

Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data

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Abstract

In this paper, we propose a quantitative risk assessment methodology for skin sensitization aiming at the derivation of ‘safe’ exposure levels for sensitizing chemicals, used e.g., as ingredients in consumer products. Given the limited number of sensitizers tested in human sensitization tests, such as the human repeat-insult patch test (HRIPT) or the human maximization test (HMT), we used EC3 values from the local lymph node assay (LLNA) in mice because they provide the best quantitative measure of the skin sensitizing potency of a chemical. A comparison of LLNA EC3 values with HRIPT and HMT LOEL, and NOEL values was carried out and revealed that the EC3, expressed as area dose, can be used as a surrogate value for the human NOEL in risk assessment. The uncertainty/extrapolation factor approach was used to derive (a) an ‘acceptable non-sensitizing area dose’ (ANSAD) to protect non-allergic individuals against skin sensitization and (b) an ‘acceptable non-eliciting area dose’ (ANEAD) to protect allergic individuals against elicitation of allergic contact dermatitis. For ANSAD derivation, interspecies, intraspecies and time extrapolation factors are applied to the LLNA EC3. For ANEAD derivation, additional application of a variable sensitization–elicitation extrapolation factor is proposed. Values for extrapolation factors are derived and discussed, the proposed methodology is applied to the sensitizers methylchloroisothiazolinone/methylisothiazolinone, cinnamic aldehyde and nickel and results are compared to published risk assessments.

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1. Introduction

In the past, allergic reactions to chemicals were often regarded as all-or-none responses that lack dose–response relationships and thresholds. This can probably be attributed to the fact that the first contact (and often repeated contacts) even with relatively high concentrations of a sensitizer go unnoticed because no signs or symptoms of allergy occur. Nevertheless, this contact can lead to sensitization, which often occurs without noticeable clinical signs and symptoms. Once sensitization is established subsequent contact with the same sensitizer—sometimes even at concentrations several orders of magnitude lower—will lead to symptoms of

allergic contact dermatitis. This illustrates the typical ‘working manner’ of the specific immune system, the main task of which is to fight microbial infections. The immune response is characterized by a ‘learning phase’ without symptoms (termed primary immune response or sensitization phase) followed by the immune response effector phase (also termed secondary immune response or elicitation phase).

From research done during the last 15 years, e.g., using experimental human sensitization to 2,4-dinitrochlorobenzene (DNCB) (Friedmann et al., 1983; White et al., 1986; reviewed in Friedmann, 1990), we know today that skin sensitization as well as allergy elicitation only occur above threshold doses and follow predictable dose–response relationships (Basketter, 1998; Basketter et al., 2002; Boukhman and Maibach, 2001; Kimber et al., 1999, 2002; Marzulli and Maibach, 1974; Roggeband et al., 2001; Scott et al., 2002; Van Och et al.,

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2001). It has become clear that skin sensitization is not different from other toxicological effects in this respect. This increasing understanding is also reflected in the way how skin sensitization is dealt with in toxicological risk assessment: while older test systems aimed at pure hazard identification and were used for classifying chemicals either as sensitizers or non-sensitizers, newly developed test systems deliver dose–response curves and potency information that will enable more adequate risk assessment (Kimber et al., 2001, 2002).

2. Potency information

During recent years, our understanding of the mechanisms underlying immune reactions to chemicals, including allergic contact dermatitis, has increased greatly (for reviews see Griem et al., 1998, 1997; Kimber et al., 2002, 1999; Smith and Hotchkiss, 2001). The dose of a chemical which is necessary to either sensitize a person or elicit an allergic reaction in a sensitized person depends on a number of parameters (listed in Table 1). These comprise (a) chemical-specific properties, e.g., skin penetration capacity and ability to bind (covalently) to amino-acid side chains of proteins (Alvarez-Sanchez et al., 2003), (b) host-specific parameters, e.g., expression and

polymorphisms of genes relevant for metabolism of a prohaptent into a haptent (Anderson et al., 1995; Smith Pease et al., 2003), and (c) availability of T lymphocytes with T-cell receptors specific for the haptent-peptide conjugates formed (Budinger et al., 2001), as well as circumstantial influences, such as preexisting dermal irritation (Allenby and Basketter, 1993) and presence of solvents enhancing skin penetration (Dearman et al., 1996).

The sensitization potency of a chemical can be experimentally determined in both animal and human tests (reviewed in Kimber et al., 2001). Tests in guinea pigs (guinea pig maximization test, Buehler test) have been used for over 30 years to identify possible sensitization hazards (OECD, 2002). However, guinea pig tests provide only poor information with regard to sensitizing potency. More recently, modified guinea pig protocols have been proposed in order to generate useful potency data (Andersen et al., 1995; Van Och et al., 2001; Yamano et al., 2001), but these protocols have not been validated up until now. The critical points with regard to the potency estimation that can be derived from guinea pig experiments, are, for example, circumventing the skin barrier by intracutaneous injection, elicitation of a local inflammatory reaction and activation of Langerhans cells by use of adjuvans, subjective endpoint determination, and the dependency of the sensitization

Table 1
Some parameters determining sensitization potency

Parameter	Explanation
Skin penetration	Only after passing the skin barrier a chemical can interact with cells of the immune system and elicit a sensitization; the penetration depends on the nature of the chemical itself (size, lipophilicity, and reactivity) as well as on circumstances, such as skin hydration, location of affected skin on the body, presence of solvents that promote penetration
Protein binding	The chemical can only be recognized specifically by T lymphocytes after binding covalently to soluble protein or membrane proteins; in the case of very protein-reactive chemicals a large amount will bind inefficiently to protein in the stratum corneum, in the case of poorly binding chemicals a large amount will be transported away from the site thus lowering the local concentration
Metabolism	Some sensitizing chemicals (prohaptens) are not protein-reactive as they come but have to be metabolized into protein-reactive intermediates (haptens) first; the efficiency of metabolic conversion depends on enzyme expression and, eventually, on genetic polymorphism
Efficiency of uptake by Langerhans cells	Only haptent bound to soluble protein, membrane proteins of Langerhans cells or other damaged/dead skin cells can be taken up by Langerhans cells and is thus available for presentation to T cells
Induction of migration and maturation of Langerhans cells	Langerhans cells must be induced to leave the skin and to migrate to the draining lymph node and to mature (e.g., upregulation of costimulatory membrane molecules) into dendritic cells; this activation can be caused by the sensitizing chemical itself (cytotoxic, irritative effect) or by circumstantial influences, such as physical injury or irritation, UV irradiation, or chemical irritation
Presentation of haptentated peptide–MHC complexes	In order to activate T lymphocytes, one or more kinds of haptentated peptides must be cut out of the haptentated protein in a partial degradation by proteases and the haptentated peptide must fulfill the peptide-binding motif (i.e., carry adequate amino acid side chains in certain positions) allowing it to bind to a class II major histocompatibility complex (MHC) molecule, so that it can be presented as a haptentated peptide–MHC complex at the DC surface
Foreignness of haptentated peptide–MHC complexes	T lymphocytes must be available that carry a T-cell receptor specific for the presented complex of MHC molecule and haptentated peptide; suitable T lymphocytes may be lacking due to genetic polymorphism in the T-cell receptor gene segments or due to immunological tolerance (inactivation or deletion of T lymphocytes with certain T-cell receptors)

rate on the challenge concentration, and these points have been discussed elsewhere (Basketter et al., 1997).

The animal test best suited for providing dose–response information is the local lymph node assay (LLNA) in mice (Dean et al., 2001; Haneke et al., 2001; Kimber et al., 2002; OECD, 2002; Sailstad et al., 2001). In the LLNA, the test substance is applied onto the mouse ear on three consecutive days. On the sixth day, the proliferation of lymphocytes in the draining lymph node, caused by the primary immune reaction, is measured, e.g., by incorporation of radioactive labeled ^3H -methyl thymidine into the DNA of proliferating lymphocytes.

The LLNA was originally used for qualitatively identifying sensitizing chemicals. A stimulation index (SI) of 3 or higher is used to differentiate sensitizers from non-sensitizers. This procedure shows good agreement with classical guinea-pig test results and human experience studies (Basketter and Scholes, 1992; Basketter et al., 1994; Haneke et al., 2001; Van Och et al., 2001). In the LLNA, the sensitizing potency is expressed as the EC3 value, which is the effective concentration of a chemical (percent of chemical in vehicle) required to produce a 3-fold (i.e., threshold level) increase in the proliferation of lymph node cells compared to vehicle-treated controls. Potency information (NOEL and/or LOEL expressed as percent of chemical in vehicle) can also be obtained from human tests, such as the human repeat-insult patch test (HRIPT) and the human maximization test (HMT) (Draize et al., 1944; Kimber et al., 2001; Kligman, 1966). In these assays, the test substance is applied topically on the skin of the back or the arm for 24 h under occlusion. Nine to 12 repeated applications are done over a two to three week period. Two to three weeks after the last induction application, a challenge patch is applied for 24 h and subsequently the skin reaction is scored.

Several experimental investigations, especially with DNCB in human subjects, revealed that the induction of skin sensitization is dependent on the dose of the test chemical per skin area (Friedmann, 1990; White et al., 1986). Therefore, it has been widely recommended that sensitization thresholds obtained in LLNA, HRIPT or HMT should be expressed as the area dose (Boukhman and Maibach, 2001; Robinson et al., 2000; Roggeband et al., 2001). The area dose can either be given as the molar area dose (i.e., in units of $\mu\text{mol}/\text{cm}^2$) or as the specific area dose (i.e., in units of $\mu\text{g}/\text{cm}^2$).

3. Current risk assessment concepts

Skin sensitization is an important occupational and consumer problem. It is mandatory to ensure that chemicals, ingredients and products do not cause allergic contact dermatitis in workers and consumers.

Current regulation uses qualitative systems to classify chemicals and mixtures/formulations as either sensitizers or non-sensitizers (Basketter et al., 1999a; Kimber et al., 2001; OECD, 1999, 2001; Roggeband et al., 2001). Hazard identification constitutes a first, fundamental step in protecting workers and consumers. However, when skin contact with a potential sensitizer cannot be avoided completely a responsibility to conduct a quantitative skin sensitization risk assessment exists in order to prevent large numbers of consumers from developing allergic contact dermatitis (Menne and Wahlberg, 2002).

For fragrance materials, against which consumers will be skin exposed during the intended use, it was suggested to assess the safety with regard to sensitization by performing a HRIPT in at least 100 normal healthy volunteers using a 10-fold higher concentration than the intended use concentration in consumer products. Before human testing, the test concentration should be tested negative in a Buehler assay in guinea pigs (Api, 2002). The advantage of this approach is that it involves direct testing in humans. While this approach may be feasible for chemicals that are intended for use in products designed to come into contact with the skin of consumers (e.g., cosmetic ingredients), it cannot be used for other chemicals. For ethical reasons, chemicals with mutagenic and carcinogenic properties cannot be tested in humans. Moreover, if the dose has been selected appropriately according to the approach by Api (2002), the HRIPT should normally give a negative result. Thus, no information is available on the margin of safety between the consumer exposure level tested and the sensitization threshold. Also it is unclear, how individuals are to be protected that are more susceptible to skin sensitization than the ‘normal healthy’ volunteers used for testing.

