within the EU area was for long 6% calculated as free base (3% when added to the oxidizing solution required to develop the colour). However, the concentration limit of PPD in hair dye products was recently changed to a maximum of 2% PPD, calculated as free base. Below, data available from European countries are presented to demonstrate the development of PPD allergy.

Studies investigating temporal changes of PPD allergy

Studies have investigated the development of PPD allergy over time. In Denmark, the prevalence of PPD allergy among dermatitis patients routinely tested revealed a near significant increase from 1.4% in 1989 to 2.4% in 2007 ($p=0.052$) (53). This increase seemed not to be explained by an increase of contact allergy to cross-reactants of PPD (54). Consecutive patch test data from Swedish dermatitis patients tested in 9 centres during the period 1991-1993 ($n=3680$) and 1999-2001 ($n=3790$) showed that the prevalence of PPD allergy increased significantly from 1.4% to 2.0% ($p<0.05$) (11). Patel et al. found an upward linear trend among consecutive patch tested dermatitis patients in London (55) (Fig. 9). Thus, a significant increase was observed from 3.8% in 1989 to 7.1% in 2004 ($p<0.001$). The authors concluded that the increase could not only be explained by the use of temporary henna tattoos together with an increased uptake of Asian patients; suggesting that the increased use of hair dyes also contributed significantly to the increase of PPD allergy. At the university clinic in Aachen Germany, an increase of PPD allergy was observed from 2.5% in 1980-86 to 5.5% in 1987-1993 and to 8.0% in 2004 (56).

A few centres have found stable and even slightly decreasing prevalences of PPD allergy. In Poland, a non-significant decrease of PPD allergy was observed between 2000 (3.7%) and 2006 (3.4%); however, it is important to note that the prevalence of PPD allergy was higher in men (4.8%) than in women (3.1%); suggesting that PPD allergy was also explained by other exposures than hair dyes (57). In Finland, the

prevalence of PPD allergy remained stable at 2.0% during the period 1995-2002 (58). More complete epidemiological studies have been conducted in recent years. A European multicentre study including 21 515 patients showed that the prevalence of PPD allergy was significantly higher in patients patch tested at clinics in Central and Southern Europe when compared to patients patch tested in Scandinavian centres (OR= 2.40; CI95%=2.07-2.78) (59) (Fig. 10). The weighted average prevalence of PPD allergy was 4.6% and the overall proportion of positive patch test reactions to PPD that were registered as being of either current or past clinical relevance was high (weighted average 53.6% and 20.3%, respectively). Consumer hair dyeing was the most prominent cause of PPD sensitization (weighted average 41.8%). Furthermore, occupational hair dye exposure (10.6%) and cross-sensitization to textile dyes (12.6%) were frequently reported (59). Finally, a review including published patch test studies revealed that the prevalence of PPD allergy in Europe seems to have reached a stable plateau with prevalences ranging between 2% and 6% (60) (Fig. 11).

Other recent PPD allergy prevalence estimates

2005/2006 clinical patch test data registered in 10 European countries and reported to the ESSCA, revealed high prevalences of PPD allergy in both Western, Southern, Central and Northeastern Europe being respectively, 3.6%, 4.2%, 4.0% and 4.2% (19). Unpublished data from the period 2004-07 revealed that the mean prevalence of PPD allergy in 11 British patch test centres was 3.8% (courtesy Dr. Statham, Singleton Hospital, Swansea, UK). By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, there seem to be an increase of PPD allergy in approximately 600-700 annually patch tested dermatitis patients during the period 1990-2009. The lowest prevalence was 5% and the highest 9%.

A study from the Czech Republic revealed that 2% of 12 058 dermatitis patients had PPD allergy (61). In Budapest, Hungary, the prevalence of PPD allergy was 7.8-12% during the period 2007-2009 (courtesy from Professor Temesvári, Department of Dermatology, Venerology and Dermatocology, Semmelweis University Budapest). The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities (Courtesy, Dr Razvigor Darlenski, Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 6.8% were PPD allergic.

Conclusion

There are consistent data to support that the prevalence of PPD allergy in European dermatitis patients is high and has been increasing in parts of Europe in recent years. There are several causes of positive patch test reactions to PPD since this chemical cross-reacts with other para group chemicals, e.g. N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD), benzocaine and textile dyes, and since many young people from the general population have tried temporary henna tattooing, sometimes resulting in cutaneous PPD exposure, allergy and dermatitis (Table 3) (62). It is important to appreciate that the prevalence of contact allergy to para group chemicals has been stable for decades; hence these compounds can not explain the increase (54). Furthermore, it has been shown that positive patch test reactions to PPD are frequently of clinical relevance (about 50-80%) making the high prevalence of PPD allergy an epidemic that should be addressed. It has been proposed that the increase of PPD allergy is explained by exposure to PPD from temporary henna tattoos. Although temporary tattoos are of clinical importance, a recent European multicentre study showed that they only explained about 5% of positive patch test

reactions to PPD (59). This study also showed that consumer and occupational hair dye exposure were the most prominent causes of PPD allergy (weighted average 41.8% and 10.6%) (59). Results from a recent German study were overall in accordance with results from the European study (63). Thus, consumer hair dye exposure caused 22% of positive PPD patch test reactions and occupational exposure 23%. It is foreseen that the prevalence of PPD allergy will remain high if PPD exposure from hair dyes is not further restricted. Taken together, the EU regulation on PPD hair dye exposure has not had a *detectable effect* on the prevalence of PPD allergy and the morbidity caused by PPD.

