

Application of the risk assessment paradigm to the induction of allergic contact dermatitis

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Abstract

The National Academy of Science (NAS) risk assessment paradigm has been widely accepted as a framework for estimating risk from exposure to environmental chemicals (NAS, 1983). Within this framework, quantitative risk assessments (QRAs) serve as the cornerstone of health-based exposure limits, and have been used routinely for both cancer and noncancer endpoints. These methods have focused primarily on the extrapolation of data from laboratory animals to establish acceptable levels of exposure for humans. For health effects associated with a threshold, uncertainty and variability inherent in the extrapolation process is generally dealt with by the application of “uncertainty factors (UFs).” The adaptation of QRA methods to address skin sensitization is a natural and desirable extension of current practices. Based on our chemical, cellular and molecular understanding of the induction of allergic contact dermatitis, one can conduct a QRA using established methods of identifying a NOAEL (No Observed Adverse Effect Level) or other point of departure, and applying appropriate UFs. This paper describes the application of the NAS paradigm to characterize risks from human exposure to skin sensitizers; consequently, this method can also be used to establish an exposure level for skin allergens that does not present an appreciable risk of sensitization.

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Introduction

In 1983, the National Academy of Sciences (NAS) established the now widely used risk assessment paradigm which describes risk assessment as a four-step process: hazard identification; dose–response assessment; exposure assessment; and risk characterization. While different regulatory agencies and government institutions have slightly different approaches and use different terminology for noncancer risk assessments, the principles are the same: typically, a NOAEL or equivalent is identified (usually from a laboratory animal study), and appropriate uncertainty factors (UFs) are applied to account for areas of extrapolation. The risk assessment paradigm has been applied widely to systemic health endpoints; this paper describes the ex-

ension of this method to allergic contact dermatitis, a site-of-contact effect.

Adverse outcomes relating to excessive occupational exposures have long highlighted the need for restricting dermal exposure to contact allergens. More recently, there has been an increased focus on the need for guidance to help prevent sensitization to the general public from exposures to household and personal care products. Particular attention has been focused on perfume raw materials, several of which are known to pose an allergenic hazard and have been associated with an increasing frequency of allergic response in the general public (SCCNFP, 1999). To ensure that these concerns are effectively addressed, this paper advocates the application of a science-based risk assessment method to establish limits for dermal exposures in the general population that would prevent the induction of sensitization.

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Many consumer products are designed for direct or indirect contact with the skin. Prior to evaluating products in consumer studies, or widespread human exposure in the marketplace, it is necessary to conduct a safety evaluation of both the ingredients and formulation as a whole to ensure that exposures to consumers will not pose undue health risks. Quantitative risk assessment (QRA) approaches are routinely used to determine if an adequate Margin-of-Safety exists for systemic health effects. Historically, however, QRAs have not been used for evaluating the potential risk for site-of-contact effects, such as allergic reactions following dermal exposure. This paper discusses the scientific rationale for extending QRA methodologies to dermal endpoints, particularly the induction of allergic reactions, and provides a case study to illustrate this application.

For the purpose of simplicity, terminology of the United States Environmental Protection Agency (USEPA) will be adopted to illustrate the extension of this methodology. The USEPA has traditionally defined a “reference dose” (RfD) as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure of a human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime”¹ (USEPA, 2002a,b). In its simplest form, the RfD is calculated as: $RfD \text{ (mg/kg/day)} = NOAEL \text{ (mg/kg/day)} / UF$. A “Margin-of-Safety” (MOS) is the ratio of the RfD to the calculated human exposure, such that a $MOS \geq 1$ (i.e., the human exposure does not exceed the RfD) is generally considered to reflect an acceptable exposure that is unlikely to pose a health risk.

The skin sensitization testing and risk assessment process for new ingredients and consumer products generally follows a stepwise approach that may involve structure–activity evaluations, analytical assessments, preclinical skin sensitization testing (e.g., the mouse local lymph node assay), confirmatory clinical testing (e.g., the Human Repeat Insult Patch Test [HRIPT]), and benchmarking of resulting data against similar ingredients and product types. The details of these various elements and the overall process have been reviewed previously (Basketter et al., 1996; Gerberick and Robinson, 2000). If the allergenic potential of a new raw material has not been determined, it is necessary to follow this stepwise approach to ensure the safety of consumers. However, the allergenic potential of many raw materials used in consumer products is well-characterized, such that it is not necessary to conduct additional skin sensitization safety testing. Rather, where potency data are available to establish a NOAEL or other point of departure, we can use a RfD approach

similar to that described above for systemic endpoints. In addition to bringing more scientific evaluation and data into the process of estimating risks from skin sensitizers, the application of this risk assessment approach also has the potential to reduce animal testing and unnecessary confirmatory human patch testing.

The application of QRA methods to sensitization has previously been introduced in the contact dermatitis literature (e.g., Gerberick et al., 2001a; Robinson et al., 2000). It is the intent of this paper to facilitate communication between risk assessors working in the field of immunology and contact dermatitis with those who are primarily focused on risk assessment methods for systemic toxicity endpoints. Following is a description of the application of the NAS paradigm and QRA methods to calculate a RfD and ultimately a MOS for skin sensitizers. This is followed by a case study which illustrates the application of this method for an allergenic perfume raw material in an antiperspirant/deodorant.