It has been proposed to group sensitizing chemicals according to their relative sensitizing potency as determined in the LLNA, i.e., their EC3 value (Kimber et al., 2002). The defined categories could then be compared to the relative sensitization potency in humans determined on the basis of clinical experience and/or prevalence of allergic contact dermatitis in the population (Dearman and Kimber, 2001). Overall, several studies report a good correlation between LLNA results and the sensitization potency in humans using this semiquantitative method (Basketter et al., 1994, 2001, 2002; Gerberick et al., 2001a,b). However, different numbers of categories and different EC3 ranges were used in these studies: Gerberick et al. (2001a) suggested five categories classifying the sensitization potential as “potent” when the measured LLNA EC3 value or the measured human NOEL value is $\leq 10 \mu\text{g}/\text{cm}^2$; “strong” for $10\text{--}100 \mu\text{g}/\text{cm}^2$; “moderate” for $100\text{--}1000 \mu\text{g}/\text{cm}^2$; “weak” for $1000\text{--}10,000 \mu\text{g}/\text{cm}^2$; and “extremely weak” for $\geq 10,000 \mu\text{g}/\text{cm}^2$. Kimber et al. (2002) defined five categories with boundaries of $<0.1\%$ ($25 \mu\text{g}/\text{cm}^2$), $0.1\text{--}1\%$ ($25\text{--}250 \mu\text{g}/\text{cm}^2$), $1\text{--}10\%$ ($250\text{--}2500 \mu\text{g}/\text{cm}^2$), $10\text{--}100\%$ ($2500\text{--}25,000 \mu\text{g}/\text{cm}^2$) and

>100% (i.e., non-sensitizing) (>25,000 $\mu\text{g}/\text{cm}^2$). Dearman and Kimber (2001) suggested classification into three categories, namely “potent” for EC3 values of <0.1% (corresponding to <25 $\mu\text{g}/\text{cm}^2$; see the following section), “moderate” for 0.1–10% (25–2500 $\mu\text{g}/\text{cm}^2$) and “weak” for $\geq 10\%$ ($\geq 2500 \mu\text{g}/\text{cm}^2$).

Based on the potency classes described by Gerberick et al. (2001a) a methodology for derivation of a ‘sensitization reference dose’ for sensitizers in consumer products has been developed (Felter et al., 2002; Gerberick et al., 2001a). As a starting point, the lower boundary of the potency category is used into which a given sensitizing chemical is grouped. Category boundaries are expressed in units of specific area dose. For “potent” sensitizers (i.e., area dose is $\leq 10 \mu\text{g}/\text{cm}^2$) 1 $\mu\text{g}/\text{cm}^2$ is used as a starting point. Uncertainty factors with values between 1 and 10 are applied for: (a) interindividual variability, accounting for susceptibility differences caused, among others, by age, sex, race, genetic factors and compromised skin; (b) product matrix, i.e., other components causing skin irritation or enhanced skin penetration; and (c) use pattern, accounting for factors affecting human exposure, such as skin site exposed, occlusion and dermal integrity. Combination of these three uncertainty factors by multiplication will result in a total uncertainty factor of up to 1000. The proposed risk assessment approach has been applied to the fragrance component cinnamic aldehyde (Gerberick et al., 2001a) and the preservative methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) (Robinson et al., 2000), for both of which LLNA and human sensitization potency data were available.

The present paper uses a more general extrapolation/uncertainty factor approach to derive two kinds of safe skin area dose levels, one for skin sensitization and one for elicitation of allergic contact dermatitis. We aimed at developing a methodology that allows to base quantitative risk assessment solely on LLNA data. We acknowledge that human potency data are valuable for risk assessment. However, for many chemicals quantitative human potency data are not available. For these and for new chemicals, LLNA results are more easily obtained, especially for chemicals with mutagenic, carcinogenic and/or reproductive toxicity hazards, as well as for chemicals not intended for consumer products. In this manuscript we argue that the LLNA EC3 not only is a measure for the relative sensitization potency of chemicals, as discussed currently in the literature, but provides absolute potency information that is applicable to quantitative risk assessment.

4. Quantitative risk assessment for sensitization

Human sensitization threshold data from HRIPT or HMT are available only for a limited set of chemicals.

Nowadays, these tests are usually only performed to confirm the safe use of potentially sensitizing chemicals in consumer products, such as cosmetics or household products (Api, 2002). They are normally not performed for industrial chemicals or contaminants in consumer products. Since the number of sensitizing chemicals for which human data exist is limited we first investigated if LLNA data could be used for quantitative risk assessment. To this end, we identified known human sensitizing chemicals, for which both, an EC3 value from LLNA and a NOEL and/or LOEL from HRIPT or HMT were available. In most of the human tests only one dose was tested, which causes problems when a high percentage of subjects were sensitized, i.e., when no LOEL (and no NOEL) was identified. Use was made of this data when the sensitization rate was below 50%; in these cases a factor of 3 for sensitization rates between 10 and 25% and a factor of 10 for sensitization rates between 25 and 50% were used to extrapolate to a LOEL value. The data set is shown in Table 2.

The reported concentrations were converted into specific and molar area dose values. For LLNA, the calculation was based on an exposed surface of 1 cm^2 per mouse ear (Robinson et al., 2000). Since according to the standard protocol, 25 μl test solution are distributed over this surface, multiplication of the concentration of the chemical in the test solution (in percent) with a factor of 250 results in the specific area dose value (in $\mu\text{g}/\text{cm}^2$). For human tests, the reported area dose was used if given in the literature. Otherwise it was calculated by dividing the amount of test substance by the area of the application site. If no quantitative information on amount of test material applied and/or the size of the application area was available, it was assumed that 400 mg of test solution was used on a $2 \times 2\text{-cm}$ Webril patch (4 cm^2) (Robinson et al., 2000), resulting in estimation of the specific area dose value (in $\mu\text{g}/\text{cm}^2$) by multiplication of the concentration of the chemical in the test solution (in percent) with a factor of 1000.

Comparison of the area doses of LLNA and human test results revealed that sensitization thresholds are very similar in mice and humans, despite of the fact that the area doses for different chemicals ranged over several orders of magnitude. Fig. 1A depicts the area dose values for NOELs in humans vs. murine EC3 values and Fig. 1B depicts LOELs in humans vs. murine EC3 values. Linear regression lines from the logarithmically transformed values were nearly identical for the NOEL and LOEL data and the slope was not significantly different from 1. This result indicates that sensitization area doses are directly comparable between mouse and man, i.e., a sensitization threshold of 10 $\mu\text{mol}/\text{cm}^2$ in mice corresponds to 10 $\mu\text{mol}/\text{cm}^2$ in humans. This observation is further supported by comparison of LOEL and NOEL values in Fig. 2A, which depicts all LOEL and NOEL data. Fourteen of 22 human LOEL-murine

Table 2
Comparison of NOEL/LOEL values for sensitization in humans with murine LLNA EC3 values

Chemical CAS No. molecular weight	Human study			Mouse study			
	Study type; effect level	Concentration [%] (solvent)	Area dose [$\mu\text{g}/\text{cm}^2$] ($\mu\text{mol}/\text{cm}^2$)	Reference	LLNA EC3 [%] (solvent) ^a	Area dose [$\mu\text{g}/\text{cm}^2$] ($\mu\text{mol}/\text{cm}^2$)	Reference
Amylcinamic aldehyde (2-(phenylmethylene)- heptanal) 122-40-7 202.3	HRIPT; NOEL	20 (diethylphthalate)	23,622 (117)	RIFM (1994b)	11 (A00)	2750 (13.6)	Smith and Hotchkiss (2001)
Aniline 62-53-3 93.1	HMT; LOEL	2 ^b (petrolatum)	1379 (14.8)	Kligman (1966)	37 (A00)	9250 (99.3)	Smith and Hotchkiss (2001)
Benzocaine 94-09-7 165.2	HRIPT; NOEL	2 (not given)	2000 (12.1)	Marzulli and Maibach (1974)	18 (DMF)	4500 (27.2)	Smith and Hotchkiss (2001)
Benzyl benzoate 120-51-4 212.2	LOEL	10 (not given)	10,000 (60.5)				
Benzylidene acetone 122-57-6 146.2	HMT; NOEL	30 (petrolatum)	20,690 (97.5)	RIFM (1970b)	17 (A00)	4250 (20.0)	Smith and Hotchkiss (2001)
Benzylidene acetone 122-57-6 146.2	HRIPT; LOEL	3 (petrolatum)	3000 (20.5)	Marzulli and Maibach (1980)	0.85 ^c (A00)	213 (1.46)	Ryan et al. (2000)
t-Butylglycidyl ether 7665-72-7 130.2	HMT; LOEL	0.3 ^b (petrolatum)	300 (2.05)	Gardiner et al. (1992)	31 (A00)	9300 (71.4)	Basketter et al. (1994)
Cinnamic alcohol (3-phenyl-2-propen-1-ol) 104-54-1 134.1	HMT; NOEL	4 (petrolatum)	4000 (29.8)	Steltenkamp et al. (1980b)	21 (not stated)	5250 (39.1)	Basketter et al. (2002)
Cinnamic aldehyde (3-phenyl-2-propenal) 104-55-2 132.2	LOEL	10 (petrolatum)	10,000 (74.6)				
Citral (trans-3, 7-dimethyl-2,6-octadienal) 5392-40-5 152.2	HRIPT; NOEL	0.5 (ethanol)	590 (4.46)	Danneman et al. (1983)	1.4 (A00)	350 (2.65)	Smith and Hotchkiss (2001)
Cobalt(II) salts 7440-48-4 58.9	LOEL	1.0 (ethanol)	1200 (9.08)		2.0 (A00)	500 (3.78)	Basketter and Scholes (1992), Basketter et al. (2000)
Diethylmaleate 141-05-9 172.2	HMT; LOEL	2.5 ^b as cobalt sulfate (petrolatum)	500 (3.29)	Steltenkamp et al. (1980a)	3.1 (A00)	780 (5.90)	Basketter et al. (2001)
DNCB (2, 4-dinitrochlorobenzene) 97-00-7 202.6	LOEL	1.0 (ethanol)	1000 (6.57)		6.6 (A00)	1700 (11.2)	Basketter and Scholes (1992)
Ethyl acrylate 140-88-5 100.1	HMT; LOEL	0.4 ^f (petrolatum)	361 ^d (6.13) (as Co)	Kligman (1966)	4.8 as cobalt chloride (DMSO)	297 ^e (5.04) (as Co)	Ikarashi et al. (1992)
Eugenol (2-methoxy- 4-allylphenol) 97-53-0 164.2	HRIPT; LOEL	0.0625 (acetone)	400 (2.32)	Marzulli and Maibach (1980)	2.5 ^g (A00)	625 (3.6)	Ryan et al. (2000)
Formaldehyde 50-00-0 30.0	one 48-h occluded patch; LOEL	8.8 (0.043)	8.8 (0.043)	Friedmann et al. (1983)	0.048 (mean) (A00)	12 (0.059)	Loveless et al. (1996)
	HRIPT; LOEL	4 (petrolatum)	4000 (40.0)	Marzulli and Maibach (1980)	28.7 (A00)	7175 (71.7)	Warbrick et al. (2001)
	HMT; LOEL	0.4 ^b (petrolatum)	400 (4.0)				
	HRIPT; NOEL	2.5 (ethanol)	1938 (11.8)	RIFM (1964)	9.8 (A00)	2500 (15.2)	Loveless et al. (1996)
	HRIPT; LOEL	8 (ethanol)	8000 (48.7)	Marzulli and Maibach (1980)			
	HRIPT; NOEL	0.037 (petrolatum)	37 (1.23)	Marzulli and Maibach (1974)	0.54 (acetone)	135 (4.50)	Hilton et al. (1998)