Figure 9. Temporal trends of PPD allergy (%) at St Johns, London, UK (1982-2004) (55)

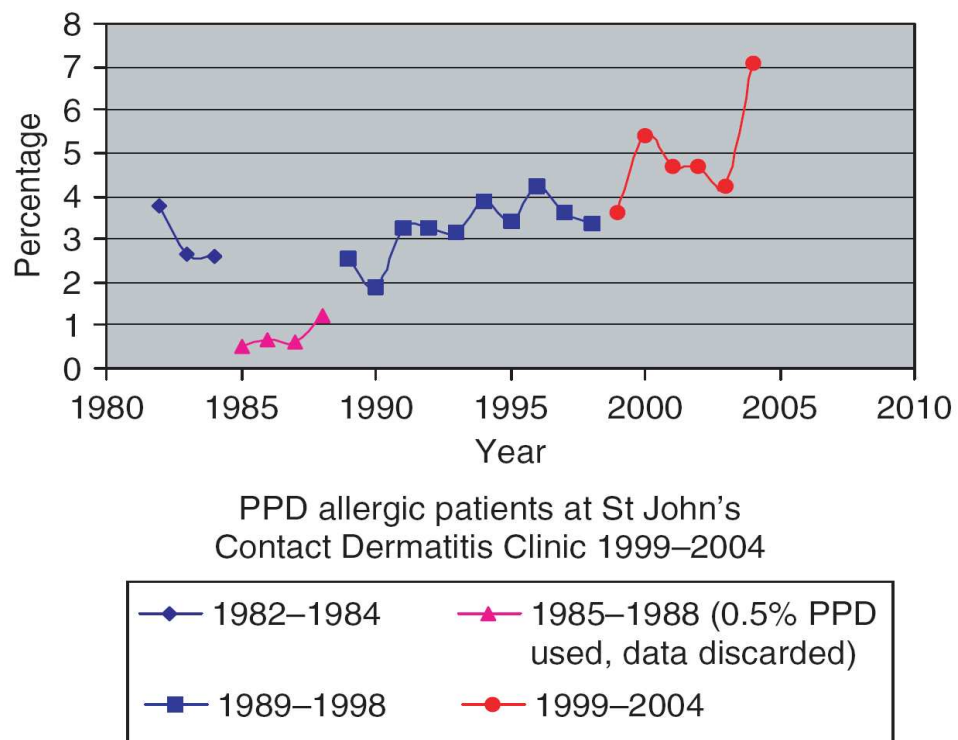


Figure 10. The prevalence (%) of positive patch test reactions to PPD among 21515 dermatitis patients from selected European patch test clinics (2003-2007) (59).

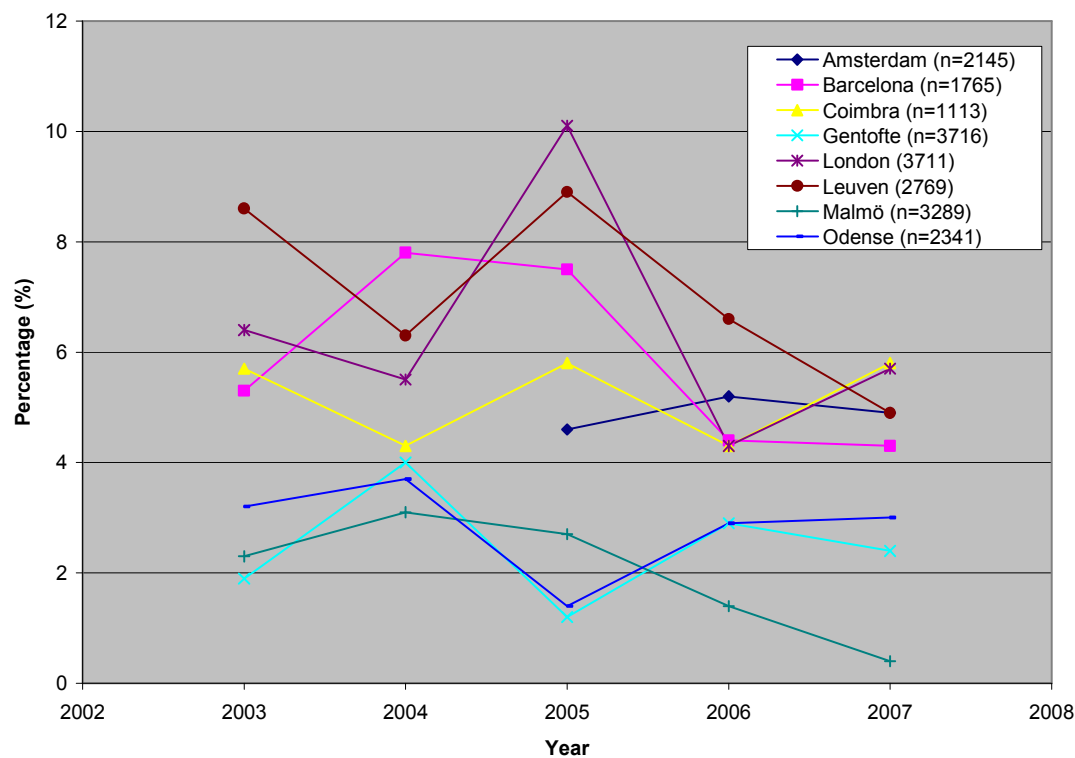


Figure 11. The prevalence (%) of positive patch test reactions to p-phenylenediamine among European patch test populations (60).

