

Dose-Response Relationships and Threshold Levels in Skin and Respiratory Allergy

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A literature study was performed to evaluate dose-response relationships and no-effect levels for sensitization and elicitation in skin- and respiratory allergy. With respect to the skin, dose-response relationships and no-effect levels were found for both intradermal and topical induction, as well as for intradermal and topical elicitation of allergenic responses in epidemiological, clinical, and animal studies. Skin damage or irritation may result in a significant reduction of the no-effect level for a specific compound. With respect to the respiratory tract, dose-response relationships and no-effect levels for induction were found in several human as well as animal studies. Although dose-response relationships for elicitation were found in some epidemiological studies, concentration-response relationships were present only in a limited number of animal studies. Reported results suggest that especially relatively high peak concentrations can induce sensitization, and that prevention of such concentrations will prevent workers from developing respiratory allergy. Moreover, induction of skin sensitization may result in subsequent heightened respiratory responsiveness following inhalation exposure. The threshold concentration for the elicitation of allergic airway reactions in sensitized subjects is generally lower than the threshold to induce sensitization. Therefore, it is important to consider the low threshold levels for elicitation for recommendation of health-based occupational exposure limits, and to avoid high peak concentrations. Notwithstanding the observation of dose-response relationships and no-effect levels, due to a number of uncertainties, no definite conclusions can be drawn about absolute threshold values for allergens with respect to sensitization and elicitation reactions in the skin and respiratory tract. Most predictive tests are generally meant to detect the potential of a chemical to induce skin and/or respiratory allergy at relatively high doses. Consequently, these tests do not provide information of dose-response relationships at lower doses such as found in, for example, occupational situations. In addition, the observed dose-response relationships and threshold values have been obtained by a wide variety of test methods using different techniques, such as intradermal exposure versus topical or inhalation exposure at the workplace, or using different endpoints, which all appear important for the outcome of the test. Therefore, especially with regard to respiratory allergy, standardized and validated dose-response test methods are urgently required in order to be able to recommend safe exposure levels for allergens at the workplace.

Keywords Dose-Response, Elicitation, Respiratory Allergy, Sensitization, Skin Allergy

I. INTRODUCTION

Allergy is a diverse family of diseases caused by untoward immune reactions that may ultimately lead to tissue inflammation and organ dysfunction. Allergy comprises a two-phase process: (1) a generally symptom-free sensitization phase and (2) a symptomatic effector phase. After initial encounters with a particular allergen, a primary immune response is mounted, resulting in a state of heightened responsiveness to this allergen

(induction or sensitisation). Upon subsequent exposures (challenge), a vigorous immune response is provoked that can result in clinically manifest adverse health effects (challenge or elicitation).

Two important types of allergic diseases at the workplace are skin and respiratory allergy. These are caused by exposure to exogenous substances, that is, certain low-molecular-weight (LMW) chemicals ($\ll 5000$ Da) or high-molecular-weight (HMW) compounds (usually proteins, > 5000 Da; Chan-Yeung and Malo, 1995). Occupational exposure of humans to LMW allergens via the skin may be considerable, such as found in auto body shop workers exposed to isocyanates despite protective clothing (Liu et al., 2000). Occupational skin allergy induced

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by allergenic proteins is less frequently reported since such chemicals do not easily penetrate the skin. Occupational asthma resulting from respiratory sensitization can be life-threatening (Fabbri et al., 1988; Ehrlich, 1994), and the majority of patients do not completely recover even when removed from exposure for several years (Saetta et al., 1992, 1995; Chan-Yeung and Malo, 1995; O'Neill, 1995). The number of known respiratory allergens is increasing and includes both HMW compounds and LMW chemicals. LMW compounds are reactive chemicals and act as an allergen when bound to an appropriate carrier protein; that is, a complete hapten-carrier antigen is formed. The antigenic determinant may be the hapten, or it may be a new antigenic determinant formed by the chemical reaction between the hapten and the carrier protein. This requirement for haptentation is an important distinction between protein and LMW-induced allergy (Cullen et al., 1990; Chan-Yeung and Malo, 1995).

Given the serious health problems of skin and respiratory allergy together with the continuous introduction of new compounds into workplaces, early identification of chemical sensitizers is very important. With respect to skin allergy, validated predictive tests exist. For respiratory allergy, however, validated tests are lacking. Although a number of animal test protocols have been published to detect respiratory allergy (see for reviews Briatico-Vangosa et al., 1994; Pauluhn et al., 1999; Pauluhn and Mohr, 2005), none of these are widely applied or fully accepted. Moreover, most predictive tests carried out are meant to detect potential rather than potency.

Determination of potency is important since the development of sensitivity to allergens and the severity of symptoms may be directly related to exposure levels. Exposure-response data are needed to assess the effective dose(s) and "no-observed-effect" or threshold levels for sensitization and elicitation upon challenge to specific skin and respiratory allergens. No-effect levels are to be used as a basis for risk assessment and management to prevent allergic sensitization and specific hyperreactivity in exposed workers. Established thresholds may thus form a basis for the recommendation of health-based occupational exposure limits.

The objective of this survey was to review the available literature on dose-response relationships and no-effect levels for both skin and airway sensitization and elicitation that could be used to obtain safe exposure concentrations for allergens at the workplace. Epidemiological, clinical, and animal studies were reviewed.

II. SKIN ALLERGY

Skin allergy generally is associated with a cell-mediated immune reaction, formerly described as type IV hypersensitivity reaction according to the classification of Gell and Coombs (Roitt et al., 1998), and is then called allergic contact dermatitis. In this type of reaction, allergens come into contact with Langerhans cells in the skin, which carry the allergen through the lymphatics to the regional or local lymph nodes, where the al-

lergen is presented to naive CD4+ T lymphocytes. After several days, clones of these T cells become sensitized to the allergen and circulate as memory cells in the bloodstream, and some reside in the skin. Upon reexposure to the allergen, the specifically sensitized memory T lymphocytes infiltrate the site of contacted skin, where they release cytokines and other factors that attract the numerous inflammatory cells that are responsible for the epidermal cell injury. Clinically, the contacted skin becomes erythematous and swollen with vesiculation within 6 hours to several days after reexposure. Hence the term "delayed reaction" for the cell-mediated response. Other allergic skin reactions require in the sensitization phase the production of allergen-vasoactive mediators from mast cell granules. Clinically, this results in urticaria (raised pruritic papules and plaques) and angioedema, which develop acutely and may fade within hours. Persistent plaques, however, can last for more than 24 hours. Because of the involvement of immunoglobulin E (IgE) antibody and the acute response, urticaria and angioedema are called antibody- or IgE-dependent, anaphylactic or immediate-type reactions, formerly called type I hypersensitivity reaction according to the classification of Gell and Coombs. Urticaria occurs in atopic as well as in nonatopic individuals.

The most common skin allergens at the workplace are a number of low-molecular-weight chemicals, such as Kathon CG, aldehydes, nickel salts, certain isocyanates, DNCB, DNFB, oxazolone, PPD, and rubber chemicals. Far less is reported on effects of skin allergenic proteins, probably because these HMW compounds do not easily penetrate the intact skin.

With respect to skin effects, a compound is usually classified as a skin sensitizer (EC risk phrase R43) upon a positive skin sensitization test such as the guinea pig maximization test (GPMT; Magnusson and Kligman, 1969) or the Buehler test (Buehler and Griffith, 1975), incorporated in the Organization for Economic Cooperation and Development (OECD) guidelines (OECD 406; OECD, 1992). However, according to European Commission (EC) guidelines, scientific justification should be given when the Buehler test is used. The sensitivity of the GPMT can be increased by means of intradermal injection with Freund's complete adjuvant. Therefore, different labeling criteria are used: A positive result in the GPMT is obtained when 30% or more of the animals react in a test involving adjuvant, or when 15% or more of the animals react without using adjuvant. Two other tests are also used: the mouse ear swelling test (MEST; Gad et al., 1986) and the local lymph node assay (LLNA; Dearman et al., 1992a, 1992b). The LLNA investigates sensitization potential by measuring cell proliferation in the lymph nodes draining the area of application that is the ear. Initially, these tests were used as a first stage in the assessment of skin sensitization potential. If a positive result was seen in either assay, a test substance was classified as a sensitizer and it was not necessary to conduct a further guinea pig test. However, if a negative result was seen, a guinea pig test still had to be conducted. Recently, the LLNA (OECD 429; OECD, 2000) has been indicated as the preferred skin sensitization test.

A. Dose-Response Relationships and Thresholds for Skin Sensitization

1. Low-Molecular-Weight Chemicals

a. *Epidemiological or Clinical Studies (Table 1).* Multiple patches for induction of sensitization, followed by a 2-week rest period and a subsequent challenge with a patch at a new skin site, are generally used to determine skin allergy in humans (Stotts, 1980). This method has shown dose-response relationships for several skin sensitizers like benzocaine, mafenamidine, bronopol, formaldehyde, glutaraldehyde, and PPD (Marzulli and Maibach, 1974). A linear relationship was found between the degree of sensitivity and the sensitizing dose (in log) using DNCB. The 50% sensitizing dose (ED_{50}) calculated from the dose-response curve was $116 \mu\text{g}$ (Friedmann et al., 1983). Patch tests with Kathon CG showed that the induction threshold for sensitization was between 10 and 20 ppm (0.001–0.002%), similar to the value found in animal studies (Cardin et al., 1986).

With respect to the dose per unit area, it was shown that high nickel concentrations applied to limited skin areas (i.e., a local high concentration, comparable to skin occlusion) were needed to sensitize a substantial number of individuals (Menné and Calvin, 1993). A nickel concentration of $0.5 \mu\text{g}/\text{cm}^2$ has been suggested as a no-effect level for sensitization, based on a wide range of studies (Menné, 1994).

The vehicle used may play a significant role. Upon testing the sensitization potential of pure hydroxycitronellal, none out of 39 subjects was sensitized at 5%, 1 out of 38 subjects was sensitized at 7.5%, while 6 out of 40 subjects were positive at 10% (Steltenkamp et al., 1980). Similar results were reported by Ford et al. (1988): No reactions were induced at 5%, while 10% sensitized 2 out of 25 subjects, and a 12% concentration sensitized 8 out of 11 subjects. In contrast, hydroxycitronellal in petrolatum at 5% or 12% did not induce sensitization (Opdyke, 1974). For cinnamic aldehyde, results also depended on the vehicle used. Using ethanol as vehicle, cinnamic aldehyde did not induce sensitization at concentrations of 0.1 or 0.5%. At 1%, 5 out of 41 subjects were positive, and at 1.25%, 5 out of 10 subjects were sensitized. Using petrolatum as a vehicle, in contrast, subjects treated with 20 (i.e., 4 sets of 5) consecutive 48-h patch test exposures responded already to 0.5% cinnamic aldehyde (Danneman et al., 1983).

b. Animal Studies (Table 1).

Skin sensitization measured by delayed-type skin reactions after dermal challenge. For several compounds, dose-response relationships were obtained. In a GPMT in which both induction and challenge application concentrations were varied in order to determine relative sensitization potencies of substances, groups of animals were sensitized by intradermal and topical induction of various concentrations of 1 of 15 chemicals. Three weeks after the initial injection, animals were challenged with one of various concentrations by an open patch test. The sensitization rate was found to be related to the sensitization dose for all 15 chemicals tested. The minimum induction concentrations that induced a positive response were, for in-

stance, 20 ppm (0.002%) DPTU, 500 ppm (0.05%) MBT, and 10 ppm (0.001%) DNCB. No-effect levels were 2 ppm (0.0002%) DPTU, <500 ppm (0.05%) MBT, and 1 ppm (0.0001%) DNCB (Nakamura et al., 1994; see also Table 1).

With respect to the type of the dose-response curve, a modified GPMT using five dose groups (multiple dose design) resulted in nonlinear, sigmoid, sensitization dose-response curves for formaldehyde, cinnamic aldehyde, MBT and Kathon CG. The intracutaneous sensitization EC_{50} s were found to be 0.96% for formaldehyde, 0.04% for cinnamic aldehyde, 0.07% for MBT, and <3 ppm (0.0003%) for Kathon CG. For MBT a no-effect level of 0.03% was found, and for the other three compounds a no-effect level could not be established (Andersen et al., 1995). A sigmoid dose-response relationship for sensitization with saturation at higher doses has also been described for other LMW chemicals like DNCB (Buehler and Griffith, 1975), sultones (Ritz et al., 1975), *p*-nitrobenzyl compounds (Roberts et al., 1983), and chlorocresol (Andersen and Hamann, 1984). Also using “Buehler’s occluded epicutaneous patch test,” a sigmoid sensitization dose-response curve was obtained for Kathon biocide. There was evidence of a very steep slope in this sigmoid response curve, resulting in a substantial increase in sensitization with relatively small changes in concentration. A no-response concentration was observed for several combinations of induction and challenge concentrations. The EC_{50} for induction was 88 ppm (0.0088%) at a challenge concentration of 2000 ppm (0.2%; Chan et al., 1983). In a cumulative contact enhancement test (CCET), a test method using multiple topical applications, a dose-related sensitization potential with a bell-shaped curve and threshold levels could be detected for MDBGN (Wahlkvist et al., 1999a). Dose-related sensitization potential (monotone or nonmonotone curves [i.e., increases at lower concentrations, decreases at higher concentrations]) was also reported for potassium dichromate and hydroxycitronellal using the CCET or the FCAT, but not the GPMT. However, for all three tests, EC_{50} values and threshold concentrations could be calculated (Wahlkvist et al., 1999b).

Different types of sensitization dose-response curves have been observed in different species using the same compounds. Guinea pigs topically sensitized with various doses of HMDI and PiCl showed a normal dose-response curve upon challenge; that is, reactions were more severe in animals receiving the higher sensitization doses. A no-observed-effect level was found in animals receiving 0.01 mg HMDI or PiCl. In mice, in contrast, the sensitization dose-response to both HMDI and PiCl showed a bell-shape curve; that is, the response increased with increasing dose, showed a plateau at higher doses, and decreased at the highest exposure concentrations tested (Stadler and Karol, 1985).