Table 2 (continued)

Chemical CAS No. molecular weight	Human study			Mouse study			
	Study type; effect level	Concentration [%] (solvent)	Area dose [$\mu\text{g}/\text{cm}^2$] ($\mu\text{mol}/\text{cm}^2$)	Reference	LLNA EC3 [%] (solvent) ^a	Area dose [$\mu\text{g}/\text{cm}^2$] ($\mu\text{mol}/\text{cm}^2$)	Reference
Geraniol 106-24-1 154.3	LOEL HRIPT; NOEL	0.37 (petrolatum) 6 (petrolatum)	370 (12.3) 6000 (38.9)	Marzulli and Maibach (1980)	57 (AOO)	14,250 (92.4)	Basketter et al. (1994)
Glutaraldehyde 111-30-8 100.1	LOEL HRIPT; NOEL	10 (ethanol) 0.1 (petrolatum)	10,000 (64.8) 100 (1.00)	Marzulli and Maibach (1974)	0.06 (acetone)	15 (0.15)	Hilton et al. (1998)
Hexylcinnamic aldehyde (2-(phenylmethylene)- octanal) 101-86-0 216.3	LOEL HRIPT; NOEL	1.7 ^b (petrolatum) 20 (diethylphthalate)	1700 (16.6) 23,662 (109)	RIFM (1944a)	14 (AOO)	3500 (16.2)	Basketter et al. (2001)
Hydroxycitronellal (7-hydroxy-3,7-dimethyl- octanal) 107-75-5 172.3	HMT; NOEL LOEL HRIPT; LOEL	5 (petrolatum) 12 (petrolatum) 2.5 (ethanol + 25% diethylphthalate)	5000 (29.0) 12,000 (69.6) 4200 (24.4)	Ford et al. (1988) Ford et al. (1988)	9 (mean) (AOO) 18 (AOO) 33 (AOO)	2250 (10.4) 4500 (26.1) 8250 (47.9)	Basketter et al. (1999b) Basketter and Scholes (1992) Basketter et al. (2001)
Isoeugenol (2-methoxy-4- propenylphenol) 97-54-1 164.2	HRIPT; NOEL LOEL	0.5 (ethanol) 1.0 (ethanol)	69 (0.42) 775 (4.72)	RIFM (1973a,b, 1980)	2.2 (mean) (AOO)	550 (3.35)	Loveless et al. (1996)
Lilial (<i>p</i> -tert-butyl- α -methyl hydrocinnamal) 80-54-6 204.3	HRIPT; NOEL	5 (diethylphthalate)	3750 (18.3)	RIFM (1988)	19 (AOO)	4750 (23.3)	Basketter et al. (2001)
Linalool (3,7- dimethylocia-1,6-dien- 3-ol) 78-70-6 154.2	HMT; NOEL	20 (petrolatum)	13,793 (89.4)	RIFM (1970a) Gerberick et al. (2001b)	30 (AOO)	7500 (48.6)	Ryan et al. (2000)
2-Mercaptobenzothiazole 149-30-4 167.2	HMT; LOEL	2.5 ^b (petrolatum)	1724 (10.3)	Kligman (1966)	3.3 ⁱ (DMF)	825 (4.93)	Basketter and Scholes (1992)
Methylchloroisothiazoli- none/methylisothiazoli- none 2682-20-4, 26172-55-4 mean 132.3	HRIPT; NOEL LOEL	0.001 (cosm. formulation) (MCI/MI) 0.00125 (cosm. formulation)	1.1 (0.0083) 1.4 (0.011)	Cardin et al. (1986)	0.0049 (AOO)	1.2 (0.0091)	Warbrick et al. (1999a)
5-Methyl-2,3-hexandione 13706-86-0 128.2	HMT; NOEL	Not given	3448 (26.9)	Gerberick et al. (2001b)	26 (AOO)	6500 (50.7)	Ryan et al. (2000)
<i>p</i> -Methylhydrocinnamic aldehyde (3-(4-methylphenyl)- propanal) 5406-12-2 148.2	HMT; NOEL	Not given	1379 (9.30)	Gerberick et al. (2001b)	22 (AOO) 13.7 (AOO)	5500 (37.1) 3425 (23.1)	Ryan et al. (2000) Basketter et al. (2001)
Nickel sulfate 7786-81-4 58.7 for Ni	HMT; LOEL	1.0 ^b (petrolatum)	154(2.62) (as Ni)	Kligman (1966)	2.5 (1% surfactant in water)	140 ^j (2.39) (as Ni)	Ryan et al. (2002)

Phenylacetaldehyde 122-78-1 120.1	HRIPT; LOEL 0.1 (ethanol)	78 (0.65)	RIFM (1973b)	3.0 (AOO)	750 (6.24)	Basketter et al. (2001)
p-Phenylenediamine 106-50-3 108.1	HRIPT; LOEL 0.01 (petrolatum)	10 (0.093)	Marzulli and Maitbach (1974)	0.07 (mean AOO)	18 (0.17)	Warbrick et al. (1999b)
Tetramethylthiuram disulfide 137-26-8 240.4	HMT; LOEL 8.3 ^h (petrolatum)	5747 (23.9)	Kligman (1966)	6.0 (AOO)	1500 (6.2)	Dearman and Kimber (2001)

^aThe EC₃ is the effective concentration of a chemical (percent of chemical in vehicle) required to produce a 3-fold (i.e. threshold level) increase in the proliferation of lymph node cells compared to vehicle-treated controls. Vehicles used are AOO, acetone-olive oil (4:1); DMF, dimethylformamide; DMSO, dimethylsulfoxide.

^bLOEL extrapolated from frank effect level (sensitization rate 25–50%) by divisor 10.

^cEC₃ estimated from stimulation index of 8.5 at 10% by divisor 10.

^dAssuming that cobalt sulfate heptahydrate (CAS No. 10026-24-1, 281.1 g/mol) was used.

^eCobalt chloride hexahydrate (CAS No. 7791-13-1, 237.9 g/mol) was used.

^fAn additional extrapolation factor of 10 was applied since a concentration of 4% induced a sensitization rate of 100% in the HMT (Marzulli and Maitbach, 1980).

^gEC₃ estimated from stimulation index of 16 at 25% by divisor 10.

^hLOEL extrapolated from frank effect level (sensitization rate 10–25%) by divisor 3.

ⁱEC₃ estimated from stimulation index of 4.5 at 10% by divisor 3.

^jAssuming the nickel sulfate hexahydrate (262.9 g/mol) was used.

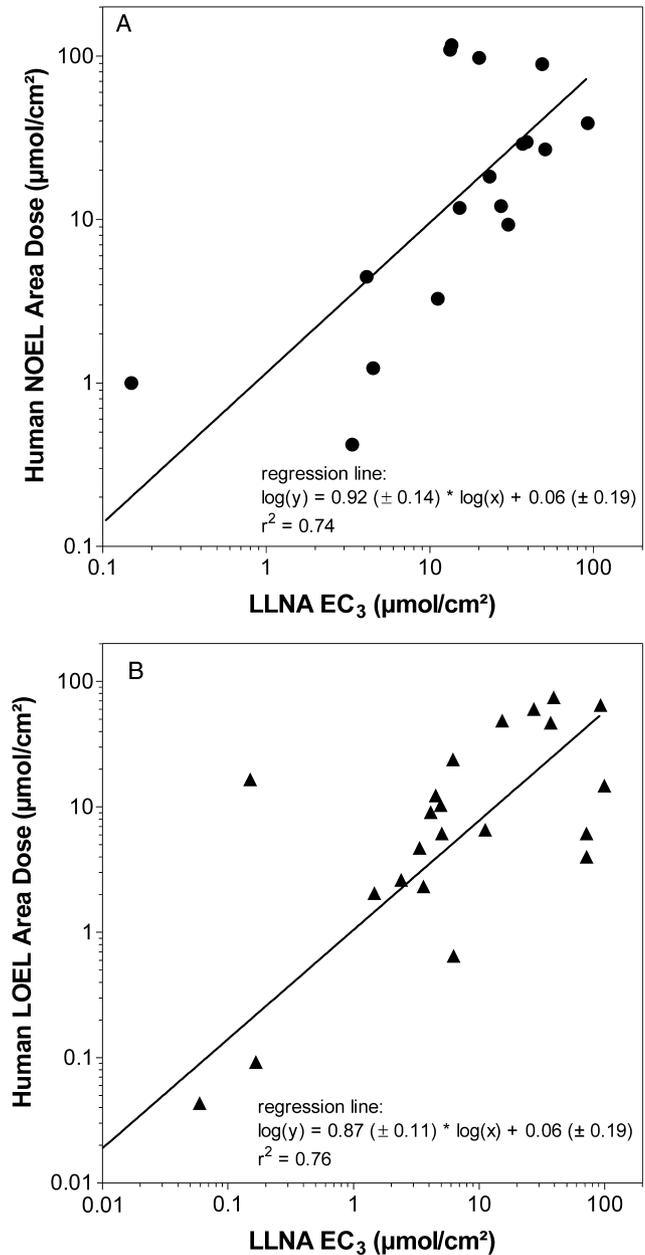


Fig. 1. Correlation of the sensitization potency in humans and mice, expressed as molar area dose. For the chemicals listed in Table 2, human NOEL values vs. murine EC₃ values (A) and human LOEL values vs. murine EC₃ values (B) are depicted. When for a chemical more than one value from experiments in humans or mice was available, the arithmetic mean value was used. Linear regression was performed on the logarithmically transformed values (the outlier in B (glutaraldehyde) was excluded from regression analysis).