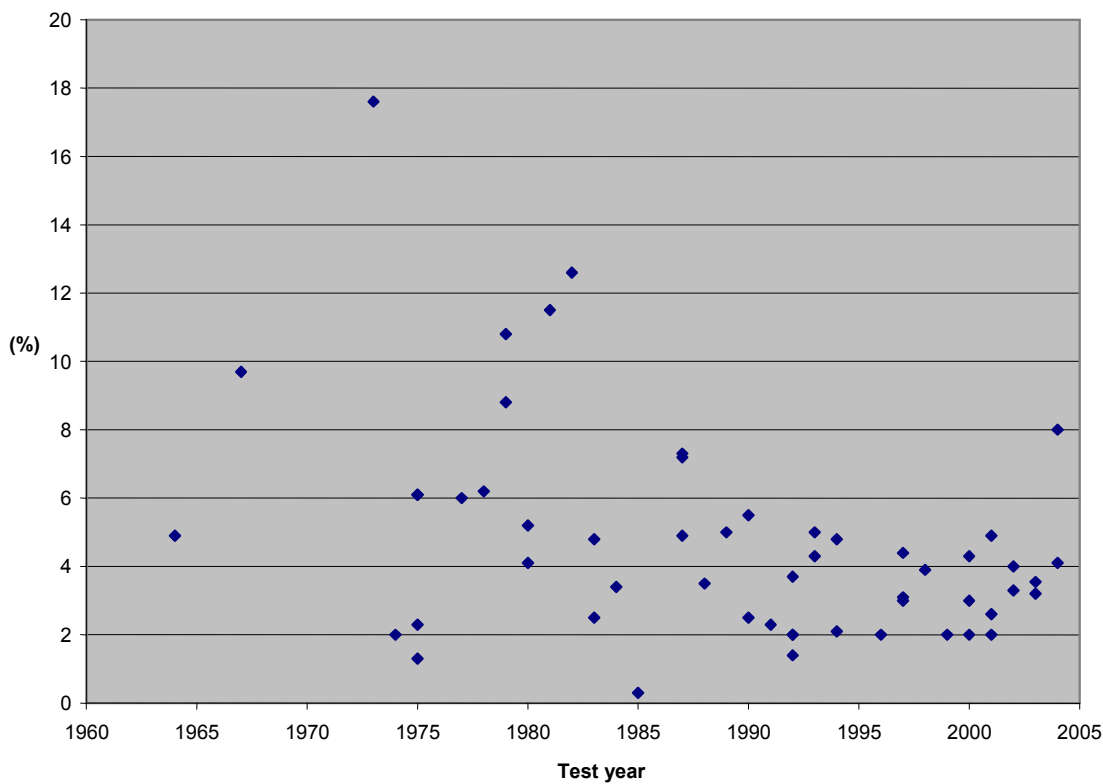


Table 3. Lifetime prevalence of temporary black tattoo in Danish adults from the general population (62).

General population n= 3 441		
Age (years)	male n=1536	female n=1905
18-24	7/77 (9.1 %)	43/129 (33.3 %)
25-34	16/165 (9.7 %)	33/221 (14.9 %)
35-70	34/1294 (2.6 %)	83/1555 (5.3 %)
Total	57/1536 (3.7 %)	159/1905 (8.3 %)

10.3.4

Methyldibromo glutaronitrile

MDBGN has been banned in stay-on products since 2003 and in rinse-off products since 2007.

Studies investigating temporal changes of MDBGN allergy

Studies have investigated the development of MDBGN allergy over time. A 10-year multicentre analysis on the prevalence of MDBGN allergy in 16 centres in 11 countries showed a rapidly increasing trend of MDBGN allergy between 1991 (0.7%) and 2000 (3.5%) (Fig. 4) (47). Also, an increase in the prevalence of MDBGN allergy was observed in the Netherlands from 0.5% in 1991 to 4% in 1994 (64). Finally, a significant increase of MDBGN allergy was observed in London between 1989 and 2000 (Fig. 5) (51).

Few studies have evaluated the effect of regulation. In Denmark, the prevalence of MDBGN allergy decreased from 4.6% in 2003 to 2.6% in 2007 ($P < 0.001$) in 19 279 dermatitis patients (Fig. 12) (65). By courtesy of Professor An Goossens, Department of Dermatology, University Hospital, Katholieke Universiteit Leuven, Belgium, a decrease of MDBGN allergy was observed in dermatitis patients who were routinely patch tested with MDBGN in recent years (3.5% in 2005 to 1.5% in 2009). Finally, IVDK data showed a rapid decrease of MDBGN allergy in recent years independent of patch test concentration (Fig. 13).

Other recent MDBGN allergy prevalence estimates

2005/2006 clinical patch test data registered in 10 European countries and reported to the ESSCA, offered prevalence estimates of MDBGN allergy in both Western, Southern, Central and Northeastern Europe being respectively, 1.2%, 0.1%, 5.6% and 1.5% (19). Unpublished data from the period 2004-07 revealed that the mean prevalence of MDBGN allergy in 11 British patch test centres was 1.0% (courtesy Dr. Statham, Singleton Hospital, Swansea, UK). In Budapest, Hungary, the prevalence of MDBGN allergy was 1.7-1.9% during the period 2007-2009 (courtesy from Professor Temesvári, Department of Dermatology, Venerology and Dermatoooncology, Semmelweis University Budapest). The Bulgarian Dermatological Society recently performed a national campaign including 5 university centres in the biggest Bulgarian cities (Courtesy, Dr Razvigor Darlenski,

Department of Dermatology and Venereology, Medical Faculty, Sofia, Bulgaria). Of 220 patch tested patients in 2009, 5.4% were MDBGN allergic.