Different results have been obtained using intradermal versus topical exposure. In the GPMT, intradermal concentrations ranging from 0.01% to 3% and topical (second induction) concentrations ranging from 0.5% to 20% formaldehyde were tested. A nonlinear, sigmoid, sensitization dose-response

TABLE 1
Summary of methods to determine dermal sensitization dose-response relationships

Human/animals	Sensitization method ^a	Elicitation method	Parameter(s) evaluated	References
Human	Multiple patches	New patch after 2 weeks	Evaluation of skin effects	Marzulli and Maibach (1974), Stotts (1980), Friedmann et al. (1983), Cardin et al. (1986)
Guinea pig (GPMT)	One intradermal, one topical induction under occlusion with or without FCA	Topical application under occlusion	Evaluation of skin effects	Andersen et al. (1985, 1995)
Guinea pig (GPMT)	One intradermal, one topical induction	Topical application (open patch)	Evaluation of skin effects	Nakamura et al. (1994)
Guinea pig (Buehler)	Three topical applications under occlusion	Topical application under occlusion	Evaluation of skin effects	Buehler and Griffith (1975), Chan et al. (1983)
Guinea pig (CCET)	Multiple (4) topical applications under occlusion with FCA prior to the 3rd application	Topical application (open patch)	Evaluation of skin effects	Wahlkvist et al. (1999a, b)
Guinea pig (FCAT)	Three intradermal injections (with FCA)	Topical application (open patch)	Evaluation of skin effects	Wahlkvist et al. (1999b)
Guinea pig	Multiple (20) topical (open) applications with 4 adjuvant injections	Topical application under occlusion	Evaluation of skin effects	Nielsen et al. (1992)
Mouse (MEST)	Multiple topical applications	Topical application	Measurement of ear thickness	Brown and Shivji (1991)
Mouse/rat (LLNA)	Multiple topical (usually 3) applications on ears	—	Measurement of proliferation of draining lymph nodes	Botham et al. (1991b), Kimber et al. (1995), Loveless et al. (1996), Arts et al. (1997), Ulrich et al. (1998), Dearman et al. (1999), Wahlkvist et al. (1999a), Warbrick et al. (1999), Woolhiser et al. (2000), van Och et al. (2000), Dearman et al. (2001), Klink and Meade (2003), Plitnick et al. (2003)
Mouse	Topical applications (2) on flanks, followed by ear applications (3)	—	Measurement of several cytokine levels (IL-4, IL-5, IL-10, IL-13, IFN- γ , TNF- α)	Ryan et al. (1998), Dearman and Kimber (1999), Vandebriel et al. (2000), Ulrich et al. (2001), Plitnick et al. (2002)
Mouse	Topical applications (2) or multiple topical applications	—	Measurement of total serum IgE content	Hilton et al. (1995), Potter and Wederbrand (1995), Klink and Meade (2003)
Rat	Multiple topical applications	—	Measurement of specific IgE and IgG	Zhang et al. (2002)

(Continued on next page)

TABLE 1
Summary of methods to determine dermal sensitization dose-response relationships (*Continued*)

Human/animals	Sensitization method ^a	Elicitation method	Parameter(s) evaluated	References
Guinea pig	Single intradermal injection	—	Measurement of antigen-specific antibodies	Blaikie et al. (1995), Zhang et al. (1998)
Guinea pig	Single intradermal injection	Inhalation challenge	Measurement of airway responses	Blaikie et al. (1995)
Guinea pig	Single intradermal injection	Intratracheal challenge	Measurement of airway responses, or plasma extravasation with Evans blue dye	Arakawa et al. (1995), Zhang et al. (1998)
Mouse	Multiple topical applications	Intratracheal challenge	Evaluation of airway histopathology	Klink and Meade (2003)

^aBy using various concentrations, dose-response relationships were obtained; see text for more details of the methods used.

relationship was obtained following challenge with 1% formaldehyde, with saturation at high induction doses. The incidence of contact sensitivity depended on the intradermal application (first induction) dose but not on the topical (second induction) dose. The formaldehyde concentration at which contact sensitivity was seen in 50% of the animals (EC₅₀) was 0.061% and 0.024%, using two guinea pig strains, respectively (Andersen et al., 1985). In guinea pigs, using an open epicutaneous application method, animals were sensitized 5 days a week for 4 weeks with 0.3–3% nickel sulfate using adjuvant. They were challenged 14 and 21 days after the last treatment using 2% nickel sulfate in water and 1% in petrolatum, respectively. A nonlinear dose-response curve in incidence was obtained indicating that there was an optimal induction dose (Nielsen et al., 1992). From the same group, another study was reported also using nickel sulfate. Six intradermal (0.01–3.0%) and six topical (0.25–10.0%) concentrations were used for induction, while 1% and 2% concentrations were used for patch test challenges. Intradermal induction was performed on day 0, topical induction on day 7, and subsequent challenges were performed on days 21 and 35. After the first challenge with 1% nickel on day 21, a linear relationship was obtained between the intradermal induction dose and the number of animals developing contact dermatitis, resulting in a significant maximum contact dermatitis rate of 42% (at an intradermal induction with 3%). The intradermal nickel dose was decisive for the development of contact allergy, while variation in the topical induction concentration had no effect. Another patch challenge with 2% nickel on day 51 revealed a nonlinear (bell-shaped) dose-response relationship following a topical booster with 10% nickel on day 35. A maximum response frequency of 45% was found after initial intradermal induction with 3%, and initial topical induction with 2% nickel. Thus, the dose-response relationship was linear at the initial challenge and nonlinear after the rechallenge following a topical booster. This test, however, was not found to be an efficient animal model for experimental nickel contact allergy,

as it was not possible to achieve strong, lasting, and sensitive nickel sensitization in the majority of the test animals (Rohold et al., 1991).

Also, the number of applications may play a significant role. In the aforementioned study of Wahlkvist et al. (1999a), using MDBGN, no statistically significant sensitization was induced in the GPMT using one intradermal application followed by a topical application. In contrast, in the CCET, using multiple topical applications, a dose-related sensitization potential could be detected for MDBGN.

Changing the dose by changing the volume is not necessarily the same as changing the concentration, which may lead to differences in surface concentrations. In a MEST, used to investigate the relationship between sensitizing dose and skin reactions to DNFB, groups of mice were sensitized on the shaved back skin with various volumes of a fixed concentration of DNFB. Upon challenge, ear swelling reactions were greater at lower DNFB sensitization doses. Reactions were even observed at the lowest dose tested (Brown and Shivji, 1991). For DNFB, DCC, and oxazolone, ear swelling dose-response relationships with a cubic trend (optimum for DNFB and DCC, plateau for oxazolone) were obtained after cutaneous sensitization with different concentrations followed by a challenge with the same compound (Flint et al., 2003).

Skin sensitization measured directly by proliferation in skin-draining lymph nodes. In the LLNA, dose-response relationships and sensitization thresholds have been obtained for Kathon CG and two other isothiazolinone-based chemicals (Botham et al., 1991b) and a number of other chemicals such as DNCB, citral, DNBS, chloromethylisothiazolone, diphenylpropenone, ethylene glycol dimethacrylate, formaldehyde, glutaraldehyde, TDI, MDI, HDI, TMA, PA, isopropyl myristate, MDBGN, PPD, PDC, AMT, nickel sulfate, eugenol, isoeugenol, geraniol, hydroxycitronellal, isopropyl myristate, linalool, cinnamic aldehyde, alpha-hexylcinnamaldehyde, BMHCA, TCSCA, squaric acid dibutyl ester, tetramethylthiuram disulfide, and a

piperidiny chlorotriazine (PCT) derivative (see Table 5). A dose-response relationship was also found for oxazolone, but no threshold was reported as more than threefold increases above control were obtained even at the lowest tested concentration of 0.0025% (Loveless et al., 1996; Ulrich et al., 1998). Thresholds were neither found for MA and HHPA (Plitnick et al., 2003). In the study of Ryan et al. (2002), different vehicles were tested, among others a vehicle for use with water-soluble compounds.

In an LLNA by van Och et al. (2000), 10 chemical allergens were tested, each chemical at 4–6 concentrations. For each compound, concentration-related increases were obtained and the dose-response data were analyzed by nonlinear regression analysis. Four different concentration-response curves were obtained: exponentially shaped curves (two types) were seen for six of the chemicals (benzocaine, diethylamine, MBT, TMTD, ZDMC, and oxazolone), three of the chemicals showed a sigmoidally shaped curve (DNCB, TDI and TMA), whereas one curve showed a logarithmic dose-response profile (phthalic anhydride). Oxazolone showed the strongest sensitizing potency, followed in this order by DNCB, TDI, TMA, PA, TMTD, ZDMC, MBT, benzocaine, and diethylamine.

In the LLNA, irritating compounds were not found to induce cell proliferation (Dearman et al., 1992a, 1992b), although some studies suggest this distinction between irritants and allergens was not always evident. For instance, the nonsensitizing skin irritant sodium lauryl sulfate induced a positive response in this assay (Loveless et al., 1996), whereas the nonsensitizing skin irritant methyl salicylate induced a nearly positive response (increases of up to 2.9-fold above control at 20%; Kimber et al., 1996). As a result, compounds with strong irritating properties could falsely be classified as allergens, or the allergenicity of chemicals with both allergenic and irritating properties could be overestimated (Robbins et al., 1991; Basketter and Scholes, 1992; Edwards et al., 1994; Montelius et al., 1994). Arts et al. (1997) reported that formaldehyde (which is both an irritant and a sensitizer) caused a dose-dependent and a slightly more than threefold activation of the lymph nodes, but irritant properties might have substantially contributed to the LLN cell proliferation observed. Basketter et al. (1996) discussed the possible mechanisms involved in low level activity in the LLNA by some but not all irritants, and noted that a weak response in the LLNA to a known strong irritant should prompt further consideration.

Skin sensitization measured directly by cytokine profiles. Increases in cytokine levels, induced by sensitizers, can be measured using different assays such as enzyme-linked immunosorbent assay (ELISA) (Ryan et al., 1998; Dearman and Kimber, 1999; Dearman et al., 1999; Vandebriel et al., 2000; Ulrich et al., 2001), reverse-transcription polymerase chain reaction (RT-PCR) (Ryan et al., 1998; Ulrich et al., 1998,) or a multiprobe ribonuclease protection assay (RPA; Plitnick et al., 2002). Changes in cytokine profiles are generally measured in mice upon flank application of the chemicals on days 0 and 5, followed by ear application on days 10, 11, and 12. At various times following ear exposure mice are sacrificed, draining lymph

nodes removed, and one of the different assays applied. In one of these studies (Plitnick et al., 2002), dose-response relationships in several cytokine levels (interleukin [IL]-4, IL-5, IL-10, IL-13) were obtained using TMA. However, this was only observed when the TMA concentration was varied during the ear exposures. Variation in concentration during flank application did not result in dose-response relationships; variation in concentration during both exposures resulted in an initial increase at lower doses followed by a subsequent decrease at higher doses. Dearman et al. (1999) found a dose-related increase in IL-10 using different concentrations of glutaraldehyde. Concentrations were similar at each flank or ear application. Interestingly, the second series of applications on days 10–12 were considered by the investigators (Plitnick et al., 2003) to be the elicitation/challenge phase of the exposure, which would imply that the cytokine profiles test should be considered a full test, including sensitization and elicitation. However, as cytokine levels are no effect parameters, that is, they do not reflect clinical symptoms whatsoever, this test was not considered to be an endpoint test and was therefore not incorporated in the elicitation section.

Skin sensitization measured by total serum IgE or specific antibody levels (as function of immediate reaction). Groups of mice received a topical application of various concentrations of the chemical under investigation. Changes in serum IgE after topical reexposure to the chemicals 7 days later enabled the examination of dose-response relationships. Exposure to TDI, MDI, HDI, IPDI, and TMA caused a significant dose-related increase in serum IgE-concentration measured 14 days after the initiation of exposure. The no-effect level was lower than 1% w/v for the diisocyanates and lower than 10% w/v for TMA. In contrast, exposure of mice to DNCB, oxazolone, or glutaraldehyde produced only a relatively small elevation in serum IgE levels at a high concentration only, whereas formaldehyde did not induce an IgE antibody response (Hilton et al., 1995; Potter and Wederbrand, 1995). In another experiment with TDI, various concentrations of TDI were administered 15 times over a 3-week period, or 30 times over a 6-week period. The apparent TDI threshold for IgE antibody production increased with an increase in the number of TDI applications. In contrast, multiple applications with high TDI concentrations increased total IgE antibody production when TDI was administered in 15 or 30 doses rather than in 2 doses (Potter and Wederbrand, 1995).

Blaikie et al. (1995) investigated a single intradermal injection model in the guinea pig to establish the relationship between the sensitizing dose and the antibody response. Four strong immediate-type (type I) allergens were screened: TMA, PA, MDI, and TDI. The skin sensitizer DNCB was used as a negative control. Guinea pigs were sensitized on day 1 and different groups were sensitized with a range of concentrations. Sensitization was assessed on day 19 by serological analysis measuring the presence of antigen-specific antibodies in the serum of treated animals. All test compounds but DNCB induced high serum levels of specific antibodies. There was a sensitization dose-related increase in specific antibody levels for TMA, PA,

and MDI (for TDI, only one sensitization dose was used). For MDI, a no-effect level for the presence of antibodies was found at a sensitization dose of 0.01%. No-effect levels were not found for TMA and PA, as the lowest tested doses of 0.003% and 0.03%, respectively, were still effective. For TDI, a threshold was not found either at the only sensitization concentration of 0.1%.

Dose-dependent increases in prevalence and concentration of specific IgE and IgG were found in BN rats treated dermally with different doses of dry powder TMA under occlusion on days 0, 7, 14 and 21. Specific antibodies were detectable 2 weeks after the first application and peaked between 3 and 4 weeks; no threshold was found (Zhang et al., 2002). Vanoirbeek et al. (2003) found a significant increase in total serum IgE following treatment with 20% of a PCT derivative but not at 10%. Klink and Meade (2003) found a dose-dependent elevation in total serum IgE upon dermal exposure to 5, 15, or 25% AMT, 5 days a week for 68 days. Significantly elevated concentrations were obtained at 25% at 26 days after initiation, and at all concentrations at 40 days after initiation.

Skin sensitization measured by pulmonary reactions after inhalation or intratracheal challenge. In the aforementioned study by Blaikie et al. (1995; see preceding subsection), the relationship between the various intradermal sensitization doses and pulmonary response (endpoint) was also investigated upon inhalation challenge (on day 22) at a fixed concentration of each chemical. No clear dose-response relationship between sensitizing dose and pulmonary reactions was noticed for TMA, PA, and MDI—that is, there was no substantial increase in the incidence or severity of these pulmonary responses with the increase in the sensitizing dose. For MDI, a no-effect level for the occurrence of pulmonary reactions was found at a sensitization concentration of 0.01%, which was in agreement with the detected no-effect level for the presence of specific antibodies. For TMA and PA again no thresholds were found; for TDI, only one sensitization dose was used.