EC₃ data points lie above the diagonal line, while 13/18 human NOEL-murine EC₃ data points lie below the diagonal line. Moreover, for 6 of the 9 chemicals for which human NOEL and LOEL values were available, the NOEL-murine EC₃ data point was below and the LOEL-murine EC₃ data point was above the diagonal line. It is obvious that more data were available for chemicals with a sensitization threshold between 1 and

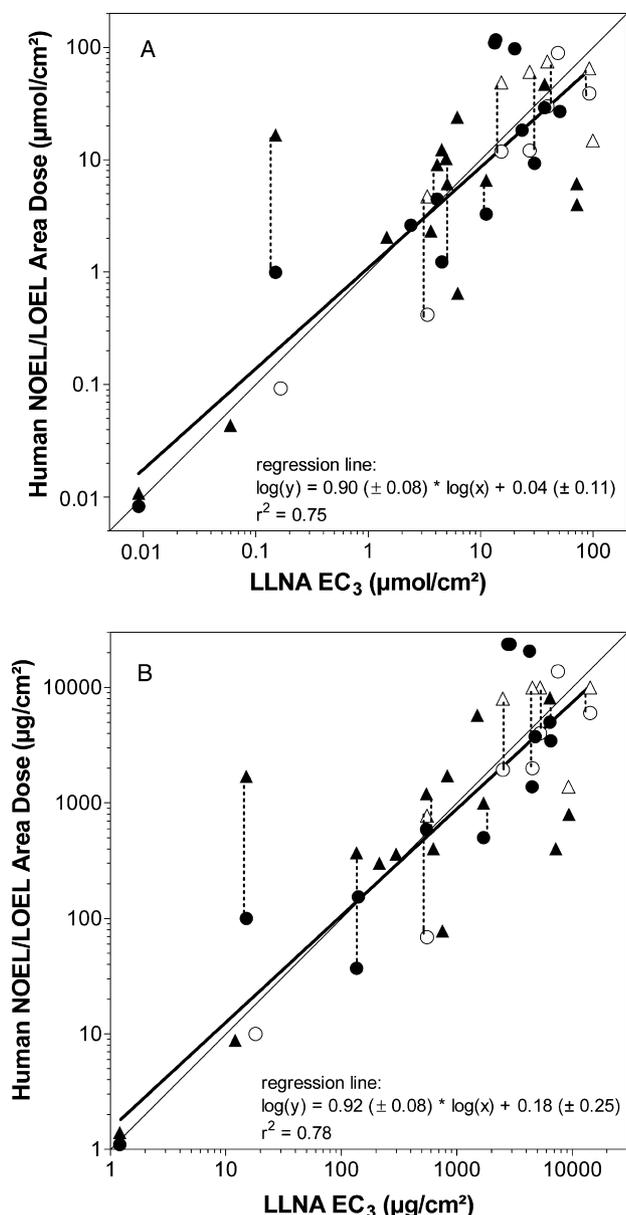


Fig. 2. Correlation of NOEL/LOEL values from human sensitization tests with murine LLNA data. The figure combines the data shown in Fig. 1 A and B. The area dose is expressed as specific area dose (A) and as molar area dose (B). LOEL (triangles) and NOEL (circles) values for one chemical are connected by a dashed line. Chemicals for which metabolism into haptens was considered necessary are depicted as open symbols, while chemicals that can directly act as haptens are depicted as closed symbols. Linear regression was performed on the logarithmically transformed values, in addition, the diagonal line is drawn.

100 $\mu\text{mol}/\text{cm}^2$ than for sensitizing chemicals with a threshold between 0.01 and 1 $\mu\text{mol}/\text{cm}^2$. The reason for this is that only data were used for which both human and murine data were available. For many potent sensitizers, such as benzoyl peroxide, chromium(VI) salts, glyoxal and tetrachlorosalicylanilide, only murine or only human data were available or the sensitization rate respectively the stimulation index was very high and no

threshold value could be obtained. Moreover, strong sensitizers are only rarely tested in human sensitization tests. Possible reasons for the dispersion of the data points are discussed in the following section.

When potency data are expressed as specific area dose (see Fig. 2B) instead of molar area dose (see Fig. 2A), a very similar picture is obtained. This was expected because most skin sensitizing chemicals have a molecular weight of about 100–200 g/mol and therefore, only data points of chemicals with a higher or lower molecular weight, e.g. formaldehyde, are shifted relative to the other chemicals. The figures also provide the linear regression analysis for all data points combined and, again, the regression line is not significantly different from the diagonal line.

The comparison of human and murine potency information revealed that the LLNA EC₃ value is a useful measure of sensitizing potency in humans. The correlation of human NOEL and human LOEL values with murine EC₃ values revealed no significant differences, i.e., the EC₃ values were not significantly closer to the human LOEL values than to the human NOEL values, although LOEL values tended to lie above the regression line and NOEL values tended to lie below the regression line. We suggest that the EC₃ value can be used as a surrogate value for the human NOEL that can be used as a starting point in quantitative sensitization risk assessment. Similarly, Gerberick et al. (2001a) correlated human NOEL values and LLNA EC₃ values in their quantitative risk assessment approach.

4.1. Extrapolation factors for the derivation of safe area doses

In order to derive safe area doses for sensitizing chemicals we applied the extrapolation factor/uncertainty factor approach. For interspecies variability, we propose to reduce the default extrapolation factor (EF) of 10 to $\sqrt{10}$ (rounded to 3) because comparison of human and murine data, as shown above, revealed that the sensitizing area doses are very similar for both species. An EF of 3 is supported by the data shown in Fig. 2A because most data points lie within a factor of 3 from the diagonal line and because data points for chemicals that require metabolism (open symbols) are not more widely dispersed than data points for chemicals that do not need to be metabolized into haptens.

The dispersion of the data points in Fig. 2A is likely caused by interspecies differences and experimental variations. Interspecies differences may, for example, be related to differences in skin penetration and metabolism. With regard to skin penetration, use of murine data is considered conservative because rodents tend to show a considerably higher skin penetration for chemicals compared to humans (a 3–10-fold higher penetration is often reported) (Boogaard et al., 2000; Barber

et al., 1992). We think that metabolism has a limited relevance for the variability between humans and mice because only local metabolism is relevant. Therefore, systemic toxicokinetic differences (e.g., allometric differences) do not play a decisive role in skin sensitization. In line with this is the observation that sensitizing chemicals that require metabolism into 'ultimate sensitizers' do not show a larger variability than chemicals that do not need to be metabolized (see Fig. 2A). Although no documented example is known to the authors, it cannot be excluded that sensitizing chemicals might exist that show a large species difference with regard to metabolism in the skin, which could translate into a large difference in sensitization potency.

The dispersion of the data points may also be caused by interlaboratory differences in experimental protocols for the LLNA and especially for the experimental human sensitization tests (HRIPT, HMT). In human tests, often only one dose was employed, which can have a large influence on the NOEL or LOEL value. Also, application frequency, amount applied and the skin site used tend to differ considerably between different laboratories. Finally, for many, but not all chemicals the HMT tends to give lower LOEL values than the HRIPT. All these factors make the human data less robust than the murine data. In contrast to the human tests, influences of LLNA parameters, such as mouse strain and vehicle (solvent) used have been thoroughly studied and were usually within a factor of two to three (Dean et al., 2001; Warbrick et al., 1999a,b). The vehicle was found not to have a substantial influence on EC3-based assessment of skin sensitization potential (Kimber et al., 2002).

Much less data is available with regard to intraspecies (interindividual) differences in susceptibility to sensitization. Several factors, listed in Table 1, may contribute to interindividual differences. From the following evaluation we concluded that an intraspecies EF of 10 is adequate for risk assessment. A limited interindividual variability is supported by the following arguments: (a) Experimental DNCB sensitization in humans indicated a factor of about 10 between the lowest effective area dose and the area dose required to sensitize all subjects. While an area dose of $8.8 \mu\text{g}/\text{cm}^2$ sensitized 8% of the subjects, $35 \mu\text{g}/\text{cm}^2$ sensitized about 80%, and $70 \mu\text{g}/\text{cm}^2$ were necessary to sensitize 100% (Friedmann, 1990; Friedmann et al., 1983). (b) Compared to men, women showed an about 2-fold higher increase in skin thickness, which was used to quantify the allergic reaction to DNCB after a single sensitization exposure (Friedmann, 1990; Rees et al., 1989). (c) Friedmann and coworkers compared dose–response curves for DNCB sensitization in three groups of subjects, one group without skin allergies, one group with nickel allergy, and one group with multiple skin allergies (Friedmann, 1990; Moss et al., 1985). Calculation of area doses leading to sen-

sitization of 50% of non-allergic, nickel-allergic and multiple-allergic subjects resulted in 21, 16, and $14 \mu\text{g}/\text{cm}^2$, respectively. Thus, subjects with existing skin allergies, which theoretically could constitute a sub-population with a higher susceptibility for sensitization against another chemical, indeed required a smaller area dose to become sensitized. However, the difference was only a factor of about two.

An argument for not reducing the default EF was that subjects with damaged skin, e.g., from preexisting inflammatory skin disease or from working repeatedly and for longer time periods with wet hands and arms, could be at a higher risk for getting sensitized because of a higher skin penetration. Although subjects with pre-existing skin diseases are normally excluded from volunteer studies, an EF of 10 was considered sufficient because in HRIPT and HMT studies, the NOEL and LOEL values are derived on the reactions of the most sensitive individuals within the test group.

While experiments with DNCB and other sensitizers have shown that a single contact can be sufficient for effective sensitization, the question arises whether a lower area dose would suffice for sensitization if repeated contacts over a longer time occurred. In this context, a few publications on so-called subclinical sensitization might be relevant. Ford et al. (1988) reported on a HRIPT using hydroxycitronellal in which groups of 66 subjects each were treated with 4200, 8400 or $12,600 \mu\text{g}/\text{cm}^2$ during induction. One subject each of the two highest exposure groups showed a positive challenge reaction. After six months, 100 of the panelists that had completed the first HRIPT took part in a second HRIPT with hydroxycitronellal. During the first and second week of the induction phase of the second HRIPT, 29% of the subjects showed signs of allergic contact dermatitis. This result indicates that, at least for hydroxycitronellal, detectable sensitization needs longer to develop than the time between induction phase and challenge in the HRIPT (10–14 days). It is unknown whether this phenomenon occurs only at small area doses, i.e., those just beneath the sensitization threshold, whether it occurs with most or only a few sensitizing chemicals, and which mechanism is involved (e.g., slow release of sensitizer initially bound to the upper skin layer (stratum corneum)). Similar observations have also been made with DNCB (Friedmann, 1990; Friedmann et al., 1990). In addition, Vandenberg and Epstein (1963) performed a sensitization test with nickel chloride and found that in a first sensitization test 16/172 (9%) previously non-nickel allergic subjects got sensitized, while upon repetition of the sensitization test four months later with 19 subjects, that had shown a negative result in the first challenge test, 5 (19%) were successfully sensitized. While it is currently difficult to describe this phenomenon quantitatively due to the limited data available, we propose to apply an additional EF of 10 to

account for a possible higher sensitizing potency of a chemical upon repeated exposure.

Combination of the discussed interspecies, intraspecies and repeated-exposure EFs by multiplication results in a total EF of $3 * 10 * 10 = 300$. Division of the LLNA EC3 area dose by this factor of 300 results in an area dose which should not induce sensitization in the vast majority of exposed humans. We termed this 'safe' area dose 'acceptable non-sensitizing area dose' (ANSAD). It refers to the cumulative amount of chemical applied to a given skin site during one day, i.e., the total daily area dose. Based on the ANSAD and combined with a reasonable exposure assessment, acceptable concentrations for sensitizing chemicals can be derived, e.g., at the workplace, in cosmetics and in household products. It should be noted that the ANSAD is designed to protect non-allergic individuals against getting sensitized, but not to protect already allergic individuals against elicitation of an allergic response. The combination of individual EFs for the derivation of ANSAD based on a LLNA EC3 or on a human HRIPT is shown in Table 4. When human data are used as a starting point, no interspecies EF is applicable, while the intraspecies and repeated-exposure EFs have to be applied. The EF values derived here are intended as default values in cases in which no additional information is available. Modifications of these default EFs should be done if additional experimental data or defined application scenarios argue for a reduced (or increased) EF value.

5. Quantitative risk assessment for allergy elicitation

For a chemical against which a part of the population is already sensitized, it would be desirable to derive a 'safe' area dose at which allergic contact dermatitis will not be elicited in individuals patients. To this end a procedure for deriving an 'acceptable non-eliciting area dose' (ANEAD) is developed in the following.