Conclusion

Data analysis from 3 large European patch test centres demonstrates a decrease of MDBGN allergy after prohibition of MDBGN in cosmetic products. It is foreseen that the prevalence of MDBGN allergy will continue to decrease in these countries and elsewhere. Taken together, the EU regulation on MDBGN exposure has had a *likely effect* on the prevalence of MDBGN allergy and morbidity caused by MDBGN.

Figure 12. Decrease of MDBGN allergy (%) in Danish dermatitis patients following regulation (65).

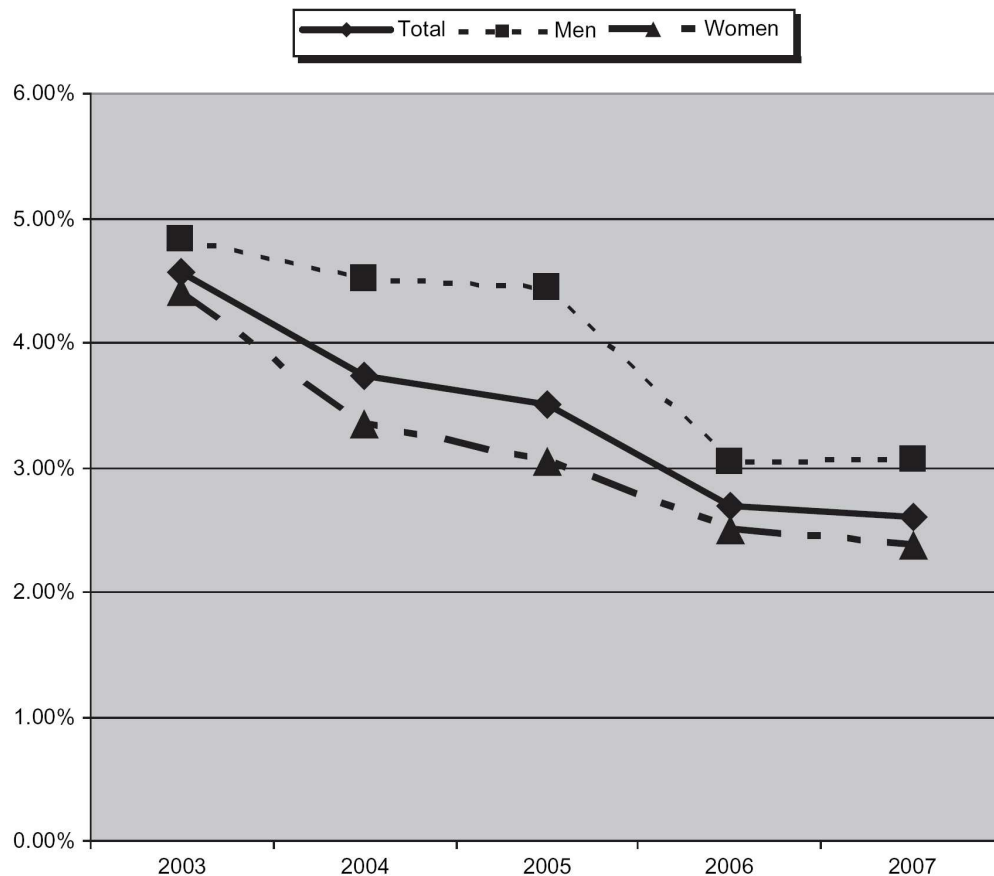
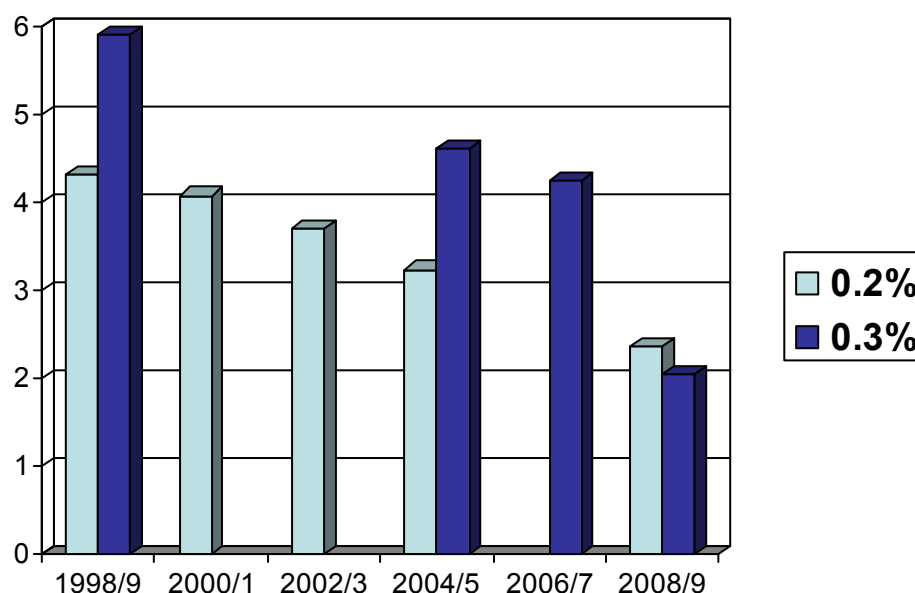


Figure 13. Time trends based on the bi-annual frequencies of sensitization to MDBGN in different concentrations, 1998–2004 as Euxyl K 400 (MDBGN 0.2% and phenoxyethanol 0.8%). In 2005 MDBGN 0.2% was tested in the monitor-series, in parallel with the baseline series. The decrease of MDBGN 0.2% and 0.3% was significant ($p < 0.0001$). The slight difference between both preparations in 2008-09 was not significant (IVDK 2010, unpublished).