Arakawa et al. (1995) used intradermal induction of sensitization to two concentrations of TMA to assess airway responses (endpoint). Guinea pigs intradermally sensitized with 300 μ g TMA and intratracheally challenged with 50 μ g of TMA (5 mg/ml) responded with both airflow obstruction and airway plasma exudation, while the low sensitization dose (3 μ g) failed to induce airway responses to TMA. Antigen-specific or total antibody levels to TMA were not measured. Using the same sensitization and challenge method, guinea pigs responded with both immediate effects on lung resistance and Evans blue dye extravasation, which was dependent on the sensitization dose of HHPA (0.05, 0.5, or 5%). A no-effect level was not found (Zhang et al., 1998).

Pauluhn (2003) showed that BN rats treated topically with 5 or 25% TMA showed a dose-related increase in the number of animals showing changes in breathing patterns and histopathological pulmonary changes upon a challenge with approximately 25 mg/m³ TMA, whereas a dose of 1% was ineffective. In

the aforementioned study of Klink and Meade (2003), it was shown that dermal exposure of BALB/c mice to concentrations of 5, 10, 15, 20, or 25% AMT, 7 days a week for 35 days, resulted in concentration-related pulmonary histopathological effects (alveolitis) following intratracheal challenge with AMT; a threshold was not found.

2. High-Molecular-Weight Compounds

a. Epidemiological or Clinical Studies. No studies have been found.

b. Animal Studies (Table 1). Arakawa et al. (1995) investigated the effect of two intradermal sensitization concentrations of *Dermatophagoides farinae* (Df: mite) allergens on airway responses (endpoint). In animals sensitized with 300 μ g of Df mite allergen, intratracheal challenge produced a significant airway plasma exudation in the airways of all animals, while no significant exudation was produced after sensitization with the lower dose of Df (30 μ g) and similar challenge. Levels of specific antibodies to Df were not measured.

Different types of vehicle may influence the results. Although guinea pigs intradermally sensitized with various doses of ovalbumin (OA) in either saline or corn oil developed dose-dependent levels of antigen-specific IgG1 antibodies, an intratracheal challenge with OA of animals sensitized with OA in corn oil induced an inversely dose-dependent airflow obstruction and airway plasma exudation. In contrast, animals sensitized with OA in saline had dose-dependent airway responses to OA (Arakawa et al., 1995).

B. Dose-Response Relationships and Thresholds for Skin Elicitation

1. Low-Molecular-Weight Chemicals

a. Epidemiological or Clinical Studies (Table 2). Concentration-dependent allergic contact dermatitis was reported for Kathon biocide (Pasche and Hunziker, 1989), DNCB (Friedmann et al., 1983), hydroxycitronellal (Ford et al., 1988), cinnamic aldehyde (Johansen et al., 1996), formaldehyde (Flyvholm et al., 1997), MBT and MBTS (Emmet et al., 1994), PPD (McFadden et al., 1998), potassium dichromate (Eun and Marks, 1989), chloroatranol (Johansen et al., 2003b), hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrå; Johansen et al., 2003a), and nickel (Allenby and Goodwin, 1983; Eun and Marks, 1989; Menné and Calvin, 1993; Uter et al., 1995).

With respect to the shape of the dose-response curves, information was limited, most probably due to the small numbers of concentrations tested. For DNCB, linear and almost linear log-dose-response curves were observed in the proportion of DNCB-sensitized subjects showing clinically detectable reactivity to an occluded patch test. Also, a proportionate increase in the degree of reactivity was detected (Friedmann et al., 1983).

For Kathon CG, the threshold for occluded challenge was in the range of 25 ppm (0.0025%; Weaver et al., 1985), 10–20 ppm (0.001–0.002%; Cardin et al., 1986), or 7 ppm (0.0007%; Pasche

TABLE 2
Summary of methods to determine dermal elicitation dose-response relationships

Human/animals	Sensitization method	Elicitation method ^a	Parameter(s) evaluated	References
Human	Sensitized subjects	Occluded patch	Evaluation of skin effects	Friedmann et al. (1983), Weaver et al. (1985), Cardin et al. (1986), Pasche and Hunziker (1989), Menné (1994), Johansen et al. (1996), Flyvholm et al. (1997)
Human	Sensitized subjects	Nonoccluded patch	Evaluation of skin effects	Flyvholm et al. (1997)
Human	Sensitized subjects	Inflamed or irritated skin under occlusion	Evaluation of skin effects	Basketter and Allenby (1990), Menné (1994)
Human	Sensitized subjects	Repeated open application (use test)	Evaluation of skin effects	Johansen et al. (1996)
Human	Sensitized subjects	Workplace exposure	Evaluation of skin effects	Jeebhay et al. (2001)
Guinea pig (open cutaneous test)	Multiple topical applications (not occluded)	Two topical applications (not occluded)	Evaluation of skin effects	Bronaugh et al. (1994)
Guinea pig (Buehler test)	Three topical applications under occlusion	Topical application under occlusion	Evaluation of skin effects	Chan et al. (1984), Bronaugh et al. (1994)
Guinea pig (GPMT)	One intradermal, one topical induction under occlusion with or without FCA	Topical application under occlusion	Evaluation of skin effects	Bronaugh et al. (1994), Nakamura et al. (1994), Wahlkvist et al. (1999a)
Guinea pig (CCET)	Multiple (4) topical applications under occlusion with FCA prior to the 3rd application	Topical application	Evaluation of skin effects	Wahlkvist et al. (1999a)
Rat	Intradermal injection	Intradermal challenge	Extravasation of Evans blue in skin and skin histology	Andius et al. (1996)

^aBy using various concentrations, dose-response relationships were obtained; see text for more details of the methods used.

and Hunziker, 1989). For cinnamic aldehyde, the minimum effect level was 0.02% (occluded patch testing) and 0.1% (repeated open application; Johansen et al., 1996). The highest concentration of MBT at which no reaction was observed was 0.0032% (1.45 $\mu\text{g}/\text{cm}^2$); the lowest concentration producing a positive reaction was 0.01% (4.5 $\mu\text{g}/\text{cm}^2$). At concentrations of 0.316% or 1% MBT, all patients reacted. For MBTS, questionable results were obtained at a concentration of 0.0316%, a positive reaction at 0.1%, and at 1%, the highest level tested, at least 5 out of 12 patients reacted (Emmet et al., 1994). In studies in nickel-sensitive patients, no-effect levels varied from 100 ppm (0.01%; Menné and Calvin, 1993) to 5 ppm (0.0005%; Uter et al., 1995), although it was stated that in the latter study, only a few weak reactions were observed at 10 ppm (0.001%) and 50 ppm (0.005%), and moderate reactions at 100 ppm (0.01%). In another dose-response study, the minimum elicitation level was approximately 112 ppm nickel (0.05% nickel sulfate) for the ma-

majority of nickel-sensitive individuals (14 out of 25; Allenby and Goodwin, 1983). Overall, the minimum threshold concentration for nonoccluded challenge in nickel-sensitized subjects has been established at 100 ppm (0.01%; Emmet et al., 1988; Uter et al., 1995; Allenby and Goodwin, 1983; Menné, 1994). There was a marked interindividual variability with regard to provocation threshold concentrations among the sensitized patients (Emmet et al., 1994; McFadden et al., 1998). It was even noted that the variability in the provocation threshold was 100-fold among 12 tested sensitive individuals (Emmet et al., 1994).

No-effect levels were not obtained for hydroxycitronellal; when 28 sensitized subjects were exposed to 1%, 3 subjects were reacting (Ford et al., 1988). One out of 20 formaldehyde-sensitive patients reacted at the lowest concentration of formaldehyde tested, that is, 250 ppm (0.025%) in the occluded patch test (Flyvholm et al., 1997). Also, for potassium dichromate a no-effect level could not be found (Eun and Marks, 1989).

In the study of Flyvholm et al. (1997), both occluded and nonoccluded patch testing were used to establish threshold levels. Although a dose-response relationship was observed using occluded patch tests, no definite positive reactions were seen in the nonoccluded patch test, indicating a different skin penetrating ability. Menné (1994) also mentioned a large difference between occluded and nonoccluded exposure; that is, concentrations to induce nickel challenge reactions were about 15–150 times lower during occluded challenge than during nonoccluded exposure. Skin penetrating ability is even more increased in damaged skin. The elicitation threshold concentration of nickel sulphate appeared 100–1000 times lower than the elicitation threshold concentration for normal skin; that is, less than 1 ppm (0.0001%) was sufficient to elicit reactions (Allenby and Basketter, 1993). Highly sensitized individuals might react to concentrations as low as 0.5 ppm (0.0075 $\mu\text{g}/\text{cm}^2$) nickel when exposed on inflamed skin under occlusion (Basketter and Allenby, 1990). Reactions were also observed in a few nickel-sensitive patients at the same concentration under occlusion after inducing a mild degree of skin irritation (Basketter and Allenby, 1990).

b. Animal Studies (Table 2).

Delayed-type skin reactions after dermal sensitization and challenge. Dose-response relationships for dermal elicitation reactions were investigated in sensitized guinea pigs using different tests. With DNCB, using the open epicutaneous test or the Buehler occlusive patch test, a gradation in response (percentage of animals showing a positive reaction) was clearly observed. Almost all animals responded at the highest DNCB challenge concentration and the observed response decreased with decreasing doses. No challenge reactions were observed at the lowest challenge concentration of 0.00001% DNCB (Buehler test; 100 animals) or 0.01% DNCB (open epicutaneous test; 20 animals). The maximization test was used to evaluate dose-response relationships for PPD. At the highest challenge dose of 6%, 93% of the 250 guinea pigs showed a positive reaction; at the lowest dose of 0.01%, only 15% of 200 guinea pigs were positive. Similar dose-response curves were obtained for both DNCB and PPD. The higher doses produced a linear relationship, but at lower doses the curves flattened out (Bronaugh et al., 1994).

The previously mentioned study by Chan et al. (1983) using Kathon CG in the Buehler test also indicated a dose-response relationship for elicitation reactions. Results showed that the sensitizing potential of the biocide (measured as the incidence of erythema) was depending on both the induction and challenge concentration. The number of animals responding increased with increasing challenge concentrations when the induction concentration was kept equal. A sigmoid dose-response curve was obtained. A no-response concentration was observed for several combinations of induction and challenge concentrations. The EC_{50} for challenge reactions at an induction concentration of 1000 ppm (0.1%) was calculated to be 429 ppm (0.0429%).

Another previously mentioned study in guinea pigs in which both induction and challenge application concentrations were varied was carried out by Nakamura et al. (1994). The dermal response, measured as the formation of erythema and/or edema, was found to be related to the sensitization dose (intra-dermal injection) as well as the challenge dose (topical application) for almost all of the 15 chemicals tested—that is, the higher the doses, the higher the number of responders.

In a third previously mentioned study, MDBGN was tested in both the GPMT and the CCET, a test method using multiple topical applications. Various challenge doses were used. Dose-related elicitation reactions (monotone curve) and threshold levels could be detected for MDBGN in the CCET; a few positive reactions were seen in the GPMT at the highest challenge concentration only (Wahlkvist et al., 1999a).

Mean ear thickness measured in sensitized mice increased dose-dependently using both DNCB and squaric acid dibutyl ester. Shallow linear increases with thresholds were found, and eventual plateaus at increasing doses. However, when also varying the sensitization concentrations, the minimum dose was not static but rather reflected a “sliding scale,” that is, as the sensitization dose was increased, the concentration required to elicit a challenge response was decreased. Correspondingly, as the challenge dose was increased, the dose required for sensitization was lessened (Scott et al., 2002).

c. Immediate-type skin reactions after dermal sensitization and challenge. In a study using BN rats, animals were sensitized by an intradermal injection of TMA at a fixed concentration. Three weeks later, Evans blue dye was given, and dermal reactions (extravasation of dye in skin) were measured 30 min after intradermal challenge with various concentrations of TMA. A dose-dependent increase of Evans blue dye extravasation was observed. In addition, skin histology revealed a significant and dose-dependent increase in eosinophils after repeated TMA injections in similarly sensitized BN rats (Andius et al., 1996).

2. High-Molecular-Weight Compounds

a. Epidemiological or Clinical Studies (Table 2). In a review on workers involved in either manual or automated processing of seafood it was found that occupational dermal exposure as a result of unprotected handling of seafood and its by-products resulted in a prevalence of occupational protein contact dermatitis from 3 to 11%. Cross reactivity between various species within a major seafood group was also found. Limited evidence from dose-response relations indicated that the development of symptoms was related to duration or intensity of exposure. It was also found that disruption of the intact skin barrier seems to be an important risk factor for occupational protein contact dermatitis. Workers in the seafood processing industry are also exposed by inhalation, which may have contributed to the adverse health effects (see also at respiratory allergy; Jeebhay et al., 2001).

b. Animal Studies. No studies have been found.

III. RESPIRATORY-TRACT ALLERGY

Occupational exposure to various LMW or HMW compounds can cause sensitization of the respiratory tract, resulting in allergic pulmonary hypersensitivity reactions upon a subsequent encounter with the same compound. It is well established that respiratory allergy to proteins in humans is associated with, and mediated by, specific IgE antibodies. There is less certainty with respect to a similar requirement for IgE antibodies in the development of respiratory allergy to LMW chemicals, not least because specific IgE antibodies could not be demonstrated in a large number of symptomatic individuals sensitized to certain diisocyanates (Karol et al., 1978, 1979a, 1979b, 1980, 1994; Butcher et al., 1980, 1983; Karol and Alarie, 1980; White et al., 1980; Karol, 1980, 1981; Baur and Fruhmann, 1981; Danks et al., 1981, 1983; Baur et al., 1984, 1994; Butcher and Salvaggio, 1986; Cartier et al., 1989; Kimber and Dearman, 1997; Beckett, 2000), as well as in a number of persons with acid anhydrides-induced asthma (Venables, 1989). Agents in the workplace that are able to induce allergen-specific airway reactions are HMW compounds such as flour allergens, grain allergens, feed dust, animal dander/urinary proteins, and a number of LMW chemicals such as diisocyanates, acid anhydrides, and reactive dyes (Kimber et al., 1996).

There are two risk phrases for respiratory effects—that is, respiratory sensitization (R42) and respiratory irritation (R37)—but for both types of effects, validated tests are lacking. Although a number of animal test protocols have been published to detect respiratory allergy (see for reviews Briatico-Vangosa et al., 1994; Pauluhn et al., 1999; Pauluhn and Mohr, 2005), none of these are widely applied or fully accepted, most probably because no large effort has yet been made for validation.

With respect to substances inducing occupational asthma, the current European Commission (EC) labeling criteria for dangerous substances (CEC, 1967; amended several times and adapted to technical progress for the 29th time recently), which are used to classify substances among others on their potential to induce respiratory allergy, include all chemicals that can induce asthma(-like) attacks. As stated: “The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.” In this guideline, it is further emphasized that “Substances that elicit symptoms of asthma by irritation only in people with bronchial hyperreactivity should not be assigned R42.” In addition, the decision on classification needs to take into account “the size of the population exposed” and the “extent of exposure.” Thus, a high-production-volume chemical found in large quantities in many workplaces throughout the world might not warrant the R42 phrase if only a few cases of asthma with its use have been reported over the years. In contrast, three cases of asthma among a workforce of 20 in contact with a specific chemical might well indicate the need for classification as a respiratory sensitizer (Evans, 1997).

