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Skin Sensitization in Chemical Risk Assessment

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SKIN SENSITIZATION IN CHEMICAL RISK ASSESSMENT

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UNCERTAINTY FACTORS AND RISK ASSESSMENT FOR SKIN SENSITIZERS[1]

Peter Griem

1. INTRODUCTION

A few decades ago, allergic reactions to chemicals were often regarded as inaccessible for quantitative risk assessment (QRA) and were seen as all-or-none responses lacking dose–response relationships and thresholds. This was probably a result of how the immune system works: its response is characterized by a "learning phase" without symptoms (termed primary immune response or sensitization phase or induction), followed by the immune response effector phase (termed secondary immune response or elicitation phase or challenge reaction). Consequently, the first contact (and often repeated contacts), even with relatively high concentrations of a sensitizer, can go unnoticed, because no signs or symptoms of allergy occur. Nevertheless, this contact may induce sensitization—that is, cause the immune system to prepare for a reaction at the next contact. Once sensitization is established, every contact with the same sensitizer—sometimes even at concentrations several orders of magnitude lower—will lead to symptoms of allergic contact dermatitis (for further reading, see references cited in the contribution by G.F. Gerberick).

In the realm of chemical regulation, current risk management measures (e.g. classification and labelling and requirement for personal protection measures) are mostly based on the classification of chemicals and mixtures/formulations into either sensitizers or non-sensitizers. Recently, suggestions for classification systems using sensitization potency categories have been put forward (see, for example, EC, 2003; ECETOC, 2003; Akkan et al., 2004; Schneider & Akkan, 2004; Basketter et al., 2005a).

From basic immunological research and experimental studies in animals and humans, we know today that skin sensitization as well as allergy elicitation occur only above threshold doses and follow predictable dose–response relationships (see, for example, Kimber et al., 1999; Boukhman & Maibach, 2001; and references cited in the contribution by G.F. Gerberick). It has been shown that skin sensitization thresholds for different chemicals are spread over at least 5 orders of magnitude. This wide range of sensitizing potency suggests that solely hazard-based risk management may not be the most adequate form of addressing skin sensitization risks, especially because skin contact with potential sensitizers, for example, from consumer products and at the workplace, cannot be avoided completely. An exposure-based QRA to determine safe exposure levels of skin sensitizing chemicals may be better suited for setting exposure levels with negligible risk, for identifying safer alternative substances, and for protecting the health of workers and consumers.

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2. MAIN POINTS

In principle, the skin sensitization QRA approach follows the same four fundamental steps as identified for general toxicology risk assessment: hazard identification, dose–response assessment, exposure assessment, and risk characterization.

2.1 Hazard identification

Hazard identification either is based on human experience or involves the use of experimental data to determine the skin sensitization potential of a substance. Typically, the murine local lymph node assay (LLNA) or the guinea-pig maximization test (GPMT) is used. The contribution by G. Patlewicz explores the possibilities of using structure–activity relationships. Criteria used to classify a substance as skin sensitizing have been published, for example, in the European Dangerous Substances Directive 67/548/EEC (EC, 1984), in the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2005), and by ECETOC (2003).

2.2 Dose–response assessment or hazard quantification

2.2.1 Dose metric for induction and elicitation of skin allergy

Convincing evidence (reviewed, for example, in QRA Expert Group, 2006) suggests that the adequate dose metric for skin sensitization is the skin area dose—that is, the amount of chemical (remaining on the skin, for example, after rinse-off) per unit area of skin, expressed as nanomoles or micrograms per square centimetre. Multiple exposures onto the same skin area can be taken into account by using the cumulative area dose per day ($\mu g/cm^2$ per day).