As has been done for the sensitizing potency above, it would again be favorable if a correlation between the 'elicitation potency' of a chemical and the sensitizing potency could be established. Especially since the latter can easily be determined in the LLNA. Theoretically, a correlation between the sensitizing and the elicitation potency of a chemical can be expected, because several of the factors influencing the sensitizing potency (see Table 1) are also relevant in elicitation, e.g. skin penetration, metabolism, protein binding, uptake by antigen-presenting cells, antigen processing and presentation. Like for the sensitization process, also for elicitation of allergic contact dermatitis thresholds and dose-responses can be defined (Hexall et al., 2002; Jerschow et al., 2001; Menne, 1994; Rees et al., 1990). The main difference between sensitization and elicitation is that during elicitation not only Langerhans' cells, but also

other class II major histocompatibility complex (MHC) molecules expressing skin cells, such as keratinocytes, are capable of presenting antigen to primed effector T lymphocytes, which are recruited to the contact site and then mediate a local inflammatory reaction (Fehr et al., 2000; Nakano, 1998; Okazaki et al., 2002).

The main problem with establishing a correlation between the elicitation and the sensitizing potency is the fact that only for a small number of sensitizing chemicals the elicitation threshold has been experimentally determined. This is caused by the fact that for diagnostic purposes often a relatively high concentration is employed in the patch test (e.g., 1% chemical in petrolatum) in order to reliably detect a sensitization. The chemicals for which both the sensitization threshold in humans and the allergy elicitation threshold in sensitized subjects were located in the literature are summarized in Table 3. The elicitation thresholds used were determined in subjects that had an established allergy for a long period of time. Tests in which elicitation thresholds were obtained using newly sensitized subjects (e.g., in HMT and HRIPT) were not used for analysis because the elicitation threshold in these subjects depends on the sensitization dose used, i.e., the higher the sensitization dose used, the lower the elicitation threshold, (Friedmann et al., 1983). This dependency of has also been found in mice (Scott et al., 2002). Moreover, the elicitation threshold decreases with the time of established allergy and/or with the number of antigen contacts.

Again, the reported concentrations were converted into specific and molar area dose values. For elicitation tests, the reported area dose was used if given in the literature. Otherwise, it was assumed that 15 μ l (or 15 mg) of the test mixture were applied on a skin area of 0.5 cm², i.e., by using 8-mm Finn Chambers (Robinson et al., 2000). On the basis of these assumptions, the area dose value in μ g/cm² can be derived by multiplication of the concentration of the chemical in the test mixture (in percent) with a factor of 300. For sensitization thresholds, the procedure and assumptions have been described above.

Comparison of the sensitization and elicitation area doses, shown in Fig. 3, reveals that no correlation between the two parameters is obvious, i.e., although the sensitization threshold area doses span five orders of magnitude, the values for the elicitation area dose mostly stay between 0.005 and 0.05 μ mol/cm². Relevant for assessing the risk of allergy elicitation is the ratio between the sensitization and elicitation threshold (see Table 3). Graphical representation of the ratio sensitization threshold/elicitation threshold versus the sensitization threshold is shown in Fig. 4. Obviously, this ratio increases with increasing sensitization threshold: for very strong sensitizers, such as MCI/MI, the area concentration necessary to elicit a skin reaction in a sensitized individual is very close or identical to the area dose that

Table 3
Comparison between sensitization and elicitation thresholds in humans

Chemical CAS-No. molecular weight	Sensitization threshold		Elicitation threshold in patch test		Reference	Area dose [µg/cm ²] (µmol/cm ²)	Ratio sensitization/ elicitation thresholds
	Effect level—test concentration [%] (solvent)	Area dose [µg/cm ²] (µmol/cm ²)	Effect level test— concentration [%] (solvent)	Area dose [µg/cm ²] (µmol/cm ²)			
Cinnamic alcohol (3-phenyl-2-propen-1-ol) 104-54-1 134.1	LOEL 10.4 (petrolatum)	4000 (29.8)	LOEL 0.025 (petrolatum)	7.5 (0.056)	Steltenkamp et al. (1980b)		532
Cinnamic aldehyde (3-phenyl-2-propenal) 104-55-2 132.2	NOEL 0.5 (ethanol)	590 (4.46)	NOEL 0.01 (petrolatum)	3 (0.023)	Johansen et al. (1996b)		197
Cobalt(II) salts 7440-48-4 58.9	LOEL 1.0 (ethanol)	1200 (9.08)	LOEL 0.02 (ethanol)	6 (0.045)	Wahlberg (1973)		200
	LOEL 2.5 ^a cobalt sulfate (petrolatum)	361 ^b (6.13)	LOEL 0.016 ^c cobalt chloride (petrolatum)	1.19 (0.020)			300
Formaldehyde 50-00-0 30.0	NOEL 0.037 (petrolatum)	37 (1.23)	NOEL 0.01 (not stated)	3 (0.10)	Flyvholm et al. (1997)		12
	LOEL 0.37 (petrolatum)	370 (12.3)	LOEL 0.025 (not stated)	7.5 (0.25)	Johansen et al. (1996a)		49
Isoeugenol (2-methoxy-4-propenylphenol) 97-54-1 164.2	NOEL 0.5 (ethanol)	69 (0.42)					
	LOEL 1.0 (ethanol)	775 (4.72)	LOEL 0.01 (petrolatum)	4 (0.024)			194
Hydroxycitronellal (7-hydroxy-3,7-dimethyloctanal) 107-75-5 172.3	LOEL 12 (petrolatum)	12,000 ^d (69.6)	LOEL 0.05 ^d (hydroalcoholic)	1.83 (0.011)	Api and Letizia (2001)		4426
	LOEL 2.5 (ethanol +2.5% diethylphthalate)	4200 (24.4)					
2-Mercaptobenzothiazole 149-30-4 167.3	LOEL 2.5 ^e (petrolatum)	1724 (10.3)	LOEL 0.01 (petrolatum)	3 (0.018)	Emmett et al. (1994)		575
Methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) 2682-20-4, 26172-55-4 mean 132.3	NOEL 0.001 (cosm. formulation)	1.1 (0.0083)	NOEL 0.0015 (soap formulation)	0.45 (0.0034)	Weaver et al. (1985) (CD)		2.4
	LOEL 0.00125 (cosm. formulation)	1.4 (0.011)	LOEL 0.0025 (soap formulation)	0.75 (0.0057)			1.9
Nickel chloride (7718-54-9) 58.7 for Ni	LOEL 25 (0.1% SDS solution)	9700 ^f (165 (as Ni))	LOEL 0.16	1.25 (0.021) (as Ni)	Menne (1994)		7760
Nickel sulfate (as nickel) 7786-81-4 58.7 for Ni	LOEL 1.0 ^e (petrolatum)	154 ^g (2.62 (as Ni))	LOEL 0.001 (petrolatum)	0.053 (0.00090)	Uter et al. (1995)		2900
	NOEL 0.001 ^b (petrolatum)	1 (0.0093)	LOEL 0.0032 (water)	0.21 (0.0036)	Hindsen et al. (1999)		730
<i>p</i> -Phenylenediamine 106-50-3 108.1	LOEL 0.01 (petrolatum)	10 (0.093)	LOEL 0.01	3 (0.028)	McFadden et al. (1998)		3.3
Pentaerythritol triacrylate 3524-68-3 298.3	LOEL 0.3 ⁱ (petrolatum)	300 (1.006)	LOEL 0.01 (petrolatum)	3 (0.010)	Dahlquist et al. (1983)		100

^a LOEL extrapolated from frank effect level (sensitization rate 25–50%) by divisor 3.^b Assuming that cobalt sulfate heptahydrate (CAS No.10026-24-1, 281.1 g/mol) was used.^c Assuming that cobalt chloride hexahydrate (CAS No. 7791-13-1, 237.9 g/mol) was used.^d Repeated open use test with hydroalcoholic eau de cologne; for this type of cosmetic formulation, a use of 0.43 g on 235 cm² of skin is assumed.^e LOEL extrapolated from frank effect level (sensitization rate 25–50%) by divisor 10.^f Assuming the nickel chloride hexahydrate (237.7 g/mol) was used and that 0.25 ml solution were put on the 2.86-cm Lintine disk.^g Assuming the nickel sulfate hexahydrate (262.9 g/mol) was used.^h NOEL extrapolated by divisor 10 from LOEL.ⁱ LOEL extrapolated from frank effect level (sensitization rate 75–100%) by divisor 30.

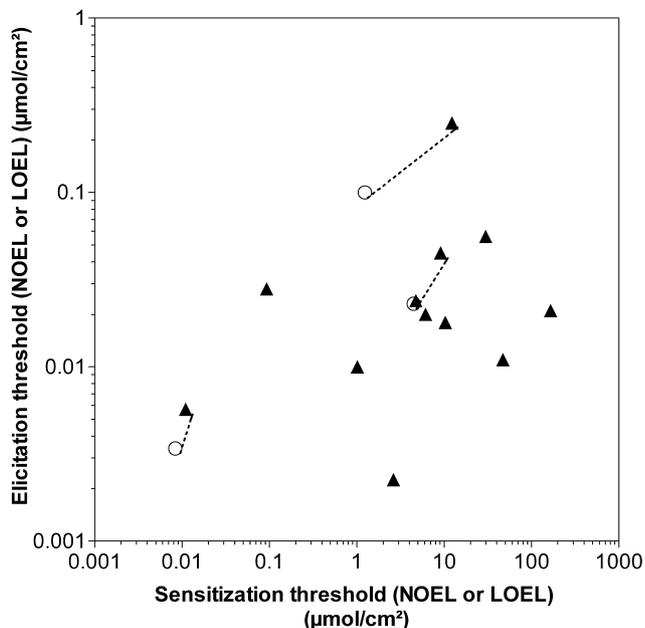


Fig. 3. Comparison of human sensitization NOEL/LOEL values with elicitation NOEL/LOEL values in allergic subjects, expressed as molar area dose. For the chemicals listed in Table 3, LOEL(sensitization)–LOEL(elicitation) (triangles) or NOEL(sensitization)–NOEL(elicitation) (circles) values are depicted. For chemicals for which both pairs of values were available, NOEL–NOEL and LOEL–LOEL points are connected by a dashed line.

will sensitize a non-allergic individual. In contrast, for a low-potency sensitizer, such as 2-mercaptobenzothiazole, a high area dose is needed to cause sensitization, but a 1000-fold lower area dose is sufficient to elicit an allergic reaction in strongly sensitized individuals. A linear correlation was used as the most simple way to describe the relationship between $\log(\text{ratio of sensitization/elicitation threshold})$ and $\log(\text{sensitization threshold})$. A regression line of $\log(\text{ratio}) = 0.84 * \log(\text{sensitization threshold } [\mu\text{mol}/\text{cm}^2]) + 1.81$ was obtained. Based on this relationship, we propose that the ratio of sensitization/elicitation threshold can be predicted on the basis of an established sensitization threshold.