A. Dose-Response Relationships and Thresholds for Respiratory-Tract Sensitization

1. Low-Molecular-Weight Chemicals

a. Epidemiological or Clinical Studies (Table 3). A few studies were found that reported dose-response relationships for respiratory-tract sensitization to LMW chemicals in workers. In a prospective study performed in TDI-exposed workers, it was shown that accidental exposure to high concentrations of TDI resulted in IgE antibody production. In contrast, exposure to low concentrations of TDI (at or below 0.02 ppm) for up to 3 years did not result in any cases of TDI sensitivity or in production of TDI-specific antibodies (Karol, 1981). In another prospective study of workers exposed to organic acid anhydrides (OAAs), 163 previously unexposed persons were exposed to epoxy resins containing HHPA, MHHPA, and MTHPA. The mean observation time was 32 (1–105 months). The levels of OAAs in air and of specific IgE and IgG in serum were recurrently monitored. The mean combined OAA exposure was 15.4 $\mu\text{g}/\text{m}^3$ (range <1–189 $\mu\text{g}/\text{m}^3$). An exposure-response relationship was demonstrated by an increasing risk of sensitization with increasing exposure. Specific IgE was demonstrated by 21 (13%) subjects with a mean induction time of 8.8 (1–35 months). The incidence of sensitization was 4.1 cases/1000 months at risk. The relative risk for atopics was 5.4 (1.9–15.3; 95% confidence interval; Welinder et al., 2001). In a cross-sectional study, also with HHPA and MHHPA, there was an increasing risk of sensitization with increasing exposure. Specific IgE was found in 13% of the workers exposed to concentrations <10 $\mu\text{g}/\text{m}^3$ HHPA, in 26% exposed to concentrations between 10 and 50 $\mu\text{g}/\text{m}^3$ HHPA, and in 21% exposed to concentrations higher than 50 $\mu\text{g}/\text{m}^3$ HHPA; for MHHPA, specific IgE levels were about similar, 15, 26, and 17%, respectively. Specific IgG levels were 2, 22, and 41%, and 4, 26, and 38% for these exposure categories of HHPA and MHHPA, respectively. Atopy did not significantly increase these risks (Nielsen et al., 2001). Drexler et al. (2000) also reported in a study of three plants using MTHPA that sensitization to OAAs increased with increasing exposure.

b. Animal Studies (Table 3).

Respiratory sensitisation measured by antibody levels. Guinea pigs were exposed to various TDI concentrations (0.12–10 ppm) for 3 hours a day on 5 consecutive days. TDI-specific antibodies were measured from day 22 onward. No antibodies were detected in animals exposed to 0.12 ppm TDI. Within the range of 0.12–0.96 ppm, a linear relationship was observed between log-concentration of TDI and the antibody response as well as the percentage of animals producing antibody to TDI. Half of the animals exposed to 0.36 ppm TDI or more showed TDI-specific antibodies in their sera. At exposure levels of 0.96 ppm or higher, all animals produced an antibody response (Karol, 1983). In a similar study (Karol et al., 1980), concentrations of 0.25 ppm also resulted in the production of antibody to TDI. No production of TDI-specific antibody was noted after exposure to a low TDI concentration of 0.02 ppm.

TABLE 3
Summary of methods to determine respiratory sensitization dose-response relationships

Human/animals	Sensitization method ^a	Elicitation method	Parameter(s) evaluated	References
Human	(Accidental) workplace exposure, assessment of exposure concentrations	—	Measurement of antibody levels, evaluation of skin prick test, evaluation of clinical symptoms	Karol (1981), Houba et al. (1996a), Musk et al. (1989), Cullinan et al. (1994a, 1994b), Halpin et al. (1994), Hollander et al. (1997a, 1997b), Baur et al. (1998a), Houba et al. (1998a, 1998b), Heederik et al. (1999a), Nieuwenhuijsen et al. (1999), Drexler et al. (2000), Welinder et al. (2001), Nielsen et al. (2001)
Human	Exposure in hospital or at home	—	Measurement of antibody levels	Anonymous (1993), Liebers et al. (1993), Michael et al. (1996), Chen et al. (1997a, 1997b), Porri et al. (1997), Custovic and Chapman (1998), Custovic et al. (1998), Lindfors et al. (1999), Platts-Mills et al. (2001)
Guinea pig	Single inhalation exposure	—	Measurement of antibody response	Hillebrand et al. (1987), Pauluhn et al. (2000)
Guinea pig/rat	Inhalation on several days	—	Measurement of antibody response	Karol et al. (1980), Karol (1983), Hillebrand et al. (1987), Ritz et al. (1993), Siegel et al. (2000), Pauluhn et al. (2002a), Warbrick et al. (2002)
Guinea pig	Intratracheal administration on several days	—	Measurement of antibody response	Ritz et al. (1993)
Guinea pig	Inhalation on several days	Inhalation challenge with hapten, protein, or hapten-protein conjugate	Measurement of airway responses	Karol (1983), Thorne et al. (1986), Pauluhn et al. (2002a)
Guinea pig	Single inhalation exposure	Inhalation challenge with protein	Measurement of airway responses	Thorne et al. (1986)
Rat	Inhalation on several days	Inhalation challenge with hapten	Measurement of airway responses	Pauluhn et al. (2002b)
Guinea pig/mouse	Inhalation on several days	Topical challenge	Evaluation of skin effects	Stadler and Karol (1984)
Guinea pig	Single intranasal application	Topical challenge	Evaluation of skin effects	Ebino et al. (2001)

^aBy using various concentrations, dose-response relationships were obtained; see text for more details of the methods used.

Animals were exposed for 6 h/day, 5 days/week, for a total of 70 days (total exposure: 8.67 ppm-h, compared to a total exposure of 3.75 ppm-h [0.25 ppm for 3 h/day for 5 days] that induced production of antibodies during short-term exposure). It was concluded that the exposure concentration (in combination with the duration of exposure) was important for establishment of antibody response, rather than total exposure (Karol, 1983). A single 15-min exposure of guinea pigs to various concentrations

of polymeric MDI (5, 12, 32, 108, or 835 mg/m³) resulted in a concentration-related increase in specific antibodies 3 weeks after induction; a threshold was not reported (Pauluhn et al., 2000). Exposure of guinea pigs to various concentrations of two trimers of HDI (HDI-isocyanurate 3.0, 15.9, 49.4, or 261 mg/m³, or HDI-biuret 2.7, 9.5, 49.4, or 142 mg/m³) 3 h/day for 5 days revealed a concentration-related increase in the number of animals showing increased antibody levels. The no-observed-effect

levels (NOELs) were about 3 mg/m³ with borderline effects at concentrations of 10–15 mg/m³ (Pauluhn et al., 2002a). However, upon a challenge with a concentration of about 85 mg/m³ with the free hapten, or upon challenge with a respective GPISA conjugate 1 week later, no specific functional or morphological pulmonary responses were seen in contrast to monomeric HDI (Pauluhn et al., 2002a).

In BN rats, sensitization–concentration–response relationships were observed using TMA. A daily 1-h exposure to 5 mg/m³ for 1, 3, or 5 days resulted in an exposure-duration-related increased number of animals responding with specific IgG and IgE antibody levels. There was also some suggestion of a dose-response effect with an increased number of rats displaying induction of specific IgE antibody, namely, 1/5 rats after daily 1-h exposures, 2/5 rats after 3-h exposures, and 3/5 rats after 5-h exposures (Warbrick et al., 2002).

Respiratory sensitization measured by pulmonary reactions after inhalation challenge. Pulmonary reactions upon challenge with 1% TDI–guinea pig serum albumin aerosol, measured as an increase in respiratory rate, were not detected in animals sensitized to 0.12 ppm TDI but were present in guinea pigs exposed to TDI concentrations of 0.36 ppm and higher (Karol, 1983). Exposure of BN rats to either 25 or 120 mg/m³ TMA 3 h/day for 5 days revealed a concentration-related increase in the respiratory response, and in lung-associated lymph node weights following challenge at a concentration of 23 mg/m³ TMA (Pauluhn et al., 2002b).

Respiratory sensitization measured by skin reactions after dermal challenge. Guinea pigs and mice were sensitized by inhalation to various concentrations of HMDI for 2 h/day on 3 consecutive days and tested for dermal reactions by means of a topical challenge at a fixed dose of HMDI, 7 days following the initial HMDI inhalation exposure. In both species, a concentration-response relationship was observed between the inhalation sensitization concentration and both the severity of the dermal response and the number of animals responding. Guinea pigs developed skin reactions following exposure to concentrations ≥ 3 mg/m³; exposure to 1.25 mg/m³ did not result in respiratory sensitization. In mice, dermal reactions were noted upon exposure to ≥ 17 mg/m³; no reactions occurred at sensitization concentrations ≤ 7 mg/m³. Specific antibody levels to HMDI were not measured (Stadler and Karol, 1984). Guinea pigs receiving a single intranasal application of TDI at various concentrations exhibited skin sensitivity in a dose-dependent manner upon a patch challenge with TDI. One of four responded to 0.6%, 2/4 responded to 1.2%, and 4/4 responded to 1.8% TDI. Specific antibody levels to TDI were not measured (Ebino et al., 2001).

2. High-Molecular-Weight Compounds

a. *Epidemiological or Clinical Studies (Table 3).* Dose-response relationships for respiratory-tract sensitization to HMW compounds were observed in several studies in work-

ers. A cross-sectional epidemiological study was carried out to assess the relationship between α -amylase exposure and allergic sensitization. All workers were categorized into groups on the basis of their job histories and α -amylase exposure levels measured in personal dust samples. The prevalence of positive IgE tests to α -amylase tended to increase with intensity of exposure. After stratification for atopy, however, there was no clear exposure-response relationship. In contrast, prevalence ratios of a positive skin-prick test increased with increasing α -amylase exposure groups. Sensitization rate increased from 1.4% in the low exposed worker group (geometric mean [GM] of 0.7 ng/m³), to 12.8% in the medium exposed worker group (GM 1.3 ng/m³), and to 30.4% in the high exposed worker group (GM 18.1 ng/m³). The investigators concluded that a strong and positive association was shown between α -amylase allergen exposure levels and α -amylase specific sensitization. Sensitization was already found at 0.25 ng α -amylase/m³ air (Houba et al., 1996a). In another study in workers in bakeries and flour mills, 5% of the workers were sensitized to α -amylase. The prevalence increased with increasing exposure to α -amylase, that is, from 3.1% in the low exposed worker group (GM 0.7 ng/m³), to 16.7% in the medium exposed worker group (GM 10.7 ng/m³), and to 15.4% in the high exposed worker group (GM 46.7 ng/m³; Nieuwenhuijsen et al., 1999).

Flour dust concentrations of 1–2.4 mg/m³ were found to be associated with a significantly elevated risk of sensitization to wheat antigens (Houba, 1996). Exposure-sensitization relationships for flour dust exposure and wheat aeroallergen exposure among workers in bakeries have been reported by Musk et al. (1989), Cullinan et al. (1994a), and Houba et al. (1998a, 1998b). It was also reported that sensitization risk will be negligible when exposure levels will be reduced to average exposure concentrations of 0.2 μ g/m³ wheat allergen, or approximately 0.5 mg/m³ inhalable dust during a work shift (Houba et al., 1998a).

In laboratory animal workers, the prevalence rate of sensitization to rat allergens was also clearly associated with exposure levels (Hollander et al., 1997a) but only in those workers with less than 4 years of working experience with laboratory animals (Hollander et al., 1997b). A positive correlation was found between the intensity and frequency of rat urinary allergen exposure and the frequency of positive skin-prick test results and specific sensitization (Cullinan et al., 1994b). Data from three cross-sectional studies in laboratory animal workers revealed similar results: The rat urinary allergen sensitization risk increased with increasing air allergen exposure. Atopic workers had a clearly elevated sensitization risk at low allergen exposure levels (Heederik et al., 1999a).

In workers in a sawmill, the prevalence of sensitization to saw dust and *Trichoderma koningii* was associated with exposure levels; that is, serum concentrations of specific IgG were significantly higher in the high exposure group when compared to the low exposure group (Halpin et al., 1994).

In latex-exposed persons, inhalation exposure to a concentration of ≥ 0.6 ng/m³ latex resulted in sensitization of 18% of

people exposed. Lower concentrations were not associated with IgE-mediated sensitization (Baur et al., 1998a).

In clinical studies it was found that the frequency of latex sensitization in spina bifida patients was strongly related to the number of surgeries undergone (Michael et al., 1996; Chen et al., 1997a, 1997b; Porri et al., 1997).

In young asthmatic children, a dose-response relationship was found between cat exposures, measured as either reported degree of cat exposure or cat allergen (Fel d 1) levels in dust samples, and sensitization as measured by the amount of IgE antibodies. High levels of cat allergen were considered to be $>8 \mu\text{g/g}$ dust. No such relationship was found between exposure and sensitization to dog (Can f 1) allergens (Lindfors et al., 1999). In another study, it was found that many children exposed to greater than $20 \mu\text{g}$ Fel d 1/g dust at home produced an IgG₁ and IgG₄ antibody response to Fel d 1 without IgE antibody. This response was not associated with symptoms and, according to the authors, should be regarded as a form of immunological tolerance (Platts-Mills et al., 2001). These studies show that the dose-response relationship between cat allergen exposure and sensitization is bell-shaped (Murray et al., 2001) and it was suggested that high exposure to cat allergen can modify the Th2 response to suppress IgE production while maintaining or increasing IgG₄ and IgG₁ antibody production (Erwin et al., 2005).

On the basis of data from several studies, a significant correlation between cumulative exposure to house dust-mite allergen and the risk of sensitization was reported (Anonymous, 1993). The dose-response relationship appears to have a linear relationship (Murray et al., 2001). A threshold level was proposed: Exposure to more than $2 \mu\text{g}$ Group 1 mite allergen/g dust should be regarded as a risk factor for the development of IgE antibody and asthma in susceptible children (Custovic and Chapman, 1998; Custovic et al., 1998). A linear dose-response relationship between exposure and sensitisation was also reported for cockroach allergen (Murray et al., 2001). Also, the frequency and extent of exposure to *Chironomidae* insect allergens (Chi t 1-9) was associated with IgE-mediated sensitization (Liebers et al., 1993).

b. Animal Studies (Table 3).