The effectiveness with which a chemical can cause skin sensitization depends on a number of factors. Of prime importance is the skin penetration of the substance—that is, the topical dose versus the dose delivered to the first layers of living cells in the skin. Besides skin penetration, other factors, such as evaporation, metabolism on/in the skin (either inactivation or activation), sequestration in the stratum corneum, binding to protein or cells in the epidermis, as well as uptake and presentation by antigen-presenting cells and recognition by T-lymphocytes, determine if and how strong an immune response is triggered (reviewed, for example, in Kimber et al., 1999; Boukhman & Maibach, 2001; Gerberick et al., 2001a; Griem et al., 2003; QRA Expert Group, 2006). Typically, there is very little information available about the bioavailability (here, the availability to cells of the immune system) of sensitizing chemicals in either the experimental situation or real-life exposure scenario. Therefore, it is suggested (QRA Expert Group, 2006; see also contribution of G.F. Gerberick) that the applied area dose be used as a dose metric and that the uncertainty in this area be accounted for by the use of uncertainty factors (more precisely, all the parameters mentioned above are implicitly covered as part of uncertainty factors for differences between species, individuals, chemical matrices in which sensitizers occur, and use regimes).

2.2.2 Induction

Typically, the dose–response for induction of skin sensitization is determined in the first instance using animal assays such as the LLNA. Confirmatory human assays, such as the

human repeat insult patch test (HRIPT), may be subsequently conducted for substances intended for skin contact to provide substantiation of the LLNA data (see contribution by A.M. Api). The aim is to define a point of departure for risk assessment. For ethical reasons, no-observed-effect levels (NOELs) or benchmark doses (BMDs) from studies in humans are normally not available. Therefore, in a number of studies, human NOELs and BMDs were compared with LLNA thresholds (EC3 values, or the effective concentrations inducing a stimulation index of 3), and it was found that the average ratio of both values is close to 1, indicating that area doses are directly comparable between mice and humans—that is, a sensitization threshold of 10 $\mu mol/cm^2$ in mice corresponds to a NOEL or BMD of 10 $\mu mol/cm^2$ in humans. Therefore, the LLNA EC3 value has been suggested as a surrogate NOEL in QRA (Basketter et al., 2000, 2005b; Gerberick et al., 2001a, 2001b; Griem et al., 2003; Schneider & Akkan, 2004). For certain substances that are intended to come into contact with the skin of consumers, such as cosmetic ingredients, confirmatory HRIPTs using an area dose not exceeding the area dose equivalent to the LLNA EC3 may be acceptable (Api, 2002; QRA Expert Group, 2006).

Guidelines to apply a weight-of-evidence approach to all available human and animal data in order to derive a point of departure for the QRA have been suggested for fragrance ingredients (QRA Expert Group, 2006). This group suggested naming the point of departure the "no-expected-sensitization induction level" (NESIL).

2.2.3 Elicitation

The dose–response for elicitation of allergic contact dermatitis can be determined in different experimental setups. In clinical patch tests on allergic patients, the concentration of the sensitizer (in a suitable vehicle such as Vaseline) can easily be varied and an elicitation threshold determined. Alternatively, the repeated open application test (ROAT) or a product use test can be employed. The patch test minimum elicitation threshold (MET)—for example, as the MET inducing a threshold response in 10% of the subjects tested (MET_{10})—and a NOEL or BMD from a ROAT or use test have been proposed as points of departure for risk assessment (Weaver et al., 1985; Sosted et al., 2006; Zachariae et al., 2006; see also contribution of G.F. Gerberick).

The elicitation thresholds are usually determined in subjects who have had an established allergy for a long period of time. Tests in which elicitation thresholds were obtained using newly sensitized subjects (e.g. in the human maximization test [HMT] and HRIPT) showed that elicitation thresholds in these subjects depend on the sensitization dose used; that is, the higher the sensitization dose, the lower the elicitation threshold (Friedmann et al., 1983). This dependency has also been found in mice (Scott et al., 2002). Thus, it seems that the elicitation threshold decreases with the time of established allergy and with the number of exposures. Although it has not been formally shown that a "minimum threshold" is finally approached, the thresholds determined in well established allergic individuals seem more reliable than those determined after experimental sensitization.