10.3.5 Isoeugenol

According to the Regulation No 1223/2009, the presence of isoeugenol must be indicated in the list of ingredients, when their concentrations exceed 0.001% in leave on products and 0.01% in rinse-off products.

Studies investigating temporal changes of isoeugenol allergy

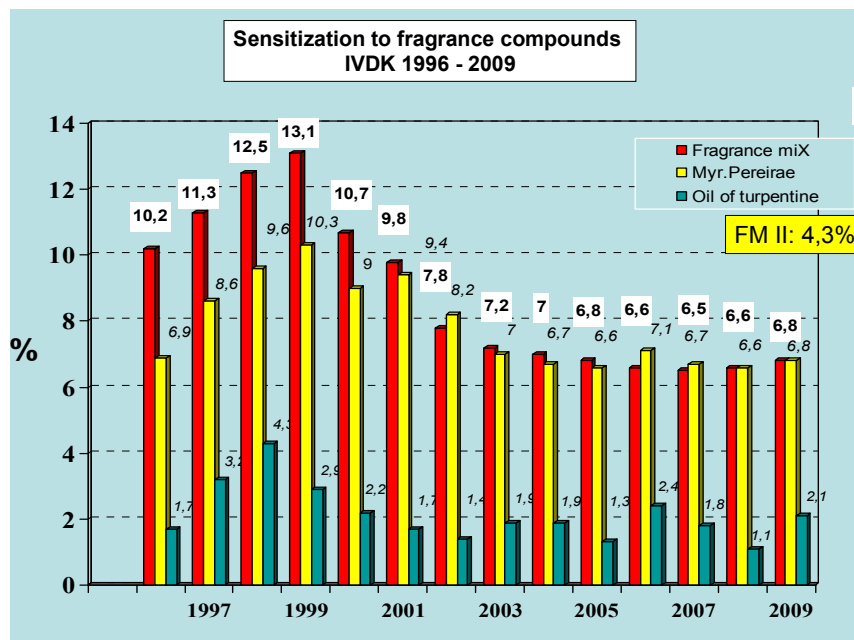
Although isoeugenol is not part of the German or European standard series, the substance has repeatedly been tested in selected and unselected patients in larger studies. Two studies covered the period before 2000: In one large European multicentre study, isoeugenol patch testing was performed in 1072 unselected patients and 1.9% had a positive reaction (66). In a UK study, the prevalence of isoeugenol allergy increased significantly over a 17-years period (67). UK patch test data collected from 3 636 patients for the period 2001-2005 were also analyzed for isoeugenol allergy (68). The overall prevalence of sensitization was 2.7%. Interestingly, there was a significant increasing trend over the years despite a reduction of the maximum recommended concentration of isoeugenol in cosmetics (please see part II) (68). IVDK-data registered between 1996 and 2002 were analyzed with regard to contact allergy to fragrances (Fig. 14) (69). Based on a reaction rate to isoeugenol of 18.9% in fragrance mix (FM) I positives, the number of isoeugenol positives can be extrapolated from the frequencies of sensitization to the FM I, ranging from 10.2% in 1996 to 7.8% in 2002, with the highest rate (13.1%) in 1999 (Fig. 14). The extrapolated frequencies for isoeugenol would then be respectively, 1.9%, 2.5% and 1.5% (Table 4). In the most recent analysis of patch test results (2005 to 2009) from the IVDK network, 18.0% of FM I allergic patients also reacted to isoeugenol (70). The prevalence of isoeugenol allergy, extrapolated from the prevalence of FM I allergy (6.8%) would then be 1.2% (70). The presented

extrapolations from the prevalence of FM I allergy were confirmed by testing isoeugenol alone in unselected patients (70). Recently, a patch test study was conducted using the 26 fragrances required to be labelled according to current European regulation, among them isoeugenol (71). The substances were tested in 2 063 unselected patients during a 6-months period. The sensitization rate found was 1.1% (CI95% 0.7–1.6) (72).

Table 4. Frequencies of sensitization to isoeugenol extrapolated from the frequencies of sensitization to the FM I and as a result of single testing (69).

Year	Sensitization to isoeugenol (%)	
1996	1.9	Extrapolated
1999	2.5	
2002	1.5	
2009	1.2	
2004	1.1	Tested (n=2063)

Figure 14. Temporal trends of fragrance allergy in >120.000 German dermatitis patients.



Conclusion:

The prevalence of contact allergy to FM I decreased significantly when the periods before 2000 and after 2000 are compared (69;72-74). This may be explained by a decreased use in products, and also by a lower concentration, after the recommended concentration had been lowered (75). A statistics of the International Nomenclature of Cosmetic Ingredients (INCI) names declared on cosmetics and household products (n= 5 451) revealed, that only ~3% of the products contained isoeugenol (76).

Extrapolated frequencies of isoeugenol allergy indicated a decrease by IVDK data (69). Opposite trends were observed in a UK study (67). In view of such a relative small proportion of products containing isoeugenol, a sensitization rate of ~ 1% seems high. This may be due to a recommended concentration still too high, and/or lack of compliance with the recommendation.