Respiratory sensitization measured by antibody levels. Guinea pigs were exposed by intratracheal administration to various concentrations of the enzyme protein Alcalase in a detergent base once a week for 10 weeks. Other groups of guinea pigs were exposed by inhalation (6 h/day, 4 days/week for 10 weeks) to 1 mg/m^3 of the aerosolized detergent base containing various concentrations of Alcalase protein. The antibody levels to Alcalase increased dose-dependently by both the inhalation and intratracheal routes of exposure. No-effect levels were not detected (Ritz et al., 1993).

A concentration-related antigen-specific antibody response was observed in guinea pigs that had been exposed for 15 min/day during 5 consecutive days to various concentrations of subtilisin up to 1.9 mg/m^3 . Antigen-specific antibody levels

were not increased further upon exposure to higher subtilisin concentrations (up to 15 mg/m^3). A separate, single 20-min exposure to a concentration of 1.9 mg/m^3 resulted in the same antibody response as that produced following 5 days of exposure to the same concentration. Long-term exposure to low levels of the enzyme (11 weeks to a concentration of 0.00068 mg/m^3 followed by 6 weeks to a concentration of 0.0051 mg/m^3 ; i.e., 1.12 mg/m^3 in total) resulted in 36% (9 of 25) of the animals producing significant levels of specific IgG or IgM antibodies (Hillebrand et al., 1987).

BN rats were exposed 30 min/wk for 6 weeks to respirable OA aerosols at concentrations of <1 , 3.3, 15.4, or 64.1 mg/m^3 . OA-specific IgE, IgG, and IgA were measured throughout the study period. Rats were sacrificed 1 day after the last exposure. Serum concentrations of OA-specific antibodies increased with both exposure concentration and number of exposures. The number of rats with measurable titers also increased with both dose and time. A no-effect level for antibody production was not present (Siegel et al., 2000).

Respiratory sensitization measured by pulmonary reactions after inhalation challenge. Guinea pigs exposed to concentrations ranging from 0.15 to 15 mg subtilisin/ m^3 for 5 consecutive days produced pulmonary reactivity upon an inhalation challenge at a concentration of 1.9 mg/m^3 for 20 min on day 10. Animals exposed to lower concentrations up to 0.041 mg/m^3 failed to demonstrate pulmonary reactivity upon a similar challenge. The single 20-min exposure to 1.9 mg/m^3 followed by a challenge at 1.9 mg/m^3 for 20 min on day 7 also resulted in pulmonary reactivity. In contrast, the 17-week exposure to a total of 1.12 mg/m^3 did not result in pulmonary reactivity upon challenge, although the total cumulative exposure administered over a short period of time regularly induced pulmonary reactivity (Thorne et al., 1986).

B. Dose-Response Relationships and Thresholds for Elicitation Reactions in the Respiratory Tract

1. Low-Molecular-Weight Chemicals

a. *Epidemiological or Clinical Studies (Table 4).* Studies were carried out to assess the concentration-response association between exposure to acid anhydrides and airway symptoms. When the concentration of TCPA in air was between 0.21 and 0.39 mg/m^3 , the prevalence of work-related respiratory symptoms was 27–39%. After installation of ventilation the TCPA-concentration dropped to 0.1 mg/m^3 and at the same time symptoms diminished considerably (Liss et al., 1993). For TMA, concentrations in air were reduced from 0.82– 2.1 mg/m^3 to 0.01– 0.03 mg/m^3 after the installation of ventilation. Subsequently, the number of workers with specific IgE antibodies and symptoms decreased (Bernstein et al., 1983).

In two condenser plants using epoxy resins containing MTHPA, 111 workers underwent a questionnaire survey and serology investigations. In plant A, air concentrations of MTHPA were higher than in plant B (geometric mean

TABLE 4
Summary of methods to determine airway elicitation dose-response relationships

Human/ animals	Sensitization method	Elicitation method ^a	Parameter(s) evaluated	References
Human	Sensitized subjects	Workplace exposure	Evaluation of respiratory symptoms	Diem et al. (1982), Bernstein et al. (1983), Flood et al. (1985), Vedal et al. (1986), Musk et al. (1989), Liss et al. (1993), Liebers et al. (1993), Cullinan et al. (1994a, 1994b), Halpin et al. (1994), Heederik et al. (1994), Smid et al. (1994), Flindt (1996), Noertjojo et al. (1996), Kruize et al. (1997), Tarlo et al. (1997), Baur et al. (1998a), Houba et al. (1998a), Post et al. (1998), Yokota et al. (1999), Vyas et al. (2000), Jeebhay et al. (2001), Nielsen et al. (2001)
Human	Sensitized subjects	Laboratory challenge	Evaluation of respiratory symptoms	Malo (1990), Nielsen et al. (1994), Vanhanen et al. (2000)
Human	Sensitized subjects	Exposure at home	Evaluation of respiratory symptoms	Custovic and Chapman (1998), Custovic et al. (1998), Alvarez et al. (2000)
Guinea pig/rat	Inhalation on several days	After several exposures, next exposure became challenge exposure	Evaluation of respiratory symptoms/lung pathology	Ritz et al. (1993), Siegel et al. (2000)
Guinea pig	Intratracheal administration on several days	After several administrations, next administration became challenge	Evaluation of respiratory symptoms	Ritz et al. (1993)
Guinea pig	Single intradermal injection	Inhalation challenge with hapten–protein conjugate	Evaluation of respiratory symptoms	Botham et al. (1989)
Guinea pig	Single intradermal injection	Inhalation challenge with hapten	Evaluation of respiratory symptoms	Botham et al. (1989), Blaikie et al. (1995)
Guinea pig	Multiple intradermal injections	Inhalation challenge with hapten	Evaluation of respiratory symptoms/lung pathology	Pauluhn and Mohr (1994)
Rat	Multiple topical applications	Inhalation challenge with hapten	Evolution of respiratory symptoms, and respiratory tract pathology	Arts et al. (1998; 2004), Zhang et al. (2004)
Mouse	Single ip injection (with adjuvant)	Inhalation challenge with protein	Evaluation of bronchial hyperresponsiveness and lung pathology	Tournoy et al. (2000)
Mouse	Multiple (2) ip injections (with adjuvant)	Inhalation challenge with protein	Evaluation of bronchial hyperresponsiveness and lung pathology	Tanaka et al. (2001)

^aBy using various concentrations, dose-response relationships were obtained; see text for more details of the methods used.

approximately 25–64 and 4.9–5.5 $\mu\text{g}/\text{m}^3$, respectively). In total, 24 workers (65%) in plant A and 38 workers (66%) in plant B had MTHPA-specific IgE. In sensitized workers in plant A, eyes, nose, and pharynx symptoms were observed at a higher incidence when compared to sensitized workers in plant B. In addition, work-related symptoms were often observed in 73% of the 26 symptomatic workers in plant A, and in only 15% of the 20 symptomatic workers in plant B. In plant B the minimum level that was associated with work-related symptoms was 15–22 $\mu\text{g}/\text{m}^3$, indicating that levels above 15 $\mu\text{g}/\text{m}^3$ should be avoided (Yokota et al., 1999).

In a previously mentioned cross-sectional study, the incidences of work-related symptoms (eyes, nasal, and lower airways) were concentration-related increased among HHPA and MHPA exposed workers. An exposure level of <10 $\mu\text{g}/\text{m}^3$ was not associated with a significant increase in symptoms (but did not prevent sensitization as measured by an increased incidence of workers with increased levels of specific IgE and IgG; Nielsen et al., 2001). Subjects who were IgE-sensitized against HHPA and who reported work-related nasal symptoms had a concentration-related increase in nasal symptoms and a concentration-related decrease in peak flow after increasing nasal challenge concentrations (1:100, 1:10 diluted or undiluted); a threshold was not found (Nielsen et al., 1994).

A case-control study was designed to compare isocyanate concentrations measured at 20 companies with 56 isocyanate-induced occupational asthma cases (total of 2256 workers) with isocyanate levels at 203 companies without occupational asthma cases (total of 4052 workers). Exposure was determined based on the highest level identified. Companies were categorized into two groups: always <0.005 ppm and ever \geq 0.005 ppm. Although the small number of case companies limited the statistical power, cases were significantly more likely to be working at companies in the higher exposure category (50% vs. 25%; $p < .05$, OR = 3.1). The estimated incidence of occupational asthma in the 4-year study period was greater in high than in low exposure companies, 2.7% and 2.2%, respectively (Tarlo et al., 1997). It was also observed that a chronic decline in airflow occurs on spirometry in groups of workers with isocyanate exposure, in relation to the intensity of the exposure (Diem et al., 1982). In a laboratory challenge test in sensitized individuals, acute airflow limitation worsened with increased isocyanate exposure (Malo, 1990).

In a toluene diisocyanate (TDI) production unit, the annual incidence of asthma induced by TDI declined from 1.8% before 1980 to 0.7% afterward. This was due to a reduction of the TDI concentration (mean 8-h TWA ranged from approximately 10, 5.5, and 2.5 ppb in jobs with potentially high, mid, and low exposure during the early years of plant operations to 3.5, 2, and 0.5 ppb, respectively in more recent years). However, neither cross-sectional nor longitudinal analyses of airflow spirometry showed significant dose-response findings relative to exposure to TDI (Ott et al., 2000).

In nurses working in endoscopy units, a significant relation was observed between peak glutaraldehyde concentrations and work-related chronic bronchitis and nasal symptoms. However, only 1 case out of 364 showed glutaraldehyde-specific IgE (Vyas et al., 2000).

b. Animal Studies (Table 4). In these studies immediate-type airway reactions after dermal sensitization and inhalation challenge were measured. Guinea pigs were sensitized by a single intradermal injection at a fixed dose of TMA in corn oil on day 1. On day 19, sera from all TMA-injected guinea pigs contained TMA-specific antibodies. Animals were challenged with TMA–GPPSA conjugate (nonirritant concentrations of 0.9, 1.5, 2.4, or 7.0 mg/m^3) or free TMA (nonirritant concentrations of 5.8, 43.7, or 52.3 mg/m^3) for 15 min on day 22. In the sensitized animals, there was no clear relationship between antibody titer and the severity of the pulmonary reactions. The TMA–protein conjugate elicited reactions in less than half of the animals at the concentrations tested, and no concentration-response relationship was observed between the challenge concentration and the number of animals responding or the severity of pulmonary responses. In contrast, for free TMA the number of responding animals seemed to be concentration-dependently related to the challenge concentration. However, the concentration gap between two of the three challenge concentrations was much larger than for TMA–GPPSA. However, even at the high concentration of free TMA, pulmonary reactions were not observed in all sensitized guinea pigs (Botham et al., 1989). In a similar study, different groups of guinea pigs were intradermally sensitized with TMA, MDI, PA, TDI, or DNCB at a single concentration. On day 22, each group of animals was subsequently challenged with various inhalation concentrations of either compound. TMA, MDI, PA, TDI, but not DNCB, induced high serum levels of specific antibodies following sensitization. Upon inhalation challenge, respiratory reactions were observed, but there was no clear relationship between the inhalation challenge concentration and pulmonary responsiveness for TMA, MDI, and PA. For TDI, there were no pulmonary reactions detectable at inhalation challenge. As expected, no specific pulmonary reactions were observed for the contact allergen DNCB (Blaikie et al., 1995). Pauluhn and Mohr (1994) sensitized guinea pigs intradermally three times at a single concentration of MDI. On day 21, groups of animals were challenged with various concentrations of MDI (3.5, 13.9, or 59.5 mg/m^3). An immediate-onset respiratory response was seen in 6/8 animals that were challenged with concentrations exceeding the irritant threshold for MDI ($> \sim 20 \text{ mg}/\text{m}^3$) because the response at 3.5 mg/m^3 was 0/8 animals, and at 13.9 mg/m^3 1/8 animals. Pulmonary eosinophilic influx was seen in 2/8, 1/8, and 7/8 animals, respectively (Pauluhn and Mohr, 1994). Arts et al. (1998) investigated specific functional and histopathological airway reactions to TMA following inhalation challenge as an extension to the IgE test. BN rats were topically exposed to a fixed sensitization dose (day 0 and 7), total serum IgE was measured (day 20 or 21), and animals were subsequently challenged by exposure to various concentrations of TMA (16, 31

and 52 mg/m³; day 21 or 22). A significant decrease in respiratory rate during challenge, followed by an increase in breathing rate with a reduced tidal volume 24 h after challenge, were observed at all levels tested. A concentration dependence of functional respiratory reactions was therefore not present. In contrast, a challenge concentration-dependent histopathological response to TMA challenge was observed in the larynx and lungs. In a more recent study, using a broader range of challenge concentrations (0.2–61 mg/m³), concentration-dependent increases in functional and histopathological changes, and aspecific airway hyperresponsiveness were observed. The no-observed-effect level was 0.2 mg/m³ (Arts et al., 2004). Interestingly, an elicitation no-observed-effect level of 0.2 mg/m³ was also found in BN rats treated dermally with TMA powder under occlusion on days 0, 7, 14, and 21, and challenged by inhalation at concentrations of 0.2–40 mg/m³ on day 35 (Zhang et al., 2004). In this study, airway responses were concentration dependent: 1 mg/m³ induced early-phase airway responses only, whereas 5 and 40 mg/m³ induced both early- and late-phase responses.

2. High-Molecular-Weight Compounds

a. Epidemiological or Clinical Studies (Table 4). Several studies in workers reported dose-response relationships for respiratory allergic reactions to HMW compounds. Work-related symptoms in workers in bakeries or flour mills seemed to be related to exposure intensity when exposure was expressed in terms of flour aeroallergen using univariate analyses but not using multivariate analyses (Cullinan et al., 1994a). Current or past exposure to bakery dust was found to be dose-dependently related to respiratory symptoms, a decline in lung function, and bronchial reactivity (Musk et al., 1989). However, in neither of these two studies were sensitized and non sensitized workers distinguished.

Houba et al. (1998a) reported that within a group of sensitized bakers, a positive relationship was found between allergen exposure and work-related symptoms. In sensitized bakers, those with an elevated allergen exposure had more often work-related symptoms (i.e., prevalence ratios for high and medium wheat allergen exposure of 3.5 (95% confidence interval of 1.6–7.5) and 2.6 (0.9–7.8), respectively, compared to workers with low wheat allergen exposure. Workers in the animal feed industry in the high exposure category showed a greater decline in lung function than workers in the low exposure category (Heederik et al., 1994; Smid et al., 1994). In addition, a decline in lung function, expressed in reductions of the forced expiratory volume in 1 second (FEV1) and in maximal mid-expiratory flow (MMEF), was found in animal feed industry workers as well as in workers in the grain processing industry. The decline in lung function was related to the extent of occupational exposure (Post et al., 1998). In employees exposed to laboratory animal allergens, work-related symptoms were related to exposure intensity (expressed either in terms of dust or aeroallergens) at the time of

onset of the symptoms (Cullinan et al., 1994b). In a retrospective cohort study in laboratory animal workers it was indicated that more people with asthmatic symptoms were found in the high exposure categories, and that more atopics than nonatopics reported asthmatic symptoms. In atopics, the mean time until development of symptoms of laboratory animal allergy was more than twice as short as in nonatopics and was shorter at a higher intensity of exposure, except for those exposed for less than 2 hours a week (Kruize et al., 1997).