2.3 Exposure assessment

Exposure to the skin sensitizer is determined using habits and practice data for products containing the substance and may be complemented by experimental measurement of skin exposure. While the importance of the exposure assessment for an adequate risk characterization cannot be overestimated, it is beyond the scope of this contribution to provide an overview of this topic.

2.4 Risk characterization

An extrapolation/uncertainty factor approach can be applied to the selected point of departure in order to derive an acceptable level of exposure to a skin sensitizing substance. It has been proposed to term this factor "sensitization assessment factor" (SAF) (QRA Expert Group, 2006). The acceptability or unacceptability of the real-life exposure situation with respect to sensitization induction or allergy elicitation can then be determined accordingly. To this end, the point of departure for risk assessment (for either induction or elicitation), expressed as area dose, is divided by the SAF to derive an acceptable exposure level. An estimated/determined exposure, expressed as area dose, below this acceptable exposure level is then considered without appreciable risk of, respectively, sensitization of non-sensitized subjects and elicitation of acute contact dermatitis in already sensitized subjects. The SAF is calculated by multiplication of individual factors that account for interspecies and intraspecies variability as well as for matrix and use.

2.4.1 Interspecies factor

Comparison of human NOELs with LLNA EC3 values suggested that a factor of 3 ($10^{0.5}$) is sufficient to cover the species variability (Griem et al., 2003), especially since vehicle differences in the human and animal exposure are also taken into account in the matrix factor. The interspecies factor can be set to 1 if the point of departure is based on human data. This applies to both induction and elicitation.

2.4.2 Intraspecies factor (interindividual variability)

This factor accounts for possible variations in the sensitivity between individuals due to factors such as genetic effects, higher susceptibility (e.g. individuals with multiple skin allergies or those with damaged skin from pre-existing skin disease), decreased inherent barrier function, age, sex, and ethnicity. These contributing factors have been discussed, for example, by Felter et al. (2002), Griem et al. (2003), and QRA Expert Group (2006). For induction, a factor of 10 has been proposed to adequately cover interindividual variability. With regard to elicitation, there is a considerable variation of the NOEL and MET both between individuals and when the test is repeated in the same individual (Jerschow et al., 2001). While this could be an argument for applying a default factor of 10, it should also be considered that the point of departure used for risk assessment is already based on the lowest MET, that is, the most susceptible individuals.

2.4.3 Matrix factor

The matrix factor has been introduced in the safety evaluation concept for sensitizing fragrance ingredients in cosmetic products (Felter et al., 2002; QRA Expert Group, 2006).

Consideration of matrix effects encompasses extrapolation from the matrix/vehicle used to determine the EC3/NOEL in the experimental situation to the product formulation containing the fragrance ingredient to which the consumer is exposed in real-life scenarios. The larger the difference between the experimental situation and real-life exposure scenario, the greater the factor will be. The two areas within vehicle/matrix effects that are especially noteworthy are irritants and penetration enhancers. Usually a value of 1, 3, or 10 is chosen for the matrix factor.

2.4.4 Use factor

The QRA Expert Group (2006) considered three key parameters when extrapolating from the controlled experimental situation (either human or animal) to the real-life scenario. These are site of contact, dermal integrity, and occlusion. The larger the difference in skin site location (e.g. compared with the test site, skin may be more easily irritated, highly follicular, or shaved), effect on barrier integrity (e.g. from diaper rash, existing dermatitis, wet work), and occlusion (e.g. from diapers, gloves, or axillary products), the greater the factor. Usually a value of 1, 3, or 10 is chosen for the use factor. As a fallback for situations in which the use scenario is unknown or cannot be accurately described, application of a repeat exposure factor of 10, instead of the use factor, has been suggested (Griem et al., 2003).