Alternatively, it could also be argued that Fig. 3 suggests the existence of an absolute elicitation threshold at about $0.01 \mu\text{mol}/\text{cm}^2$ which would make the use of linear regression unnecessary. However, an absolute elicitation threshold does not seem biologically plausible. As discussed above, the factors listed in Table 1, such as skin penetration, metabolism, protein binding, uptake by antigen-presenting cells, antigen processing and presentation, can be expected to have influence on the elicitation threshold and it could not be explained why they should influence the sensitization potency, but should be without influence on the elicitation potency. Thus, biologically it seems more plausible that these factors affect both the sensitization and elicitation thresholds and, therefore, a correlation between these

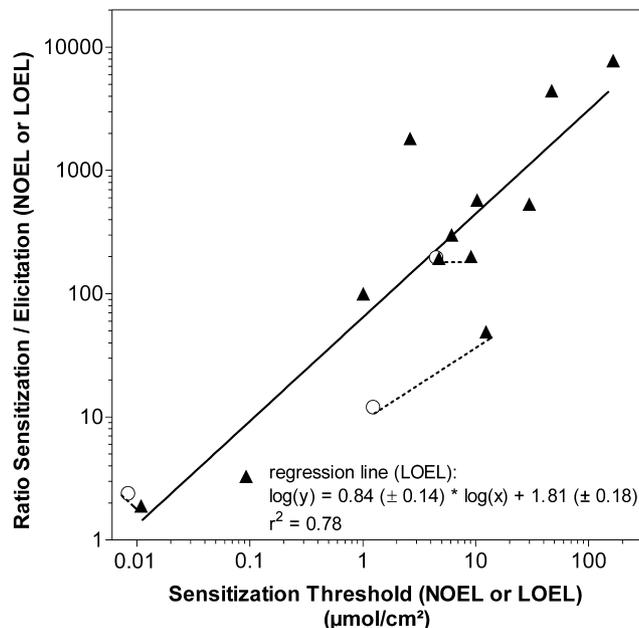


Fig. 4. Correlation of human sensitization/elicitation ratio with sensitization NOEL/LOEL values in allergic subjects, expressed as molar area dose. For the chemicals listed in Table 3, sensitization LOEL/elicitation LOEL ratio vs. LOEL(sensitization) (triangles) or sensitization NOEL/elicitation NOEL ratio vs. NOEL(sensitization) (circles) are depicted. For chemicals for which both pairs of values were available, corresponding points are connected by a dashed line. Linear regression was performed on the logarithmically transformed values.

thresholds seems more likely. For this reason, the relationship between the ratio of sensitization/elicitation threshold and the sensitization threshold derived in Fig. 4 will be used.

5.1. Extrapolation factors for derivation of an acceptable non-eliciting area dose (ANEAD)

When risk assessment is based on an EC3 LLNA value, a factor of $\sqrt{10}$ (rounded to 3) is proposed as interspecies EF to account for experimental variability, as discussed above.

Hindsen et al. (1999) have determined elicitation thresholds in 18 female subjects with allergic contact dermatitis to nickel in four independent tests spread over a period of seven months. A considerable variation of the LOEL in some of the individuals was observed. The minimal eliciting concentrations for the whole group differed less between the four tests (between 0.008 and 0.02% nickel sulfate, corresponding to $0.21\text{--}1.33 \mu\text{g Ni}/\text{cm}^2$). No comparable studies are available for other sensitizing chemicals. With regard to the intraspecies EF, it has to be taken into account that the elicitation thresholds given in Table 3 represent the most sensitive individuals examined in the respective studies, i.e., the LOEL values constitute the lowest concentrations at which positive reactions were observed in the subject

panel. Basing risk assessment on the most susceptible individuals argues for a reduced intraspecies EF. Moreover, the occlusion used in the patch test procedure tends to result in lower elicitation thresholds than open, unoccluded application, as has been shown in nickel-allergic individuals (Allenby and Basketter, 1994). However, as discussed above for sensitization, preexisting skin damage might facilitate the elicitation reaction through an increased skin penetration of the chemical. For example, nickel-allergic individuals showed about 10-fold lower elicitation thresholds when patch testing was performed on skin that showed slight inflammatory changes and dryness due to repeated contact with detergent solution (Allenby and Basketter, 1993). In the absence of more definitive data, we consider a value of 10 as adequate for the intraspecies EF.

As has been discussed for sensitization above, also for elicitation an additional EF has to be considered for repeated exposure. Two lines of evidence argue for such a factor. First, in repeated open application tests (ROAT), in which a solution or consumer product containing a small concentration of the relevant sensitizer is applied to a skin test site repeatedly, positive reactions often take 5–10 days of repeated application to develop. This has been shown, for example, in individuals allergic to formaldehyde (Flyvholm et al., 1997), isoeugenol (Johansen et al., 1996a), and cinnamic aldehyde (Johansen et al., 1996b). Secondly, recent experiments by Hextall et al. (2002) in subjects allergic to *p*-phenylenediamine have shown that concentrations too low to elicit a positive response in the first patch test may do so after repeated daily patch testing at the same skin site. In view of the small data basis that is available about effects of repeated/prolonged exposure on the elicitation of allergic skin reactions, we suggest to apply an additional EF of 10 to account for a possible allergy elicitation at lower concentrations of a chemical upon repeated exposure.

When derivation of an ANEAD is to be based on a NOEL for *elicitation* in a one-time patch test in sensitized humans, a total EF of $10 \times 10 = 100$, i.e., the product of the intraspecies EF and the repeated-exposure EF, is proposed to account for interindividual variability, possible higher penetration through the skin at certain anatomical sites, influences of existing skin damage and eventually repeated/prolonged exposure. When an ANEAD is to be derived on the basis of the *sensitization*–*elicitation* EF of $10^{(0.84 \times \log(\text{NOEL sensitization threshold}[\mu\text{mol}/\text{cm}^2]) + 1.81)}$ (as derived in Fig. 4) and an interspecies EF of 3 have to be applied additionally. Table 4 shows possible combinations of EFs depending on what kind of experimental data the risk assessment is based on. Again, as for the ANSAD, the ANEAD refers to the cumulative amount of chemical applied to a given skin site during one day, i.e., the total daily area dose.

Table 4
Proposed extrapolation factors (EF) for derivation of acceptable non-sensitizing area doses (ANSAD) and acceptable non-eliciting area doses (ANEAD) from different toxicological data

Available toxicological information (area dose)	Proposed EF for derivation of ANSAD				Proposed EF for derivation of ANEAD				
	Interspecies		Repeated exposure		Interspecies		Repeated exposure		Relationship sensitization/elicitation threshold
	Interspecies	Intraspecies	Repeated exposure	LOEL to NOEL	Interspecies	Intraspecies	Repeated exposure	LOEL to NOEL	
LLNA EC3	3 ^a	10	10	n.a. ^b	3 ^a	10	10	n.a.	1.3–3090 ^c
NOEL HRIPT or HMT	n.a.	10	10	n.a.	n.a.	10	10	n.a.	1.3–3090 ^c
LOEL HRIPT or HMT	n.a.	10	10	3 ^a –10	n.a.	10	10	3 ^a –10	1.3–3090 ^c
NOEL for elicitation in humans with for allergy	Cannot be derived				n.a.	10	10	n.a.	n.a.
LOEL elicitation in humans with for allergy	Cannot be derived				n.a.	10	10	3 ^a –10	n.a.

^a Value rounded from $\sqrt{10}$, thus multiplication of 3 by 3 gives 10.

^b n.a., Not applicable.

^c The EF is calculated from the sensitization NOEL using the equation $\text{EF} = 10^{(0.84 \times \log(\text{area dose}[\mu\text{mol}/\text{cm}^2]) + 1.81)}$. The range given reflects the range into which values will usually fall. For its calculation an area dose of 0.01 $\mu\text{mol}/\text{cm}^2$ (potent sensitizer) has been assumed as the lower value and 100 $\mu\text{mol}/\text{cm}^2$ (weak sensitizer) as the upper value. As sensitization NOEL, the LLNA EC3, HRIPT NOEL or HMT NOEL are used.

Based on the ANEAD and combined with a reasonable exposure assessment, acceptable concentrations for sensitizing chemicals against which individuals in the population are already sensitized, e.g., at the workplace, in cosmetics and in household products, can be derived. As has been stated above for the sensitization risk assessment, these default EFs (most likely affected EFs are those for intraspecies variability and repeated exposure) should be adjusted on the basis of experimental data and knowledge of exposure and intended use.

6. Examples of application of the proposed risk assessment to sensitizing chemicals

In this section, we present three example chemicals. These have been chosen because they cover a range of different potency and extensive data on sensitization and allergy elicitation in humans has been published and can

be used for comparison with the outcome of the risk assessment procedure discussed in the manuscript.

6.1. Chloromethylisothiazolinone/methylisothiazolinone (MCI/MI)

MCI/MI is used as a preservative in many rinse-off and leave-on cosmetics as well as in household products and pharmaceuticals. The high concentrations initially used after introduction onto the market have led to the development of allergy in a significant number of consumers (Hanuksela, 1986). MCI/MI is a strong sensitizer, i.e., it has a high sensitizing potency, as evidenced by a LLNA EC3 value of 0.0049% (corresponding to 1.2 µg/cm² or 9.1 nmol/cm²) (Warbrick et al., 1999a,b). The application of the proposed quantitative risk assessment for sensitizing chemicals and the comparison with human experience is shown in Table 5. The ANSAD is obtained by application of a total EF

Table 5
Risk assessment for chloromethylisothiazolinone/methylisothiazolinone (MCI/MI)

Derivation of ANSAD based on LLNA EC3 of 0.0049% (Warbrick et al., 1999a) corresponding to an area dose of		9.1 nmol/cm ²
Interspecies EF	3	
Intraspecies EF	10	
Repeated exposure EF	10	
Application of a total EF of 300 results in ANSAD of		0.030 nmol/cm ²
Derivation of ANSAD based on HRIPT NOEL of 0.001% (Cardin et al., 1986) corresponding to an area dose of		8.3 nmol/cm ²
Intraspecies EF	10	
Repeated exposure EF	10	
Application of total EF of 100 results in ANSAD of		0.083 nmol/cm ²
Human experience		
Lowest reported exposure leading to sensitization: 7 ppm in moisturizing cream ^a (Hanuksela, 1986) corresponding to an area dose (0.019 µg/cm ²) of		0.14 nmol/cm ²
Acceptable exposure limit (0.004 µg/cm ² for face cream) derived in published risk assessment (Robinson et al., 2000)		0.030 nmol/cm ²
Derivation of ANEAD based on LLNA EC3 of 0.0049% (Warbrick et al., 1999a) corresponding to an area dose of		9.1 nmol/cm ²
Interspecies EF	3	
Intraspecies EF	10	
Repeated exposure EF	10	
Sensitization–elicitation EF	1.2	
$10^{(0.84 * \log(0.0091) + 1.81)}$		
Application of total EF of 360 results in ANEAD of		0.025 nmol/cm ²
Derivation of ANEAD based on NOEL of 0.0015% in patch tests (Weaver et al., 1985) corresponding to an area dose (0.45 µg/cm ²) of		3.4 nmol/cm ²
Intraspecies EF	10	
Repeated exposure EF	10	
Application of total EF of 100 results in ANEAD of		0.034 nmol/cm ²
Human experience		
Lowest reported concentration leading to positive reaction in ROAT: 7 ppm in cream ^b (Hanuksela, 1986) corresponding to an area dose (0.007 µg/cm ²) of		0.053 nmol/cm ²

^a Assuming use of 0.8 g face cream twice daily to 600 cm² facial surface (corresponding to 2.67 mg cream/cm²) (Robinson et al., 2000).