The prevalence of occupational asthma in workers exposed to various constituents of seafood, due to aerosolization of seafood and cooking fluid during processing, ranges from 7 to 36%. There was great variability of aerosol exposure within and among various jobs, with reported allergen concentrations ranging from 0.001 to 5.1 µg/m³. These health outcomes are mainly due to high-molecular-weight proteins in seafood causing an IgE-mediated reaction. Cross-reactivity between various species within a major seafood group was also found. Limited evidence from dose-response relations indicated that the development of symptoms was related to duration or intensity of exposure. The evidence for atopy as a risk factor seemed to be supportive. Dermal exposure as a result of unprotected handling of seafood also occurred, which may have contributed to the adverse health effects (Jeebhay et al., 2001).

In workers at a sawmill, the concentration of (past) exposure to wood dust (unspecified) was found to be associated with a higher prevalence of work-related cough and nasal and eye symptoms (Halpin et al., 1994). The concentration and duration of (past) exposure to wood dust (Western red cedar) were also associated with a decline in lung function (Vedal et al., 1986; Noertjojo et al., 1996).

Exposure to latex aeroallergen levels of ≥0.6 ng/m³ revealed pulmonary reactions in 15.5% of people exposed. Exposure to concentrations <0.6 ng/m³ were not associated with respiratory symptoms (Baur et al., 1998a). In 11 cellulase-sensitized persons, challenge tests were performed. Four different enzyme-lactose mixtures starting from a 0.03% mixture were used. Concentration-related nasal, pharyngeal, or bronchial symptoms could be elicited at cellulase air concentrations between 1 to 1300 µg/m³ (Vanhanen et al., 2000). Studies with detergent enzymes derived from *Bacillus* revealed significant differences between minimum and maximum exposure groups with regard to a decline in lung function (Flindt, 1996; Flood et al., 1985). The extent of exposure (calculated by frequency and amount of material) to Chironomidae insect allergens was also associated with respiratory symptoms. With increasing exposure, the percentage of subjects with asthmatic symptoms increased (Liebers et al., 1993).

There were also a few clinical studies in which dose-response relationships for respiratory allergic reactions to HMW compounds were reported. A dose-response relationship between dust-mite allergen exposure and sensitization has been confirmed by several studies. Sensitized subjects are likely to have more severe asthma if exposed to high allergen levels

when compared to exposure to low exposure levels. A threshold level for elicitation of asthmatic symptoms has not been clearly defined (Custovic and Chapman, 1998; Custovic et al., 1998). In another study, 31 mild and recently diagnosed (12–24 months) asthma patients sensitized to mites (*Dermatophagoides pteronyssinus*; Der p 1, Der 2) completed an asthma symptom questionnaire, and underwent skin tests, methacholine bronchial challenge, and sputum induction. Allergenic exposure was assessed by a commercial assay based on monoclonal antibodies carried out on the dust samples collected from the patients' beds in a standardized way. Most patients were exposed to Der p 1 levels under 2 $\mu\text{g/g}$ of dust. Der p 1 levels showed a trend toward correlation with asthma symptoms ($p = .066$) and correlated with sputum tryptase levels ($p = .032$). No relationship between bronchial hyperresponsiveness, eosinophilic inflammation, and allergenic exposure was found (Alvarez et al., 2000).

b. *Animal Studies (Table 4)*. In these studies, airway reactions were measured directly after intracheal/inhalation exposure. In the previously mentioned study of Ritz et al. (1993), the relationship between intratracheal or inhalation exposure (dose and concentration) and respiratory responses to Alcalase were investigated in guinea pigs. Evaluation of respiratory responses immediately following each intratracheal administration revealed a significant ($p < .05$) dose-response in respiratory symptoms measurable after the fourth administration, which continued throughout the study. During weeks 4 through 10 of the inhalation experiment, respiratory reactions were observed that were dependent upon both the concentration of enzyme and the total exposure to the enzyme/detergent atmosphere. For both intratracheal and inhalation routes of exposure, the initial appearance of respiratory symptoms coincided with the first appearance of measurable specific antibodies (Ritz et al., 1993).

BN rats were exposed 30 min/week for 6 weeks to respirable OA aerosols at concentrations of <1, 3.3, 15.4, or 64.1 mg/m^3 (see also earlier discussion). Pulmonary inflammatory changes were observed at the high concentrations only (15.4 and 64.1 mg/m^3). Increased in vitro tracheal reactivity to methacholine was not found (Siegel et al., 2000). BALB/c mice were ip sensitized to OA with adjuvant on days 0 and 12, and challenged by inhalation to various concentrations of OA (0.01, 0.1, or 1% w/v diluted in saline), 30 min/day for 3 weeks. A concentration-related increase in bronchial hyperresponsiveness and pathology was observed 24 h after the last challenge (Tanaka et al., 2001). Because in this study no sham-sensitized but challenged control group was used, a threshold level could not be established. In a comparable study, C57Bl/6 mice were ip sensitized with house dust mite (Der p 1) with adjuvant. From day 14 to 20, the mice were exposed 30 min daily to aerosolized mite allergen at 3, 30, or 300 $\mu\text{g/ml}$ for 7 consecutive days. There was an increase in aspecific airway hyperresponsiveness, but this was not concentration related. Eosinophil influx was seen at the highest concentration only. A concentration-related increase in histopathological changes was, however, observed. The threshold level was 3 $\mu\text{g/ml}$ (Tournoy et al., 2000).

IV. DISCUSSION

For a considerable number of occupational as well as some environmental chemicals and proteins, dose-response relationships have been observed in skin and airway allergy testing.

For the skin, dose-response relationships have been found in epidemiological, clinical, and animal studies for both intradermal and topical sensitization as well as intradermal and topical elicitation by LMW chemicals (Table 5). No-observed-effect levels for induction of sensitization or elicitation under the test conditions used were also found for several of these compounds (Table 5), although a high variability of exposure exists in human exposure (Merget et al., 2003). The vehicle used, the integrity of the test compound in the vehicle, and the ability to penetrate the skin are all important variables in the induction of skin sensitization; the surface concentration and the absolute amount of allergen were considered to be the most important. Obviously, the dose per unit area is dependent on patch size/application area and the applied volume. This may indicate that the magnitude of dose to an individual dendritic (antigen-presenting) cell may be of more importance than the total number of dendritic cells sensitized. Or in other words, a few dendritic cells encountering many molecules will induce a more vigorous response than many dendritic cells encountering only a few molecules. Therefore it is important that, when comparing dose-response studies, the tested dose will be evaluated on a dose per unit area basis (White et al., 1986; Rees et al., 1990; Upadhye and Maibach, 1992; Boukhman and Maibach, 2001; Gerberick et al., 2001). However, in practice, the percentage concentration is the preferred option, as being used in the LLNA to calculate the EC3 value (Kimber et al., 2005). If tests are standardized, the percentage concentration can indeed very well be used.

Results for the elicitation of skin allergy are also dependent on the ability to penetrate the skin. Skin damage or irritation resulted in a significant decrease of the elicitation thresholds for reactions to some allergens. Persons with irritated skin are apparently at a higher risk at exposure levels not harmful to others (Boukhman and Maibach, 2001). Also, abraded skin due to shaving may have an additional effect in the case of allergenic fragrances (Johansen et al., 2003a).

Little is known about the concentrations of HMW compounds necessary for the induction of skin sensitization and the elicitation of skin allergy. A sensitization dose-dependent relationship was found for OA and mite allergen in guinea pigs (Table 6) (Arakawa et al., 1995).

In the respiratory tract, dose-response relationships in the induction and the elicitation phase have also been found for LMW chemicals, although only a limited number of (predominantly) epidemiological studies were available on this issue (Table 7). No-observed-effect levels for induction of sensitization under the test conditions used were also found in both human and animal studies (Table 7). In animal studies, functional pulmonary reactions were found following inhalation challenge (Table 7), but these reactions were not clearly concentration-dependently related (Botham et al., 1989; Blaikie et al., 1995; Arts et al.,

TABLE 5
Presence of dose-response relationships and threshold levels in dermal sensitization and/or dermal elicitation of low-molecular-weight chemicals

Compound	Dose-response	Threshold	References
Dermal sensitization			
3-Amino-5-mercapto-1,2,4-triazole (AMT)	X	X	Klink and Meade (2003)
Benzocaine	X		Marzulli and Maibach (1974), Van Och et al. (2000)
Bronopol	X		Marzulli and Maibach (1974)
<i>p-t</i> -Butyl- α -methylhydroxy-cinnamic aldehyde (BMHCA)	X	X	Lalko et al. (2004)
Chlorocresol	X		Andersen and Hamann (1984)
(Chloro)methylisothiazolinone	X	X	Warbrick et al. (1999b)
Cinnamic aldehyde	X	X	Danneman et al. (1983), Basketter and Scholes (1992), Andersen et al. (1995)
Citral	X	X	Basketter and Scholes (1992)
Dibutylthiourea (DBTU)	X	X	Nakamura et al. (1994)
Dicyclohexylcarbodiimide (DCC)	X		Flint et al. (2003)
Diethylamine	X		Van Och et al. (2000)
Diethylthiourea (DETU)	X	X	Nakamura et al. (1994)
Dinitrobenzene sulfonic acid (DNBS)	X	X	Ryan et al. (2002)
Dinitrochlorobenzene (DNCB)	X	X	Buehler and Griffith (1975), Friedmann et al. (1983), Nakamura et al. (1994), Hilton et al. (1995), Kimber et al. (1995), Loveless et al. (1996), Van Och et al. (2000), Scott et al. (2002)
Dinitrofluorobenzene (DNFB)	X		Flint et al. (2003)
Diphencyclopropenone	X	X	Basketter et al. (2000)
Diphenylthiourea (DPTU)	X	X	Nakamura et al. (1994)
Ethyleneglycol dimethylacrylate	X	X	Gerberick et al. (2000)
Eugenol	X	X	Loveless et al. (1996), Lalko et al. (2004)
Formaldehyde	X	X	Marzulli and Maibach (1974), Andersen et al. (1985), Andersen et al. (1995), Arts et al. (1997), Dearman et al. (1999), Ryan et al. (2002)
Geraniol	X	X	Lalko et al. (2004)
Glutaraldehyde	X	X	Marzulli and Maibach (1974), Hilton et al. (1998), Dearman et al. (1999)
Hexabromocyclododecane (HBCD)	X		Nakamura et al. (1994)
Hexamethylene diisocyanate (HDI)	X		Hilton et al. (1995)
Dicyclohexylmethane-4,4'-diisocyanate (HMDI)	X	X	Stadler and Karol (1985)
Hexylcinnamic aldehyde	X	X	Loveless et al. (1996), Dearman et al. (1998), Woolhiser et al. (2000), Dearman et al. (2001)
Hexahydrophthalic anhydride (HHPA)	X		Zhang et al. (1998), Plitnick et al. (2003)
Hydroxycitronellal	X	X	Steltenkamp et al. (1980), Ford et al. (1988), Basketter and Scholes (1992), Wahlkvist et al. (1999b), Lalko et al. (2004)
Isoeugenol	X	X	Loveless et al. (1996)
Isophoronediiisocyanate (IPDI)	X		Hilton et al. (1995)
Isopropyl myristate	X	X	Ryan et al. (2000)
<i>N</i> -Isopropyl- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine (IPPD)	X		Nakamura et al. (1994)
Isothiazolinone-based compounds	X		Botham et al. (1991b)

(Continued on next page)

TABLE 5
Presence of dose-response relationships and threshold levels in dermal sensitization and/or dermal elicitation of
low-molecular-weight chemicals (*Continued*)

Compound	Dose-response Threshold		References
Kathon CG	X	X	Chan et al. (1983), Cardin et al. (1986), Botham et al. (1991b), Andersen et al. (1995)
Linalool	X	X	Ryan et al. (2000)
Mafenamide	X		Marzulli and Maibach (1974)
Maleic anhydride (MA)	X		Plitnick et al. (2003)
2-Mercaptobenzothiazole (MBT)	X	X	Nakamura et al. (1994), Andersen et al. (1995), Van Och et al. (2000)
Dibenzothiazyl disulfide (MBTS)	X		Nakamura et al. (1994)
Methyldibromoglutaronitrile (MDBGN)	X	X	Wahlkvist et al. (1999a)
Diphenylmethane-4,4'-diisocyanate (MDI)	X	X	Blaikie et al. (1995), Hilton et al. (1995)
Nickel	X	X	Rohold et al. (1991), Nielsen et al. (1992), Menné and Calvin (1993), Menné (1994); Ryan et al. (2002)
Oxazolone	X		Hilton et al. (1995), Loveless et al. (1996), Ulrich et al. (1998), Van Och et al. (2000), Flint et al. (2003)
Phenylisocyanate (PIC)	X		Nakamura et al. (1994)
Phenylisothiocyanate (PITC)	X		Nakamura et al. (1994)
Phosgene (2,5-dichlorophenyl) hydrazone	X	X	Nakamura et al. (1994)
Phthalic anhydride (PA)	X	X	Blaikie et al. (1995), Van Och et al. (2000), Plitnick et al. (2003)
<i>p</i> -Phenylenediamine (PPD)	X	X	Marzulli and Maibach (1974), Warbrick et al. (1999a)
Picrylchloride (PiCl)	X	X	Stadler and Karol (1985)
1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-bis (2,2,6,6-tetramethyl-4-piperidiny) (PCT)	X	X	Vanoirbeek et al. (2003)
Potassium dichromate	X	X	Kimber et al. (1995), Wahlkvist et al. (1999b), Ryan et al. (2002)
<i>p</i> -Nitrobenzyl compounds	X		Roberts et al. (1983)
Methylbenzylphenol (SP)	X		Nakamura et al. (1994)
Squaric acid dibutyl ester	X	X	Scott et al. (2002)
Sultones	X		Ritz et al. (1975)
Tetramethylthiuram disulfide	X	X	Gerberick et al. (2000)
Toluene diisocyanate (TDI)	X	X	Hilton et al. (1995), Potter and Wederbrand (1995), Van Och et al. (2000)
3,3',4',5-Tetrachlorosalicyl-anilide (TCSA)	X	X	Ulrich et al. (1998)
Trimellitic anhydride (TMA)	X	X	Arakawa et al. (1995), Blaikie et al. (1995), Hilton et al. (1995), Van Och et al. (2000), Zhang et al. (2002), Plitnick et al. (2003), Pauluhn (2003)
Tetramethylthiuramdisulfide (TMTD)	X		Van Och et al. (2000)
Zincdibutyldithiocarbamate (ZDBC)	X	X	Nakamura et al. (1994)
Zincdimethyldithiocarbamate (ZMDC)	X		Van Och et al. (2000)
Dermal elicitation			
Chloroatranol	X		Johansen et al. (2003b)
Cinnamic aldehyde	X	X	Johansen et al. (1996)
DNCB	X	X	Friedmann et al. (1983), Bronaugh et al. (1994), Scott et al. (2002)
Formaldehyde	X		Flyvholm et al. (1997)
Hydroxycitronellal	X		Ford et al. (1988)