2.5 Examples of risk assessments

- Cosmetic ingredients (e.g. fragrance ingredients and preservatives) (Gerberick et al., 2001a; Felter et al., 2002; QRA Expert Group, 2006), as well as hand wash detergents and fabric softeners (Schütte & Kern, 2005)
  End-point: Induction
  Point of departure: Confirmatory HRIPT NOEL based on LLNA EC3
  SAF: Interindividual factor (10) × matrix factor (1–10) × use factor (1–10)

- Sensitizing chemicals in general (Griem et al., 2003)
  End-point: Induction
  Point of departure: HRIPT NOEL, HMT NOEL, or LLNA EC3
  SAF: Interspecies factor (3) × interindividual factor (10) × repeated exposure factor (10)
  End-point: Elicitation
  Point of departure: Patch test NOEL
  SAF: Interindividual factor (10) × repeated exposure factor (10)

- Metals in household consumer products (Basketter et al., 2003)
  End-point: Induction
  Point of departure: LLNA EC3
  SAF: Interspecies factor (1) × interindividual factor (10) × matrix factor (1–10) × use factor (1–10)

- Hexavalent chromium (Nethercott et al., 1994)
  End-point: Elicitation
  Point of departure: Patch test 10% MET
  SAF (not explicitly stated, but implicitly used for deriving acceptable Cr(VI)

concentration in soil): Intraspecies factor (1) × matrix/vehicle factor (1)

- Pesticides (hexavalent chromium) (USEPA FIFRA-SAP, 2004)
  End-point: Induction
  Point of departure: Human NOEL (LLNA EC3 seen as promising)
  SAF: (Interspecies factor (1–10) ×) intraspecies factor (1–10) × matrix/vehicle factor (1–10) × repeated exposure factor (1–10)
  End-point: Elicitation
  Point of departure: Patch test 10% MET or ROAT 10% MET (as BMD_{10})
  SAF: Intraspecies factor (1–10) × matrix/vehicle factor (1–10) × exposure factor (1–10)

3. CONCLUSIONS AND FUTURE DIRECTIONS

Risk assessment of skin sensitizers is not principally different from that for other toxicological end-points. 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 systemic toxicity end-points is that for skin sensitization, the adequate descriptor of exposure is dose per skin area, expressed as nanomoles or micrograms per square centimetre per day. The extrapolation/uncertainty factor approach can be used to derive acceptable non-sensitizing and non-eliciting area doses for induction and elicitation, respectively. However, up to now, this concept has been used in isolated cases and for limited, well defined fields of application. The concept might gain and be improved through discussion involving all stakeholders (academia, industry, clinic, authorities) of issues such as points of departure for risk assessment, extrapolation/uncertainty factors, fields of application, and regulatory implications.

REFERENCES

Akkan Z, Kalberlah F, Oltmanns J, Schneider K (2004) *Beurteilung der Wirkstärke hautsensibilisierender Chemikalien anhand des Local Lymph Node Assay*. Dortmund, Berlin, Dresden, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (Forschungsbericht Fb 1009).

Api AM (2002) Sensitization methodology and primary prevention of the Research Institute for Fragrance Materials. *Dermatology*, 205: 84–87.

Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, Harvey P, Evans P, White IR, Rycroft RJG (2000) Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Dermatitis*, 42: 344–348.

Basketter DA, Angelini G, Ingber A, Kern PS, Menné T (2003) Nickel, chromium and cobalt in consumer products: Revisiting safe levels in the new millennium. *Contact Dermatitis*, 49: 1–7.

Basketter DA, Andersen KE, Liden C, Van Loveren H, Boman A, Kimber I, Alanko K, Berggren E (2005a) Evaluation of the skin sensitizing potency of chemicals by using the

existing methods and considerations of relevance for elicitation. *Contact Dermatitis*, 52: 39–43.

Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, Dearman RJ, Kimber I (2005b) Predictive identification of human skin sensitization thresholds. *Contact Dermatitis*, 53: 260–267.

Boukhman MP, Maibach HI (2001) Thresholds in contact sensitization: Immunologic mechanisms and experimental evidence in humans—an overview. *Food and Chemical Toxicology*, 39: 1125–1134.