As isoeugenol was recognized as an important allergen, industry is increasingly using derivatives, ethers and esters of isoeugenol (77). However, patients sensitized to isoeugenol reacted to esters, and less to ethers, due to cross-reactions or to metabolism of the esters in the skin (78;79). Some of the products contained derivatives exceeding the maximum permitted concentration considerably (77).

10.3.6 Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

According to the Regulation No 1223/2009, the presence of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) must be indicated in the list of ingredients, when their concentrations exceed 0.001% in leave on products and 0.01% in rinse-off products.

Studies investigating temporal changes of HICC allergy

Some European patch test clinics added HICC to their baseline series in 2005 or 2006, and yielded positive reactions to HICC in 0.5% (Southern Europe) to 2.7% (Central Europe) of patients tested (19). Between 2000 and 2001, the German Contact Dermatitis Research Group tested HICC in a 'monitor series' together with the standard series in unselected patients, and included HICC 5% pet. in the German patch test baseline series in January 2002 (80). Since then, HICC persistently elicits positive reactions in 2.2-2.5% in the departments of dermatology of the IVDK. Nardelli et al. from Leuven, Belgium, reported positive patch test reactions to HICC as part of the baseline series in 2.1% (62 out of 2901) of consecutive patients tested between 2002 and 2005 (72). The Danish Contact Dermatitis Group (DCDG) observed sensitization to HICC in 2.1% of the patients tested in 2003 and in 2.8% of those tested in 2007 (81).

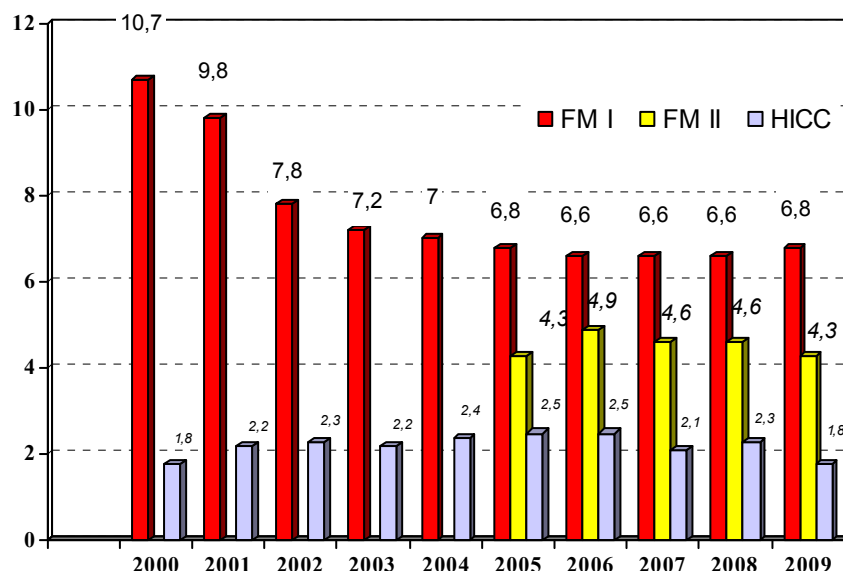
In 2008, Bruze et al. on behalf of the European Society of Contact Dermatitis (ESCD) and the European Environmental Contact Dermatitis Research Group (EECRDG) reviewed patch test experience with HICC and recommended to include this fragrance chemical in the European baseline patch test series (82).

Exposure to HICC

With regard to the concentration of exposure, there are different types of data, which are based on systematic and sporadic analyses (83). Products with high concentrations belong to the group of fine fragrances, and lesser to deodorants. Domestic products do not seem to be a major source of exposure to HICC. During the 1990's, the concentration in fine fragrances exceeded in average 3% whereas in

the years after 2000, they were lower, but still exceeding 0.1 and 0.5% (83). The high concentrations may explain the high sensitization rates found.

Figure 15. Temporal trends of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), FM I and fragrance mix II contact allergy.(IVDK 2000 – 2009; frequencies standardized for sex and age)



Conclusion:

Sensitization to HICC remains frequent in European patch test populations. In Germany, Austria and Switzerland there was a (non-significant) increase with frequencies between 1.8% (OR 1.02 (CI95% 0.995 – 1.042) and 2.5% (Fig. 15). Until 2008, none of the sensitization data mentioned above indicated decreasing trends of sensitization to HICC. The IFRA recommendation was established in 2003. In the year 2009, the first decrease down to 1.8% was noted. However, it is too early to attribute this decrease unequivocally to measures taken to reduce the use concentration of HICC.

10.4 Dose-response test data

A recent meta-analysis of elicitation threshold values obtained from patch testing showed a small variation between the allergens. This knowledge may stimulate the thoughts on introducing a generic approach for limitations of well-known allergens (Fig. 16 and Table 5) (84). No clear relationship was found between levels which induce sensitization according to data from the Local Lymph Node Assay (LLNA) and elicitation thresholds. This means that individuals who have developed a contact allergy, will not all be protected by limits based on LLNA data.

Figure 16. Logistic dose-response curve for 16 patch test elicitation dose-response studies with MCI/MI, formaldehyde, nickel, cobalt, chromium, isoeugenol, HICC and MDBGN (84).