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TABLE 5
Presence of dose-response relationships and threshold levels in dermal sensitization and/or dermal elicitation of low-molecular-weight chemicals (Continued)

Compound	Dose-response	Threshold	References
Hydrocyisohexyl 3-cyclohexene carboxaldehyde (Lyrac [®])	X		Johansen et al. (2003a)
Kathon GC	X	X	Chan et al. (1983), Cardin et al. (1986), Weaver et al. (1985), Pasche and Hunziker (1989)
2-Mercaptobenzothiazole (MBT)	X	X	Emmet et al. (1994)
2,2-Dithio-bis-MBTS	X		Emmet et al. (1994)
MDBGN	X	X	Wahlkvist et al. (1999a)
Nickel	X	X	Allenby and Goodwin (1983), Emmet et al. (1988), Eun and Marks (1989), Menné and Calvin (1993), Menné (1994), Uter et al. (1995)
PPD	X	X	Bronaugh et al. (1994), McFadden et al. (1998)
Potassium dichromate (PDC)	X		Eun and Marks (1989)
Squaric acid dibutyl ester	X	X	Scott et al. (2002)
TMA	X		Andius et al. (1996)

1998). From these studies it seemed that challenge with the hapten gave better results than those with the hapten-protein conjugate (Botham et al., 1989), but this can be a matter of dose, as the gap between the lowest and highest concentration used was larger for the haptens than for the hapten-protein conjugate. Also, in rats, a broader range of concentrations resulted in concentration-related functional changes including a no-effect level (Arts et al., 2004) in comparison to a smaller range (Arts et al., 1998). Interestingly, exposure to TDI, although inducing high serum levels of specific antibodies did not induce specific pulmonary reactions upon inhalation challenge, in contrast to MDI, PHA, and TMA (Blaikie et al., 1995). This was most probably due to the physical form of the compound; that is, MDI, PHA, and TMA were present as aerosol particles, whereas TDI was present as a vapor. Because of its high reactivity, it may be assumed that TDI did not reach the bronchi to induce functional

reactions. At the workplace, in contrast, TDI vapor may settle on dust particles, thereby reaching more distant parts of the airways. It was therefore suggested (Pauluhn and Mohr, 2005) that the choice of challenge testing, namely, as hapten or as hapten conjugate, depends on the irritant potency and the physical form of the hapten.

Also interestingly, in several of these studies the dermal route was used as the sensitization route (Botham et al., 1989; Arakawa et al., 1995; Blaikie et al., 1995; Arts et al., 1998, 2004; Pauluhn, 2003), indicating that skin and respiratory allergy are not separated entities. This also strengthens the suggestion that skin exposure in the workplace may be very important in the development of subsequent heightened respiratory responsiveness. Skin exposure can indeed be substantial as found in auto body shop workers exposed to isocyanates despite protective clothing (Liu et al., 2000).

Dose-response relationships in the induction and elicitation phase of respiratory allergy have also been found in humans and animals for a number of HMW compounds (Table 8). No-observed-effect levels for induction of sensitization or elicitation under the test conditions used were found for several of these compounds (Table 8). Several studies have also shown that clear differences in potency of the different allergens seem to exist. Sensitization to rat urinary allergens and fungal alpha-amylase occurred already in the picograms and nanograms per cubic meter range (Heederik et al., 1999b). On the other hand, the spectrum of elicitation response may change with concentration. For instance, in an ip sensitization study with OA (coupled to aluminum) in mice, 25 ng OA-alum did not develop atopic antibodies, airway hyperresponsiveness, eosinophilia, or pulmonary Th2 responses, but the 25-ng-group animals developed IgA responses. The mice sensitized with 100 ng OA-alum developed

TABLE 6
Presence of dose-response relationships and threshold levels in dermal sensitization and/or dermal elicitation of high-molecular-weight compounds

Compound	Dose-response	Threshold	Reference(s)
	Dermal sensitization		
House dust mite	X		Arakawa et al. (1995)
Ovalbumin	X		Arakawa et al. (1995)
	Dermal elicitation		
Seafood	X		Jeebhay et al. (2001)

TABLE 7
Presence of dose-response relationships and threshold levels in airway sensitization and/or airway elicitation of low molecular-weight chemicals

Compound	Dose-response	Threshold	Reference(s)
Airway sensitization			
1,6-Hexamethylene diisocyanate–biuret (HDI-biuret)	X	X	Pauluhn et al. (2002a)
1,6-Hexamethylene isocyanurate (HDI isocyanurate)	X	X	Pauluhn et al. (2002a)
Hexahydrophthalic anhydride (HHPA)	X		Nielsen et al. (2001)
HMDI	X	X	Stadler and Karol (1984)
MDI	X		Pauluhn et al. (2000)
Methylhexahydrophthalic anhydride (MHHPA)	X		Nielsen et al. (2001)
Organic acid anhydrides (OAA)	X		Welinder et al. (2001)
Toluene diisocyanate (TDI)	X	X	Karol (1981; 1983), Ebino et al. (2001)
Trimellitic anhydride	X		Pauluhn et al. (2002b), Warbrick et al. (2002)
Airway elicitation			
Glutaraldehyde	X		Vyas et al. (2000)
Hexahydrophthalic anhydride (HHPA)	X	X	Nielsen et al. (1994, 2001)
Isocyanate (not further specified)	X		Diem et al. (1982), Malo (1990), Tarlo et al. (1997)
Diphenylmethane-4,4'-diisocyanate (MDI)	X		Pauluhn and Mohr (1994)
Methylhexahydrophthalic anhydride (MHHPA)	X	X	Nielsen et al. (2001)
Methyltetrahydrophthalic anhydride (MTHPA)	X	X	Yokota et al. (1999)
Tetrachlorophthalic anhydride (TCPA)	X		Liss et al. (1993)
Toluene diisocyanate (TDI)	X		Ott et al. (2000)
Trimellitic anhydride (TMA)	X	X	Bernstein et al. (1983), Botham et al. (1989), Arts et al. (1998, 2004), Zhang et al. (2004)

airway hyperresponsiveness in the absence of detectable disease, while the mice sensitized with 250 ng–2 μ g of OA–alum developed the full spectrum of allergic disease, that is, eosinophilia, IgE, IgG1, pulmonary Th2 cytokine responses, and airway hyperresponsiveness (Schneider et al., 2001). Although the route of sensitization in this study was ip, it is to be expected that such a different spectrum may also occur following respiratory-tract sensitization. On the other hand, the bell-shaped dose-response curve of sensitization with cat allergen suggests that high exposure to cat allergen can modify the Th2 response to suppress IgE production while maintaining or increasing IgG₄ and IgG₁ antibody production, which decreases the risk of asthmatic reactions (Custovic and Murray, 2002; Platts-Mills, 2002; Erwin et al., 2005). Reasons why exposure to cat allergen induces a bell-shaped curve whereas exposure to house dust mite does not are suggested by Platts-Mills (2002). Two important issues are: Exposure to cat allergen is much greater than that to mite allergen, and the size of the particles carrying cat allergen is smaller than those carrying mite allergens; these smaller particles may be inhaled further into the lungs, possibly influencing the immune response.

Notwithstanding the already indicated information on the existence of dose-response relationships and no-observed-effect levels in dermal and respiratory allergy, several points of discussion exist. These relate to measurement methods, concentration and route of exposure, exposure and effect quantification, and intra- and interspecies variation.

A. Measurement Methods

The method chosen to assess sensitization and/or elicitation can have a significant influence on the established threshold dose for a particular allergen. Many factors, such as animal strain, application method and site of application, concentration, frequency, and duration of exposure, concentration and type of vehicle, the integrity of the test compound in the vehicle, size of the exposed area, permeability of the site, occlusion, heat, moisture, vapor pressure, particle size distribution, condition of the exposure site, and irritancy potential may all affect dose-response relationships and threshold levels (Emmet et al., 1994; Basketter et al., 1997; Wahlkvist et al., 1999a, 1999b; Boukhman and Maibach, 2001; Lalko et al., 2004). With regard to skin irritation

TABLE 8
Presence of dose-response relationships and threshold levels in airway sensitization and/or airway elicitation of high-molecular-weight compounds

Compound	Dose-response	Threshold	Reference(s)
Airway sensitization			
Alcalase	X		Ritz et al. (1993)
α -Amylase	X		Houba et al. (1996a), Nieuwenhuijsen et al. (1999)
Cat allergen	X	X	Lindfors et al. (1999), Murray et al. (2001), Platts-Mills et al. (2001)
Cockroach allergen	X	X	Murray et al. (2001)
Houset dust mite	X	X	Anonymous (1993), Custovic and Chapman (1998), Custovic et al. (1998), Liebers et al. (1993), Murray et al. (2001)
Latex	X	X	Michael et al. (1996), Chen et al. (1997a, 1997b), Porri et al. (1997), Baur et al. (1998a)
Ovalbumin (OA)	X		Siegel et al. (2000)
Rat allergen	X		Cullinan et al. (1994b), Hollander et al. (1997a, 1997b), Heederik et al. (1999a)
Subtilisin	X	X	Thorne et al. (1986), Hillebrand et al. (1987)
<i>Trichoderma koningii</i>	X		Halpin et al. (1994)
Wheat allergen	X	X	Musk et al. (1989), Cullinan et al. (1994a), Houba et al. (1998a, 1998b)
Airway elicitation			
Alcalase	X		Ritz et al. (1993)
Animal feed	X		Heederik et al. (1994), Smid et al. (1994), Post et al. (1998)
<i>Bacillus</i> detergent enzymes (not further specified)	X		Flood et al. (1985), Flindt (1996)
Cellulase	X		Vanhanen et al. (2000)
Chironomidae insect allergens	X		Liebers et al. (1993)
House dust mite	X	X	Custovic and Chapman (1998), Custovic et al. (1998), Alvarez et al. (2000), Tournoy et al. (2000)
Latex	X	X	Baur et al. (1998a)
Laboratory animals	X		Cullinan et al. (1994b), Kruize et al. (1997)
Ovalbumin (OA)	X	X	Siegel et al. (2000), Tanaka et al. (2001)
Seafood	X		Jeebhay et al. (2001)
Western red cedar	X		Vedal et al. (1986), Noertjojo et al. (1996)
Wheat	X		Musk et al. (1989), Cullinan et al. (1994a), Houba et al. (1998a)
Wood dust (unspecified)	X		Halpin et al. (1994)

potential of the compound under investigation, it seems that this property promotes the bioavailability. Due to the differences in methods used, obtained sensitization or challenge thresholds, therefore, cannot be directly compared for the various compounds tested. In addition, most studies in humans are difficult to interpret because of the variability in patch testing techniques and diagnostic criteria used (Baur et al., 1998b). It was concluded by Kimber et al. (2005) that although there is an influence of the vehicle matrix on skin sensitizing activity, and the vehicles used are, therefore, of relevance in the context of risk

assessment, they have little impact on the accuracy of hazard identification when properly conducted standard test methods are used.

As to the respiratory tract, irritants can influence the occurrence, severity, duration and type of allergic reactions in man (Venables and Chan-Yeung, 1997) or can cause asthma-like reactions and inflammation of the airways in the absence of prior exposure (Chan-Yeung and Malo, 1995). In real life, individuals are exposed not only to a mixture of allergens but also to other pollutants and irritants and little is known about

synergistic effects, if any (Murray et al., 2001). Discrimination between LMW allergens and irritants is very important since generally valid occupational exposure limits may be assessed for LMW irritants, whereas such levels cannot easily be assessed for LMW allergens due to the large interindividual variation in threshold for sensitization and provocation of an allergic response (Briatico-Vangosa et al., 1994). In addition, it has been observed in animals that changes in breathing parameters may also be caused by the irritant properties of the allergen, making distinction of irritant-induced alterations from specific allergen-induced alterations not easy (Karol, 1991; Briatico-Vangosa et al., 1994; Pauluhn et al., 1999), but for TMA these could be distinguished on the basis of breathing patterns during exposure (Arts et al., 2003). In a concentration-response study with TMA in BN rats (Arts et al., 2004), significant nonspecific hyperresponsiveness to methacholine was only seen in sensitized BN rats after challenge with relatively high, irritating concentrations of TMA, whereas pure sensitization-dependent responses were only evoked at lower concentrations. So, apparently a combined irritant and allergic reaction seemed to be required for inducing airway hyperresponsiveness: that is, irritation seems to be required for exacerbation of the airway effects.

B. Concentration and Route of Exposure

Predictive tests should preferably closely simulate anticipated exposure conditions with regard to concentrations of chemicals encountered and routes of chemical exposure. The current guinea pig methods for skin sensitization testing, however, are not designed to determine threshold levels of a particular sensitizer (hazard characterization), but to assess whether a substance has the potential to cause sensitization (hazard identification; Basketter et al., 1997; Kimber et al., 2003). By increasing the exposure dose by one or two orders of magnitude, the sensitizing potential of a chemical can be determined more accurately and reactions to a weak sensitizer may not be overlooked. On the other hand, it has also been reported that high concentrations of skin sensitizers shift the expected Th1 response to a Th2 phenotype. The production of anti-inflammatory cytokines could then, for instance, decrease the ear swelling response (Hosken et al., 1995). For hazard identification, test procedures frequently include (repeated) skin injury, intradermal administration of the chemical to bypass the protective skin barrier, or pretreatment of the skin with detergents or irritants. These procedures appear appropriate for assessment of relative potencies but are less well suited for determination of absolute potencies and for extrapolation to anticipated exposure situations.

In addition, several respiratory sensitizers have been tested in the LLNA using dermal application, but there is hardly any information available whether their skin-sensitizing potency is equal to their respiratory sensitizing potency. Also, in respiratory allergy testing, several test methods use the skin as the induction route (Botham et al., 1989; Dearman et al., 1992a; Arakawa et al., 1995; Blaikie et al., 1995; Arts et al., 1998, 2004), or as elicitation route (Stadler and Karol, 1984; Ebino et al., 2001).