^b Using 0.1 g cream (Warbrick et al., 1999a) on about 100 cm² (area estimated by author) (corresponding to 1 mg/cm²).

of 300 to the LLNA EC3 which results in an area dose of 0.030 nmol/cm^2 ($0.004 \text{ }\mu\text{g/cm}^2$). A similar value of 0.083 nmol/cm^2 ($0.011 \text{ }\mu\text{g/cm}^2$) can be derived from human data, i.e., when a total EF of 100 is applied to the HRIPT NOEL of 0.001% (corresponding to 8.3 nmol/cm^2) (Cardin et al., 1986). In a thorough risk assessment (Robinson et al., 2000) using available animal and human data, an acceptable exposure limit of $0.004 \text{ }\mu\text{g/cm}^2$ (corresponding to 0.030 nmol/cm^2) was derived, which is identical to the EC3-derived ANSAD. A report on the lowest exposure that has caused sensitization in humans further support the ANSAD derivation: Hanuksela (1986) reported sensitization of consumers that used a popular moisturizing cream in Finland containing first 19 ppm and later 7 ppm MCI/MI. Usual application of this cream corresponds to an estimated area dose of about 0.14 nmol/cm^2 , which is higher than the derived ANSAD (see Table 5).

As shown in Table 5, derivation of an ANEAD based on the LLNA EC3 using a total EF of 360 results in 0.025 nmol/cm^2 . A similar value of 0.034 nmol/cm^2 can be derived from the NOEL of 0.0015% (corresponding to $0.45 \text{ }\mu\text{g/cm}^2$) obtained in human patch tests using a total EF of 100. The derived ANEAD is below the lowest reported concentration (7 ppm in cream, corresponding to an area dose of about 0.053 nmol/cm^2) leading to a positive reaction in repeated open application tests in MCI/MI-allergic individuals (Hanuksela, 1986). In conclusion, the available data on MCI/MI support our proposal that ANSAD and ANEAD can be derived on the basis on an LLNA EC3 using the extrapolation factor approach presented in this paper. In addition, the data presented for MCI/MI demonstrate that for this potent sensitizer, the thresholds for elicitation of allergic contact dermatitis in already sensitized subjects is very close to the threshold for sensitization of non-sensitized individuals.

6.2. Cinnamic aldehyde

Cinnamic aldehyde is an important fragrance and flavor ingredient and a known human sensitizer. Its sensitizing potency is lower than that of MCI/MI as evidenced by a LLNA EC3 of 2.0% (corresponding to a molar area dose of 3800 nmol/cm^2) (Basketter and Scholes, 1992). As shown in Table 6, based on the LLNA EC3 an ANSAD of 13 nmol/cm^2 ($1.7 \text{ }\mu\text{g/cm}^2$) can be derived. A comparable value of 45 nmol/cm^2 is obtained if risk assessment is based on a HRIPT NOEL (see Table 6). In a published risk assessment an acceptable exposure limit for prevention of sensitization of 7.6 nmol/cm^2 ($1.0 \text{ }\mu\text{g/cm}^2$) was derived for cinnamic aldehyde on the basis of human and animal data and extensive exposure calculations (Gerberick et al., 2001a). The 'area reference dose' derived by Gerberick and coworkers is very similar to the ANSAD derived here on the basis of the LLNA EC3. The per-

centage of positive patch test reactions to cinnamic aldehyde in screening tests on the general population tested decreased from 60% at the beginning of the 1980s to about 10% during the 1990s (Buckley et al., 2000). This correlates with a reduction of use concentrations of cinnamic aldehyde in fragrances. In 1996 the deodorants on the European market contained mean concentrations of about 50 ppm (max. value 400 ppm) cinnamic aldehyde (Rastogi et al., 1998). Together with the reduction of allergy prevalence, this observation supports the conclusion that a concentration of about 50 ppm cinnamic aldehyde in a deodorant (corresponding to about 1.9 nmol/cm^2 ; see Table 6) can be considered safe.

Starting from the LLNA EC3 and using a total EF of 59,400 (see Table 6), an ANEAD of 0.064 nmol/cm^2 can be derived. A slightly higher value of 0.23 nmol/cm^2 is obtained when a total EF of 100 is applied to the lowest concentration that caused positive patch test reactions in allergic subjects (see Table 6). The LLNA-based ANEAD is in very good agreement with the observation that 0.1% cinnamic aldehyde in ethanol used in a ROAT (corresponding to an area dose of 0.061 nmol/cm^2) caused allergy elicitation only in the most sensitive subjects (Johansen et al., 1996b). In conclusion, the available data on cinnamic aldehyde support our proposal that ANSAD and ANEAD can be derived on the basis on an LLNA EC3 using the extrapolation factor approach presented in this paper.

6.3. Nickel

Nickel is an allergen with a relatively low sensitizing potency, but a high prevalence in the general population. As shown in Table 2, the LOEL in HRIPT was about $13 \text{ }\mu\text{mol Ni/cm}^2$ ($740 \text{ }\mu\text{g Ni/cm}^2$) as nickel sulfate (Kligman, 1966) and about $165 \text{ }\mu\text{mol Ni/cm}^2$ ($9700 \text{ }\mu\text{g Ni/cm}^2$) as nickel chloride (Vandenberg and Epstein, 1963). Although nickel is the most common chemical allergen in humans (Mortz et al., 2001), most people do not develop allergic contact dermatitis to nickel, given that virtually everyone is exposed to nickel throughout life, e.g., through ear rings, other jewelry, rivets, coins, and other consumer products.

This observation is in line with the explanation that nickel salts are weak sensitizers because nickel(II) ions are rather non-toxic, do not cause local tissue damage and inflammation, and do not activate Langerhans' cells in the skin (cf., Table 1) (Artik et al., 1999). As in humans, nickel salts are weak sensitizers in animals, often giving negative results in standard sensitization tests. In contrast, sensitization of mice was easily achieved by concomitant administration of inflammation-inducing substances or inflammatory mediators (Artik et al., 1999). Consistent with these animal findings, clinical experience in humans indicates that nickel allergy preferentially develops after nickel exposure on irritated or inflamed, but not on

Table 6
Risk assessment for cinnamic aldehyde

Derivation of ANSAD based on LLNA EC3 of 2.0% (Basketter and Scholes, 1992) corresponding to an area dose of		3800 nmol/cm ²
Interspecies EF	3	
Intraspecies EF	10	
Repeated exposure EF	10	
Application of a total EF of 300 results in ANSAD of		13 nmol/cm ²
Derivation of ANSAD based HRIPT NOEL of 0.5% (Danneman et al., 1983) corresponding to an area dose of		4500 nmol/cm ²
Intraspecies EF	10	
Repeated exposure EF	10	
Application of a total EF of 100 results in ANSAD of		45 nmol/cm ²
Human experience		
Deodorants on the European market contained mean concentrations of about 50 ppm (max value 400 ppm) ^a in 1996 (Rastogi et al., 1998). The percentage of positive patch test reactions to cinnamic aldehyde among fragrance mix-positive individuals has decreased considerably in the 1990s (to about 10%) compared to the 1980s (Buckley et al., 2000).		1.9 nmol/cm ² (max. 15)
Acceptable exposure limit to prevent sensitization derived in published risk assessment (1.0 µg/cm ²) (Gerberick et al., 2001a)		7.6 nmol/cm ²
Derivation of ANEAD based on LLNA EC3 of 2.0% (Basketter and Scholes, 1992) corresponding to an area dose of		3800 nmol/cm ²
Interspecies EF	3	
Intraspecies EF	10	
Repeated exposure EF	10	
Sensitization–elicitation EF	198	
$10^{(0.84 * \log(3.8) + 1.81)}$		
Application of total EF of 59,400 results in ANEAD of		0.064 nmol/cm ²
Derivation of ANEAD based on NOEL of 0.01% in patch tests (Johansen et al., 1996b) corresponding to an area dose of		23 nmol/cm ²
Intraspecies EF	10	
Repeated exposure EF	10	
Application of total EF of 100 results in ANEAD of		0.23 nmol/cm ²
Human experience		
Highest reported consumer exposure from use of fragrance products with max. 50 ppm without reported allergies in 1823 subjects (Danneman et al., 1983) ^b corresponding to an area dose of about 0.13 µg/cm ²		0.98 nmol/cm ²
Lowest reported concentration leading to positive reaction in ROAT: 0.1 % (NOEL 0.02%) in ethanol ^c (Johansen et al., 1996b) corresponding to an area dose of 0.008 µg/cm ²		0.061 nmol/cm ²

^aThe reported mean concentration of cinnamic aldehyde in deodorants of 50 ppm (max. value 400 ppm) (Rastogi et al., 1998) corresponds to an area dose of 0.25 (max. 2.0) µg/cm² (1.9 (max. 15) nmol/cm²) assuming the exposure scenario in Gerberick et al. (2001a).

^bThe reported concentration range of cinnamic aldehyde in fragrance blends of 0.002–50 ppm (Danneman et al., 1983) corresponds to an area dose of 0.88 fg/cm² to 0.13 µg/cm² assuming that 0.44–2.58 mg/cm² of eau de toilette are applied to the skin (Gerberick et al., 2001a).

^cUsing an atomizer pump, 0.1 ml cinnamic aldehyde in ethanol were applied twice daily on a 5 × 5 cm test area on the outer aspect of the upper arm; cinnamic aldehyde concentrations were stepwise increased from 0.02% in the first 2 weeks, to 0.1% in the next 2 weeks and to 0.8% in the last 2 weeks (Johansen et al., 1996b).

healthy skin. For example, ear-piercing for the purpose of wearing nickel-containing custom jewelry resulted in a high rate of nickel sensitization (Schubert et al., 1987). Also in HRIPT local inflammation was (unintentionally) achieved by high nickel concentration, occlusion, and addition of detergent to the nickel solution (Kligman, 1966; Vandenberg and Epstein, 1963).

Similarly, previously false negative results with nickel salts in the LLNA, could recently be overcome by the addition of a detergent to the nickel test solution (Ryan

et al., 2002). The reported EC3 value of 2.5% nickel sulfate corresponds to an area dose of 2400 nmol Ni/cm² (140 µg Ni/cm²). This value is in good agreement with the human LOEL for sensitization of 2600 nmol Ni/cm² (154 µg Ni/cm²) obtained in HMT (Kligman, 1966). Based on the LLNA EC3 an ANSAD of 8.0 nmol Ni/cm² (0.5 µg Ni/cm²) can be derived (see Table 7), which is similar to values of 2.6 and 165 nmol Ni/cm² derived on the basis of human sensitization data with nickel sulfate and nickel chloride, respectively.