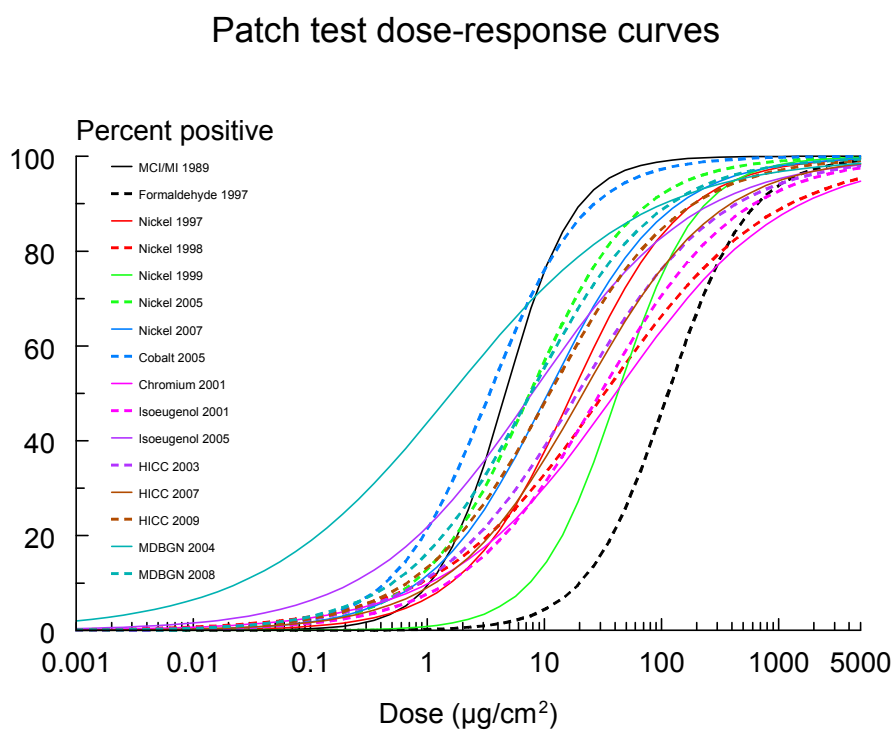


Table 5. ED₁₀ patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens MCI/MI, formaldehyde, nickel, cobalt, chromium, isoeugenol, HICC and MDBGN (84).

Study	Number patients	ED₁₀ (µg/cm²)	95 % interval
MCI/MI	12	1.05	0.17 – 2.27
Formaldehyde	20	20.1	4.09 – 43.9
Nickel 1997	24	1.58	0.32-4.04
Nickel 1998	19	0.8	0.078-2.59
Nickel 1999	26	7.49	2.42-14.5
Nickel 2005	13	0.74	0.066-2.38
Nickel 2007	20	0.82	0.13 – 2.37
Cobalt 2005	11	0.44	0.033-1.3
Chromium	17	1.04	0.0033 – 5.55
Isoeugenol 2001	24	1.48	0.22 – 4.74
Isoeugenol 2005	13	0.23	0.0073-1.32
HICC 2003	18	0.85	0.062 – 3.26
HICC 2007	14	1.17	0.043 – 5.05
HICC 2009	17	0.66	0.052-2.35
MDBGN 2004	19	0.025	0.00021-0.19
MDBGN 2008	18	0.50	0.052 – 1.69

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Conclusions for part III

The EU regulation of chromium in cement is the most successful regulation of exposures to a potent contact allergen. Chromium contact allergy and severe chronic hand eczema caused by chromium, one of the most common occupational skin diseases, have been minimized within the EU member states. In countries without a similar regulation e.g. Asia, the disease remains frequent. One of the reasons for the remarkable success is that chromium in cement is a by-product in the production and not an essential element for the function and quality of the final product. It was therefore possible to aim at the lowest technical possible level of hexavalent chromium, close to the elicitation level in human dose response studies performed in already sensitized individuals.

Even if chromium is a ubiquitous trace metal, the regulation of a specific exposure has had a major influence on morbidity caused by chromium. This observation added to the general understanding, i.e. contact sensitization is caused by specific occupational and/or domestic exposures and for this reason, it is practical and technical preventable. The main chromium problem today relates to leather goods exposure, in particular shoes. This problem needs to be targeted with replacement of the chromium tanning with more modern technologies as e.g. enzymatic.

The EU nickel directive was introduced in year 2000 but had already been partially enforced in Denmark since 1990. While the Chromium Directive targeted a specific production method, the Nickel Directive targeted various consumer products such as buttons, cheap jewellery etc. It is therefore noteworthy that it has been possible, in several EU member states, to obtain a significant effect with a decrease in both frequency of nickel contact sensitization and the related clinical diseases in the youngest generation. Several steps can improve the effect of the Nickel Directive, e.g. introduction of more accurate control methods and information campaigns. In geographical areas as the US and Asia, where a similar regulation has not been implemented, the frequency of nickel allergy is increasing markedly in the young generation in contrast to the EU. Both the Chromium (cement) and the Nickel Directive have had documented major positive health effects. Also, there has been no major technical or economic consequence for industry. The price for cement has increased marginally. Nickel has not been replaced by other contact sensitizing metals e.g. cobalt and palladium in jewellery.