In addition, chemical-protein conjugates are used during airway provocation (among others; Botham et al., 1989; Arakawa et al., 1995; Blaikie et al., 1995); these methods are appropriate for hazard identification, but may hamper the determination of absolute no-effect levels and, consequently, extrapolation to occupational exposure situations. In animals, repeated inhalation sensitization with slightly irritant concentrations of TMA (6 times 30 mg/m³ for 30 min each week, resulting in a cumulative challenge dose of approximately 5 mg/kg body weight) seemed to be more effective than a total TMA dose required for 2-times topical sensitization (83 mg/kg body weight; Pauluhn, 2003). Inhalation sensitization of reactive chemicals at various concentrations may result in different deposition within the respiratory tract—that is, at low concentrations they may be scrubbed in the nasal passages, whereas increased penetration of the compounds in the lower respiratory tract only may occur at (very) high concentrations. This means that sensitization by inhalation may increase the susceptibility to irritant stimuli, may therefore hamper the discrimination between acute, irritant- or challenge-related injury, and thus may confound the selection of appropriate challenge concentrations (Pauluhn and Mohr, 1994, 2005; Arts et al., 2003, 2004). It may also imply that during challenge, each sensitized group exposed to a different challenge concentration needs its own nonsensitized control group (Arts et al., 2004).

However, in the human situation, short-term inhalation exposures to high LMW allergen concentrations, possibly during just one or a few occasions, are believed to be important in the induction stage of sensitization (Karol, 1989), but actual exposure levels are rarely measured during these peak episodes (Tarlo and Broder, 1989). In addition, the exposure concentration of the free LMW chemical to elicit pulmonary responses in short-term animal models is in general much higher than that recognized to elicit responses in humans (Pauluhn and Mohr, 2005). Therefore, when results of high-dose animal models are extrapolated to the low-dose human exposure situation, nonlinear dose-response relationships may lead to an underestimation of the response at lower concentrations (Karol et al., 1993; Bronaugh et al., 1994). With regard to HMW compounds, it has been shown that high allergen concentrations may induce immunological tolerance (Nielsen et al., 2002). Also, models involving short-term high-level exposure of sensitized animals to antigen have significant limitations for investigating the pathogenesis of the lesions of chronic asthma (Kumar and Foster, 2002).

One would expect that the threshold for the induction of a pulmonary response would at least be higher than the threshold required for the induction of antibody production, as an antibody response provides the potential for the elicitation of a pulmonary response (Blaikie et al., 1995). The results of the studies by Hillebrand et al. (1987), Thorne et al. (1986), and Nielsen et al. (2001) indeed indicated that the antibody levels, or prevalences, reflected antigen exposure concentrations and occurred at exposures below those necessary for pulmonary reactions. However, the threshold concentration for the elicitation of allergic disease in sensitized subjects is generally lower than the

threshold to induce sensitization. Sensitized individuals may exhibit symptoms following inhalation challenge to extremely low concentrations of a respiratory allergen (Briatico-Vangosa et al., 1994). Therefore, it may be particularly important to consider threshold levels for elicitation when, for example, health-based occupational exposure limits are recommended. No sensitization occurred in workers exposed to low allergen concentrations for a long period of time (Karol, 1981, 1983; Thorne et al., 1986). Also, in a murine LLNA with prolonged exposure duration (treatment for 2 months at 7-day intervals) it was shown that concentrations inducing a threefold increase in proliferation did not essentially differ from those obtained in the standard 3-day LLNA (van Och et al., 2003). This may indicate that no-effect levels can be established and that prevention of exposure to high peak concentrations will prevent sensitization. In addition, at low concentrations (but above a threshold) it may take longer before sensitization will occur, whereas peak exposures may result in faster sensitization.

With respect to skin allergy, a lower threshold concentration for elicitation than for sensitization also seems to be valid (Bronaugh et al., 1994; Emmet et al., 1994; Nakamura et al., 1994; Andersen et al., 1995). Also, the exposure concentration (in combination with the duration of exposure) seemed to be important for establishment of antibody response and pulmonary reactivity, rather than total exposure. On the other hand, it should be taken into account that the minimum dose required eliciting sensitization or challenge was not static—that is, the degree to which skin sensitization has developed influences the amount of chemical required to provoke a challenge reaction. The higher the level of sensitization, the lower is the required dose to elicit a challenge response, and vice versa (Scott et al., 2002). These findings may indicate that there is a need to consider dose-response relationships for sensitization and challenge in establishing minimum exposure levels for chemicals that cause allergic contact dermatitis.

C. Exposure and Effect Quantification

In human studies, methods to quantify exposure are far from being standardized yet, which hampers comparison of results obtained by different methods. For instance, exposure concentrations to allergenic proteins have been assessed by measurement of total dust or by measurement of allergen concentrations. Although increases in dust exposure in general will also lead to increases in allergen exposure, specific assays need to be established for an accurate estimation of the allergen exposure dose (Wegman, 1991). Moreover, concentration measurements are often lacking in retrospective studies. In addition, the evidence for the presence of exposure-sensitization relationships in human populations hardly allows analysis of the shape of the dose-response relationships—that is, different curves might have to be used to describe exposure-effect relationships (Heederik and Doekes, 1999).

Another major difficulty in the determination of dose-response relationships is the endpoint to be used. In animal stud-

ies like the guinea pig maximization test, responses are not based on the severity of the reactions, which is an intrinsic property of the chemical, but rather based on the incidence of sensitization which depends on the activity of the chemical and the exposure conditions.

D. Intra- and Interspecies Variation

Atopics, that is, subjects that are inheritably predisposed to produce elevated amounts of IgE antibodies, are more easily sensitized to HMW compounds than nonatopic subjects are (Heederik et al., 1999b). In young (4-week-old) rats, airways seem to be more susceptible to allergen-induced inflammatory and structural airway changes than in 13-week-old rats (Palman et al., 2002). In mice, dermal exposure to TMA resulted in increased total serum IgE levels in B6C3F1, C57BL/6, and BDF1 mice, but not in BALB/c mice (Guo et al., 2002). Zhang et al. (1997) found that genetic background had a selective effect on the phenotype of murine allergic pulmonary disease. Because of the existence of different subgroups it is difficult to establish safe exposure levels (Nielsen et al., 2002). Also, besides variations within the same species, interspecies variation may hamper the interpretation of potency. Attempts to correlate animal and human dose-response studies are only available for skin sensitization. Until a few years ago, the widely used guinea pig tests such as the GPMT were almost exclusively used as a test to determine sensitization potential. When, in these tests, besides the number of positive animals, the doses tested and the vigor of the reactions also are taken into account, a classification like that being used in acute toxicity (LC50/LD50) testing may be suggested, varying from no sensitization potential, to low, high, and very high sensitization potential. More recently developed skin sensitization tests such as the LLNA and the MEST seem, however, more appropriate to assess potencies than the guinea pig tests. In the murine LLNA, there is overwhelming evidence that for a wide range of contact allergens there is a concentration below which no significant response is induced (Boukhan and Maibach, 2001), and this test was shown to provide quantitative estimates of relative skin-sensitizing potency for a range of 21 chemicals. These correlated closely with relative potencies established from human repeat patch testing (both expressed as a function of dose per unit area of exposed skin that is $\mu\text{g}/\text{cm}^2$; Gerberick et al., 2001). Progress in potency estimation has been made using EC3 values, that is, the mathematical estimation of the concentration of a chemical necessary to obtain a threefold stimulation of proliferative activity compared with concurrent vehicle-treated controls (Basketter et al., 1999; Gerberick et al., 2001; Schneider and Akkan, 2004). An attempt has also been made on how to grade allergen potency based on existing methods (LLNA, GPMT, and human experience) and on whether such grading could be translated into practical thresholds for both induction and elicitation. It was concluded by the authors, that it would be reasonable to assign chemicals into one of three categories according to sensitization potency: moderate, strong, and extreme. Accordingly, preparations containing

moderate, strong, or extreme sensitizers should be listed on the label of the product when present at or above a certain concentration (Basketter et al., 2005). However, the same authors also concluded that it would be inappropriate to define elicitation thresholds as a function of skin sensitizing potency (Basketter et al., 2005) because elicitation thresholds correlate only poorly with induction potency (Scott et al., 2002).

For respiratory allergy, the available data indicate that various factors, both genetically and environmentally determined, may influence the development of allergy. Consequently, test results will largely depend on the specific animal strain used and there is up to now hardly any evidence that relative potency in guinea pigs, rats or mice is similar to that in humans. Specific factors related to species selection have been summarized by Pauluhn and Mohr (2005). Various factors also include the relative sensitivity of the endpoints used. Standardized and validated methods are thus needed to predict the sensitization potency and dose-response relationship of occupational chemicals (Karol et al., 1993). In our opinion, animal models for respiratory allergy should at least include measurement of functional airway changes, histopathology of the respiratory tract, and measurement of serum antibody levels, in order to be sensitive, selective and biologically relevant. Moreover, these should include a nonsensitized but challenged concurrent control group in order to discriminate between irritant-induced and allergen-induced effects. In case of animal models to study chronic asthma, rechallenge (repeated challenge) protocols have received increased attention (Pauluhn and Mohr, 2005).

V. CONCLUSION

In conclusion, most clinical and animal tests carried out to date are more focused on the potential of a chemical to induce skin and/or respiratory allergy rather than on its potency. Besides, extrapolation problems exist, such as high dose in test situations versus low dose in occupational situations, and intradermal exposure versus topical or inhalation exposure. Moreover, the observed dose-response relationships and threshold levels have been obtained by a wide variety of test methods and were largely dependent on differences in techniques used. Also, animal models, by their nature, involve experiments of short duration that are not typical for chronic human allergy. Therefore, no definite conclusions can be drawn about absolute threshold values for individual allergens with respect to sensitization of and elicitation reactions in the skin and respiratory tract.

In recommending health-based occupational exposure limits, caution is needed when no-effect levels obtained by different exposure methods, different exposure concentrations, different vehicles, different endpoints, and different animal strains are applied to the occupational situation. Models for skin allergy testing should preferably be standardized with respect to animal strain(s), concentration and type of vehicles used, application area, application or administration method, occlusion, and, most

importantly, concentration, frequency, and duration of exposure. The current OECD test guideline 429 (LLNA; OECD, 2000), which recently became the preferred test for skin sensitization, will overcome many of these problems, but it is noted that this test is not very well suited to test metal compounds or HMW compounds.

With respect to respiratory allergy, despite the fact that no overall accepted testing strategy is yet available, progress in predictive testing in relation to concentration-response relationships has been made (Karol, 1983; Botham et al., 1989; Dearman et al., 1992a; Blaikie et al., 1995; Pauluhn, 1997, 2003; Arts et al., 1998, 2004; Arts, 2001). As for skin allergy methods, respiratory allergy tests should be standardized. These should be standardized with respect to animal strain(s), concentration and type of vehicle used, exposure route and method, particle size distribution and concentration, and frequency and duration of exposure. Also, immunological tolerance should be taken into account. For risk assessment, physical properties of substances with sensitizing potential also have to be taken into account to assess the actual risk for respiratory allergy (Arts et al., 1998), as those factors may influence the ability of the compound to become airborne and as such may be inhaled.

In addition, studies need to be carried out with respect to dose-response relationships and no-effect levels in the elicitation phase of respiratory allergy, as the current information is limited due to the small number of studies reported. As far as we know, no-effect levels for the elicitation phase of respiratory allergy have been reported in animal studies only a few times, that is, for TMA (Table 7; Arts et al., 2004; Zhang et al., 2004), for OA (Table 8; Siegel et al., 2000), and for house dust mite (Table 8; Tournoy et al., 2000), but no-effect levels may be expected based on findings for the elicitation phase of skin allergy. Moreover, further research needs to be carried out, especially with regard to determination of potencies, not least in view of the production and use of an increasing number of potentially allergenic LMW compounds. Only by developing appropriate test methods can safe occupational exposure threshold levels be established, taking appropriate safety margins into account.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Dutch Ministry of Social Affairs and Employment for financial support, and Dr. C.F. Kuper and Dr. G.F. Houben for critically reading the manuscript.

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APPENDIX 1: KEY TO ACRONYMS

AMT	3-amino-5-mercapto-1,2,4-triazole	Ig	immunoglobulin
BMHCA	p-t-butyl-a-methylhydroxycinnamic aldehyde	ip	intraperitoneally
BN	brown Norway	IL	interleukin
CCET	cumulative contact enhancement test	IPDI	isophoronediiisocyanate
DBTU	dibutylthiourea	IPPD	N-isopropyl-N'-phenyl-p-phenylenediamine
DCC	dicyclohexylcarbodiimide	LLNA	local lymph node assay
Df	<i>Dermatophagoides farinae</i>	LMW	low molecular weight
DNBS	dinitrobenzene sulfonic acid	MA	maleic anhydride
DNCB	dinitrochlorobenzene	MBT	2-mercaptobenzothiazole
DNFB	dinitrofluorobenzene	MBTS	dibenzothiazyl disulfide
DPTU	diphenylthiourea	MDBGN	methyl dibromoglutaronitrile
EC	effective concentration	MDI	diphenylmethane-4,4' -diisocyanate
ED	effective dose	MEST	mouse ear swelling test
ELISA	enzyme-linked immunosorbent assay	MHHPA	methylhexahydrophthalic anhydride
FCA	Freund's complete adjuvant	MTHPA	methyltetrahydrophthalic anhydride
FCAT	Freund's complete adjuvant test	OA	ovalbumin
GM	geometric mean	OAA	organic acid anhydrides
GPMT	guinea pig maximization test	OR	odds ratio
GPSA	guinea pig serum albumin	PA	phthalic anhydride
HBCD	hexabromocyclododecane	PCT	1,3,5-triazine-2,4-diamine-6-chloro-N,N'-bis(2,2,6,6-tetramethyl-4-piperidinyl)
HDI	hexamethylenediiisocyanate	PDC	potassium dichromate
HHPA	hexahydrophthalic anhydride	PIC	phenylisocyanate
HMDI	dicyclohexylmethane-4,4' -diisocyanate	PiCl	picrylchloride
HMW	high molecular weight	PITC	phenylisothiocyanate
		PPD	p-phenylenediamine
		RPA	ribonuclease protection assay
		RT-PCR	reversed transcriptase-polymerase chain reaction
		TCPA	tetrachlorophthalic anhydride
		TCSA	3,3',4',5-tetrachlorosalicylanilide
		TDI	toluene diisocyanate
		Th1	Thelper 1
		Th2	Thelper 2
		TMA	trimellitic anhydride
		TMTD	tetramethylthiuramdisulfide
		TWA	time weighted average
		ZDMC	zincdimethylthiocarbamate