EC (1984) *Classification, packaging, and labelling of dangerous substances.* European Commission (European Dangerous Substances Directive 67/548/EEC).

EC (2003) *Meeting of the Sensitisation Expert Group, Ispra, 4–6 November 2002.* European Commission (ECBI/81/02 Rev. 2, 8 January 2003).

ECETOC (2003) *Contact sensitisation: Classification according to potency.* European Centre for Ecotoxicology & Toxicology of Chemicals (Technical Report No. 87).

Felter SP, Robinson MK, Basketter DA, Gerberick GF (2002) A review of the scientific basis for uncertainty factor for use in quantitative risk assessment for the induction of allergic contact dermatitis. *Contact Dermatitis*, 47: 257–266.

Friedmann PS, Moss C, Shuster S, Simpson JM (1983) Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clinical and Experimental Immunology*, 53: 709–715.

Gerberick GF, Robinson MK, Felter SP, White IR, Basketter DA (2001a) Understanding fragrance allergy using an exposure based risk assessment approach. *Contact Dermatitis*, 45: 333–340.

Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, Wright Z, Marks JG (2001b) Contact allergenic potency: Correlation of human and local lymph node assay data. *American Journal of Contact Dermatitis*, 12: 156–161.

Griem P, Goebel C, Scheffler H (2003) Proposal for a risk assessment methodology for skin sensitization potency data. *Regulatory Toxicology and Pharmacology*, 38: 269–290.

Jerschow E, Hostynek JJ, Maibach HI (2001) Allergic contact dermatitis elicitation thresholds of potent allergens in humans. *Food and Chemical Toxicology*, 39: 1095–1108.

Kimber I, Gerberick GF, Basketter DA (1999) Thresholds in contact sensitization: Theoretical and practical considerations. *Food and Chemical Toxicology*, 37: 553–560.

Nethercott J, Paustenbach D, Adams R, Fowler J, Marks J, Morton C, Taylor J, Horowitz S, Finley B (1994) A study of chromium induced allergic contact dermatitis with 54 volunteers: Implications for environmental risk assessment. *Occupational and Environmental Medicine*, 51: 371–380.

QRA Expert Group (2006) *Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients*. Technical Dossier, 15 March 2006, revised 26 May 2006 (http://www.rifm.org/pub/publications.asp).

Schneider K, Akkan Z (2004) Quantitative relationship between the local lymph node assay and human skin sensitization assays. *Regulatory Toxicology and Pharmacology*, 39: 245–255.

Schütte K, Kern PS (2005) Skin safety of perfumes in detergents and fabric softeners in light of the labeling requirements of the new detergent regulation. *Chimica oggi/Chemistry Today*, Supplement, October, pp. 55–58.

Scott AE, Kashon ML, Yucesoy B, Luster MI, Tinkle SS (2002) Insights into the quantitative relationship between sensitization and challenge for allergic contact dermatitis reactions. *Toxicology and Applied Pharmacology*, 183: 66–70.

Sosted H, Menné T, Johansen JD (2006) Patch test dose–response study of *p*-phenylenediamine: Thresholds and anatomical regional differences. *Contact Dermatitis*, 54: 145–149.

United Nations (2005) *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 1st rev. ed. New York, NY, and Geneva (http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html).

USEPA FIFRA-SAP (2004) *Consultation on dermal sensitization issues for exposures to pesticides*. United States Environmental Protection Agency, Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel, Minutes of Meeting on 4–6 May 2004, SAP Report No. 2004-02 (http://www.epa.gov/scipoly/sap/meetings/2004/index.htm; see also FDMS Docket System located at http://www.regulations.gov under docket ID No. EPA-HQ-OPP-2006-0693).

Weaver JE, Cardin CW, Maibach HI (1985) Dose–response and diagnostic patch testing with sensitized humans. *Contact Dermatitis*, 12: 141–145.

Zachariae C, Lerbaek A, McNamee PM, Gray JE, Wooder M, Menné T (2006) An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients. *Contact Dermatitis*, 55: 160–166.