Table 7
Risk assessment for nickel chloride and nickel sulfate

Derivation of ANSAD based on LLNA EC3 of 2.5% (Ryan et al., 2002), corresponding to an area dose of	2400 nmol Ni/cm ²
Interspecies EF	3
Intraspecies EF	10
Repeated exposure EF	10
Application of a total EF of 300 results in ANSAD of	8.0 nmol Ni/cm ²
Derivation of ANSAD based on HRIPT LOEL of 25% nickel chloride (Vandenberg and Epstein, 1963), corresponding to an area dose of	165 μmol Ni/cm ²
Intraspecies EF	10
LOEL–NOEL EF	10
Repeated exposure EF	10
Application of a total EF of 1000 results in ANSAD of	165 nmol Ni/cm ²
Derivation of ANSAD based HRIPT LOEL of 3.3% nickel sulfate ^a (Kligman, 1966), corresponding to an area dose of	2.6 μmol Ni/cm ²
Intraspecies EF	10
LOEL–NOEL EF	10
Repeated exposure EF	10
Application of a total EF of 1000 results in ANSAD of	2.6 nmol Ni/cm ²
Derivation of ANEAD based on LLNA EC3 of 2.5% (Ryan et al., 2002), corresponding to an area dose of	2400 nmol Ni/cm ²
Interspecies EF	3
Intraspecies EF	10
Repeated exposure EF	10
Sensitization–elicitation EF $10^{(0.84 * \log(2.4) + 1.81)}$	135
Application of total EF of 40,500 results in ANEAD of	0.059 nmol Ni/cm ²
Derivation of ANEAD based on HRIPT LOEL of 25% nickel chloride (Vandenberg and Epstein, 1963), corresponding to an area dose of	165 μmol Ni/cm ²
Intraspecies EF	10
LOEL–NOEL EF	10
Repeated exposure EF	10
Sensitization–elicitation EF $10^{(0.84 * \log(165) + 1.81)}$	4700
Application of total EF of 4,700,000 results in ANEAD of	0.035 nmol Ni/cm ²
Derivation of ANEAD on based HRIPT LOEL of 1% nickel sulfate ^a (Kligman, 1966), corresponding to an area dose of	2.6 μmol Ni/cm ²
Intraspecies EF	10
LOEL–NOEL EF	10
Repeated exposure EF	10
Sensitization–elicitation EF $10^{(0.84 * \log(2.6) + 1.81)}$	144
Application of total EF of 144,000 results in ANEAD of	0.018 nmol Ni/cm ²
Human experience (all studies used nickel sulfate)	
LOEL patch test (1.25 μg Ni/cm ²) (Menne, 1994)	21 nmol Ni/cm ²
LOEL patch test (0.21 μg Ni/cm ²) (Hindsen et al., 1999) (NOEL 0.07 μg/cm ²)	3.6 nmol Ni/cm ²
LOEL patch test (0.053 μg Ni/cm ²) (Uter et al., 1995) (NOEL 0.026 μg/cm ²)	0.90 nmol Ni/cm ²
LOEL patch test plus detergent ^b (0.026 μg Ni/cm ²) (Uter et al., 1995)	0.44 nmol Ni/cm ²
LOEL patch test (0.15 μg Ni/cm ²) (Allenby and Basketter, 1993) (NOEL 0.05 μg/cm ²)	2.6 nmol Ni/cm ²
LOEL patch test on detergent-compromised skin ^c (0.015 μg Ni/cm ²) (Allenby and Basketter, 1993) (NOEL 0.005 μg/cm ²)	0.26 nmol Ni/cm ²
LOEL patch test with nickel-containing alloys (stainless steel) ^d (0.002 μg Ni/cm ²) (Menne et al., 1987)	0.034 nmol Ni/cm ²
EU nickel directive limits nickel-release from products, such as earrings, necklaces, bracelets, finger rings, wrist-watches, rivets and zippers to 0.5 μg/cm ² /week (EU, 1994)	1.2 nmol Ni/cm ²

^a See Table 2.

^b Test solution contained 1% of a commercial household detergent (aqueous solution of 25% alkyl benzenesulfonate and alkyl sulfonate plus 0.7% betaines).

^c Slight skin inflammation was induced by repeated immersion of forearm into 0.5% aqueous solution of sodium dodecyl sulfate at 35 °C for 10 min.

^d Patch testing was performed using 15-mm nickel-containing alloy disks that were fixed to the skin with Scanpor tape without further occlusion for 48 h; nickel release was determined by incubation of alloy disks in synthetic sweat. The release of the stainless steel was 0.01–0.04 μg Ni/cm² /week, which corresponds to a daily dose of about 0.002 μg/cm².

Starting from the LLNA EC3 and using a total EF of 40,500, an ANEAD of $0.059 \text{ nmol Ni/cm}^2$ ($0.0035 \text{ } \mu\text{g Ni/cm}^2$) can be derived (see Table 7). Similar values of 0.018 and $0.035 \text{ nmol Ni/cm}^2$ are obtained on the basis of human data for nickel sulfate and nickel chloride, respectively, using considerably higher total EFs. The LLNA-based ANEAD is well supported by extensive human data on elicitation of nickel allergy: Several studies that used titration of nickel concentration on nickel-allergic subjects reported LOEL values between 21 and $0.90 \text{ nmol Ni/cm}^2$ (1.25 and $0.053 \text{ } \mu\text{g Ni/cm}^2$; see Table 7) (Allenby and Basketter, 1993; Hindsen et al., 1999; Menne, 1994; Uter et al., 1995). When patch testing was performed on detergent-compromised skin, i.e., forearm skin showing slight inflammation induced by repeated immersion of the arm into a commercial household detergent solution, a 10-fold lower LOEL of about $0.26 \text{ nmol Ni/cm}^2$ was observed (Allenby and Basketter, 1993). The most sensitive nickel-allergic individuals reacted to stainless steel with an estimated nickel release of $0.034 \text{ nmol Ni/cm}^2$ (Menne et al., 1987).

Nickel is one of the few sensitizing chemicals, for which limit values have been derived on the basis of human potency data. In order to minimize the number of newly sensitized persons and to reduce the number of allergic reactions to nickel, the EU nickel directive limits the nickel-release from products, such as earrings, necklaces, bracelets, finger rings, wrist-watches, rivets and zippers to $0.5 \text{ } \mu\text{g Ni/cm}^2/\text{week}$ (EU, 1994), which corresponds to a daily area dose of about 1.2 nmol Ni/cm^2 ($0.07 \text{ } \mu\text{g Ni/cm}^2$). From the ANSAD of 8.0 nmol Ni/cm^2 and the ANEAD of $0.059 \text{ nmol Ni/cm}^2$ derived above, we would conclude that the EU limit should provide protection against sensitization, but not against allergy elicitation in nickel-allergic individuals, which is in line with observations described in the literature (see Table 7). In conclusion, also for the 'more difficult' allergen nickel useful ANSAD and ANEAD values can be derived on the basis on an LLNA EC3 using the extrapolation factor approach that we proposed in this paper.

7. Conclusions

A quantitative risk assessment methodology for skin sensitization is proposed in this paper. A scientifically sound risk assessment provides a suitable basis for selection of personal protection equipment and measures at the workplace. This is mandatory because allergic contact dermatitis is one of the most prevalent occupational skin diseases. Moreover, also the general population is exposed to sensitizing chemicals which are present, e.g., as preservatives, fragrance material, dye stuffs or impurities, in personal care and household products, textiles, and other consumer items. Based on a quantitative risk assessment, industry can set appropri-

ate upper use limits for preservatives (Robinson et al., 2000) and fragrance compounds (Api, 2002) and regulatory agencies can set limit values (e.g., for nickel; EU, 1994) or decide on ingredient labeling (as recently done in the EU for sensitizing fragrance compounds under the European Cosmetics Directive; EU, 2003) and warning labels (e.g., for oxidative hair dyes in the EU).

Since no species extrapolation step is necessary, it is normally preferable to base risk assessment on human data. However, with regard to the sensitization potency only a limited number of chemicals have been evaluated in human sensitization tests and the data which are available show a rather large variability due to the low number of doses tested (often only one dose is used) and differences in the test protocols used. With respect to quantitative sensitization risk assessment, the local lymph node test (LLNA) performed in mice is most relevant because by means of its EC3 value it provides a quantitative measure of sensitizing potency. A comparison of LLNA EC3 values with HRIPT and HMT LOEL and NOEL values was carried out in this study and revealed that the EC3, expressed as area dose, can be used as a starting point (as a surrogate NOEL) in risk assessment. In their sensitization risk assessment procedure for consumer products Gerberick and coworkers (Felter et al., 2002; Gerberick et al., 2001a) used the same boundaries (expressed as specific area dose) for sensitization potency categorization of either LLNA EC3 values or human NOEL values and, thereby, implied that EC3 and human NOEL values are directly comparable.

In our approach, we decided to use the 'classical' EFs for interspecies and intraspecies variability together with a factor for repeated exposure (in other toxicological risk assessment a time extrapolation factor is sometimes used) and a variable factor correlating elicitation and sensitization potency, when an ANEAD is to be derived on sensitization potency data. Using this approach, the risk assessment is independent of the product or situation resulting in human exposure. In a later step, the knowledge of exposure and intended use can be used to set appropriate use concentrations for the sensitizing chemical in, for example, a skin cream, a clothes washing powder or a metal working fluid. For example, when setting a use concentration for a sensitizing oxidative hair dye, it could be justified to reduce the EF for repeated exposure to 1 when evaluating consumer exposure because these products are recommended to be used only once a month.

The quantitative risk assessment concept by Gerberick and coworkers (Felter et al., 2002; Gerberick et al., 2001a) uses uncertainty factors of up to 10 for inter-individual variability, product matrix and use pattern. This approach is mainly focused on cosmetic and household products and integrates exposure considerations into the risk assessment. The resulting value, although termed 'area reference dose' is only valid for the

evaluated exposure scenario. In contrast, the ANSAD and ANEAD values proposed here, are in itself independent of the exposure scenario. Moreover, Gerberick and coworkers did not discuss possible interspecies differences and, thereby, implicitly used a factor of 1 without further justification. Also, by using the lower area dose boundary of the potency category, an additional arbitrary factor between 1 and 10 is introduced, depending on whether the value for the sensitization NOEL of the chemical to be evaluated is closer to the lower or the upper category boundary. In comparison, we consider our approach to be more generally applicable and transparent than that of Gerberick and coworkers.

Risk assessment for sensitization is not principally different from that for other toxicological endpoints. Both, induction of sensitization and elicitation of allergic responses, follow dose–response relationships and show thresholds below which no reactions occur. The main difference between sensitization and usual systemic endpoints is that the adequate descriptor of exposure is the area dose, expressed as nmol/cm^2 or $\mu\text{g}/\text{cm}^2$. We propose here to use the extrapolation/uncertainty factor approach to derive acceptable non-sensitizing area dose (ANSAD) and acceptable non-eliciting area dose (ANEAD) values. Compared to toxicological risk assessment for other endpoints, high EFs are applied to the LLNA EC3 value in some cases. Nevertheless, as shown for the three well-studied sensitizers MCI/MI, cinnamic aldehyde and nickel (Tables 5–7), the derived ANSAD and ANEAD values are in good agreement with clinical experience and experimental data.

Deviation from the EF values derived here (see Table 4) is explicitly encouraged during application of the methodology, provided that it is based on sound scientific reasoning. The given values for the EFs are intended as default values in cases in which no additional information, such as definitive experimental data, is available. Adjustments which we think will often be considered are deviations from the default 10's for intraspecies variability and repeated exposure, based on knowledge of the subpopulations exposed and the intended use. The adjustment means selecting and justifying a factor between 1 and 10 for the two individual EFs, which is done routinely in toxicological risk assessment. In the future, increasing knowledge and a growing body of experimental data will provide a basis for modifications to the proposed methodology, that will especially improve derivation of the ANEAD, which is currently based on a very limited set of experimental data.

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