Identical preservatives are present in consumer products (e.g. cosmetics) and industrial products (e.g. liquid soap, paints). Despite regulation and ban of certain preservatives the total burden of contact allergy caused by preservatives is increasing. The most obvious explanation is the liberal regulation of this group of highly reactive chemicals. The concentration limits are typically far higher than the concentration needed for the preservation of a standard product and much higher than the minimal elicitation concentration established in human dose response studies and consequently higher than the induction concentration for contact allergy. Most safety failures relate to the introduction of preservatives in cosmetics and paints. The most obvious being the isothiazolinones and methyl-dibromo glutaronitrile. The main problem lies in the interpretation of the Quantitative Risk Assessment method which tends to underestimate the contact sensitizing risk as the effect of repeated exposure from even moderate to low concentration of contact sensitizers in consumer products is underestimated. The most recent failures in this area relates to the permission of (methyl) isothiazolinones as preservative in cosmetics in 2007. The chemical is a well recognised contact sensitizer. A steep increase in the prevalence of sensitization is reported from several EU countries. The wisdom is clear; no sensitizing chemical should be permanently permitted in mass-market product types such as cosmetics.

The fragrance industry has within recent years recommended a significant decrease in the maximum use concentration of two chemicals, isoeugenol and HICC. Preliminary data indicate that this initiative has had a significant effect on the prevalence of isoeugenol contact allergy but hitherto not on the prevalence of HICC allergy. Perfumes contain many chemicals and there are many closely related alternatives. A decrease of some fragrance allergens should be carefully balanced out by an increase of alternative fragrance chemicals. As for the preservatives, an indication for successful prevention of fragrance allergy should be the total

number contact sensitized for the group of chemicals with an identical use e.g. defined product types such as deodorants.

Taken together, it is difficult to evaluate whether introduced regulation of both preservatives and perfume allergens have had a measurable effect.

The permitted use concentration of free PPD in hair dyes has recently been reduced from 3% to 2%. As PPD is an extreme potent sensitizing chemical, this decrease is not expected to have any effect on skin diseases caused by hair dyes. A safe concentration of permanent hair dye chemicals has not yet been defined, at least not in a concentration range where any shade of hair colour can be achieved.

Annex I

Organisation and member states contacted in relation to the project

Standard letter:

Re: Skin allergies

I am coordinator of an EU-project concerning a review of methodologies and approaches to assess the inherent skin sensitization potential of chemicals.

The project is performed in collaboration with Prof. Anders Boman from Stockholm, Prof. Jean-Pierre Lepoittevin from Strasbourg and Prof. Axel Schnuch from Goettingen.

One of the aims of the project is to assess the possible effect of limits/thresholds for allergens in the reduction/prevention of the incidents and mobility of contact allergy cases in EU.

I therefore on behalf of the project group, kindly ask you to consider if you have information, which will be valuable for us in this assessment in particular concerning national regulations which limits chemicals, which may cause skin allergy

In this case we would like to receive information on the substances concerned, the limits and the methods used to arrive at the limits.

If you have other information, you think could be of value, we will of course be very happy to receive it. We are aware of scientific studies published on PubMed.

Due to a very tight project schedule we would very much appreciate to receive information before July 15th 2010. Please answer to: susse@geh.regionh.dk or by ordinary mail to me (address as above). If you have any questions concerning the project I will be happy to answer these.

Kind regards,

Prof. Torkil Menné

The European Trade Union federation - Textiles, Clothing and Leather, Member States European Union:

Austria

Dr Brigitte MAGISTRIS
Bundesministerium für Gesundheit,
Familie und Jugend

Croatia

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Greece

Mr Theodoros PAPADIMITRIOU

Hellenic Centre for Infectious Diseases
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Mrs Chrisoula BOTSI

Hellenic Centre for Infectious Diseases
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Mr Konstantinos KAMPOURAKIS

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Department of Prevention and Communication - Office III

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Community Care
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Ministry of Health the Elderly &
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Department of International Research
Programs and Institutional relations
Ministry of Health and Consumer Affairs

Annex II

Comments and data received from the following parties contacted by the standard letter. Data incorporated in the text.

Members of European Society of Contact Dermatitis

Comments and data received

Members of IVDK

Comments and data received

Members of ESSCA

Comments and data received

Members of SCDRG

Comments and data received

Members of GERDA

Comments and data received

Members of COLIPA

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Identifying and characterizing chemical skin sensitizers without animal testing: Colipa's research and method development program.

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Abstract

The sensitizing potential of chemicals is usually identified and characterized using one of the available animal test methods, such as the mouse local lymph node assay. Due to the increasing public and political concerns regarding the use of animals for the screening of new chemicals, the Colipa Skin Tolerance Task Force collaborates with and/or funds research groups to increase and apply our understanding of the events occurring during the acquisition of skin sensitization. Knowledge gained from this research is used to support the development and evaluation of novel alternative approaches for the identification and characterization of skin sensitizing chemicals. At present one in chemico (direct peptide reactivity assay (DPRA)) and two in vitro test methods (cell based assays (MUSST and h-CLAT)) have been evaluated within Colipa inter-laboratory ring trials and accepted by the European Centre for the Validation of Alternative Methods (ECVAM) for pre-validation. Data from all three test methods will be used to support the development of testing strategy approaches for skin sensitizer potency prediction. The replacement of the need for animal testing for skin sensitization risk assessment is viewed as ultimately achievable and the next couple of years should set the timeline for this milestone.

Belgium

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International Relations Assistant

FPS Health, Food Chain Safety and

Environment

Comments and data received

Bulgaria

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Expert, Political Cabinet

Ministry of Health

Comments and data received

Hungary

Ms Brigitta GYEBNÁR

Department for Public Health

Ministry of Health

Comments and data received

Sweden

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Swedish National Institute of Public
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Comments received

United Kingdom

Sheffield Teaching Hospital – David Gowkrodger,

Comments received

National Health Service – Barry Stratham, dermatologist, Wales

Comments and data received

Acknowledgement

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