Allergic contact dermatitis: an overview

Dermal sensitization represents an immunological response to a chemical allergen, which typically develops over a period of time. The biological sequelae of the sensitization process have recently been reviewed by Basketter et al. (1999a). For a chemical to pose a sensitization risk, it must be able to cross the stratum corneum to gain access to the viable epidermis. The chemical must also be able to be recognized and processed by epidermal Langerhans cells (LC), which then migrate to the lymph nodes where the antigen is presented to responsive naïve T lymphocytes. The selective proliferation of these lymphocytes leads to the formation of an immunological “memory” and the individual is said to be “sensitized.” Following a subsequent exposure to the allergen, the specific T lymphocytes recognize the chemical at the site-of-contact, and are activated to release cytokines and other inflammatory mediators that lead to a reaction known as allergic contact dermatitis (ACD).

It is important to distinguish between the induction and elicitation phases of ACD. The induction phase represents the initial exposures that eventually lead to an immune response of sufficient magnitude such that the individual is said to be sensitized. The elicitation phase, then, represents dermal exposures in already-sensitized individuals such that the exposure results in a cutaneous allergic reaction (e.g., erythema, edema, vesiculation, pruritis). While both induction and elicitation are dose-dependent phenomena that exhibit thresholds (Basketter et al., 1997; Kimber et al., 1999), fewer studies have been conducted examining elicitation thresholds. It is also generally recognized that elicitation thresholds may be lower than induction thresholds, such that it becomes

¹ It is acknowledged that USEPA’s Risk Assessment Forum is currently re-evaluating the RfD/RfC process (USEPA, 2002b).

increasingly difficult to protect individuals who are already sensitized. The USEPA (2002c) recognized this in calculating an RfD for soluble nickel salts: “The RfD is believed to be set at a level which would not cause individuals to become sensitized to nickel; however, those who have already developed a hypersensitivity (e.g., from a dermal exposure) may not be fully protected. Accordingly, the present paper addresses the risk assessment process specifically with a goal of preventing the induction of sensitization.

The NAS paradigm applied to skin sensitization: hazard identification

The identification of a sensitization risk can be made several ways:

Physico-chemical parameters: The presence of structural alerts (e.g., α -, β -unsaturated ketones (Barratt et al., 1994)) in a chemical possessing physico-chemical properties (e.g., molecular weight, lipophilicity) that will allow dermal penetration can alert the risk assessor to a potential sensitization risk.

Laboratory animal tests: Laboratory animals models for sensitization include the guinea pig maximization test (GPMT), the Buehler test and more recently, and the mouse local lymph node assay (LLNA). Traditionally, sensitization hazard tests have been conducted in guinea pigs, with the 2 most common methods being the GPMT (Magnusson and Kligman, 1969, 1970) and the Buehler test (Buehler, 1965, 1985). Sensitizing activity in these methods is measured as a function of challenge-induced erythema and edema in previously exposed animals.

The LLNA is a recently validated and accepted method for assessing skin sensitization potential (Gerberick et al., 2000; ICCVAM, 1999; OECD, 2001). In the assay, contact allergens are identified as a function of lymphocyte proliferative responses induced in the draining lymph nodes following open, topical application of the test material (Kimber et al., 1986). A quantitative measure of the proliferative activity is made by determination of tritiated thymidine incorporation into the draining lymph nodes. The degree of proliferation has been shown to correlate closely with the skin sensitizing capacity of the chemical (Kimber and Dearman, 1991). Thus, in the LLNA, sensitization potential is determined during the induction phase of the allergic response, rather than at the elicitation phase as with the traditional guinea pig methods. In the LLNA, at least 3 concentrations of the test chemical are evaluated, along with a concurrent vehicle control. A test material that causes a 3-fold or greater increase in proliferation relative to the vehicle control (stimulation index (SI) ≥ 3) is considered to be a sensitizer. The LLNA has gained favor because the route of exposure is dermal; it has a

faster turn-around time and uses fewer animals than the guinea pig tests; and it generates dose–response data that can be used to estimate relative potency, which is described further below.

Human experience: Human case reports of ACD can also provide evidence of a sensitization hazard. Indeed, many of the most common potent allergens have been identified from human experience (e.g., urushiol, the active compound in poison ivy). Of course, for ethical reasons, humans should not be used for testing intended to evaluate unknown hazards. However, HRIPT studies can be used to provide confirmatory data that a chemical or formulation would not pose a risk for the induction of sensitization.

Dose–response assessment: determination of allergenic potency

Once an allergenic hazard has been identified, the next step is to determine its potency. The LLNA assay provides dose–response data that allow new opportunities for the objective and quantitative estimation of skin sensitization potency (Basketter et al., 1999b). From the dose–response data, it is possible to mathematically derive an estimate of the concentration of test chemical required to induce an SI = 3, or a threshold positive response. This estimated concentration is called the “EC3” value and is given as a percent concentration.

Calculation of the EC3 values is carried out by linear interpolation according to the equation:

$$EC3 = c + [(3 - d)/(b - d)] \times (a - c),$$

where the data points lying immediately above and below the SI value of 3 on the LLNA dose–response plot have the co-ordinates (a, b) and (c, d), respectively (Fig. 1) (Basketter et al., 2000). Experience to date with this approach has been encouraging; clear differences between skin sensitizing chemicals can be discerned and such differences correlate well with the ability of the materials to induce contact allergy in experimental models. Additionally, it has been shown that LLNA EC3 values for the same chemicals correlate well with clinical NOAELs calculated from the literature (Basketter et al., 2000; Gerberick et al., 2001b). These investigations demonstrate that the LLNA can be used to provide quantitative estimates of relative skin sensitizing potency (EC3 values) that correlate closely with NOAELs established from human repeat patch testing and from clinical experience.

In addition to animal models, review of the published literature for reports of dose–response induction studies in humans can also reveal valuable information on the sensitizing potency of a variety of chemicals. Using available HRIPT data, together with expert judgment, numerous compounds have been classified as strong,

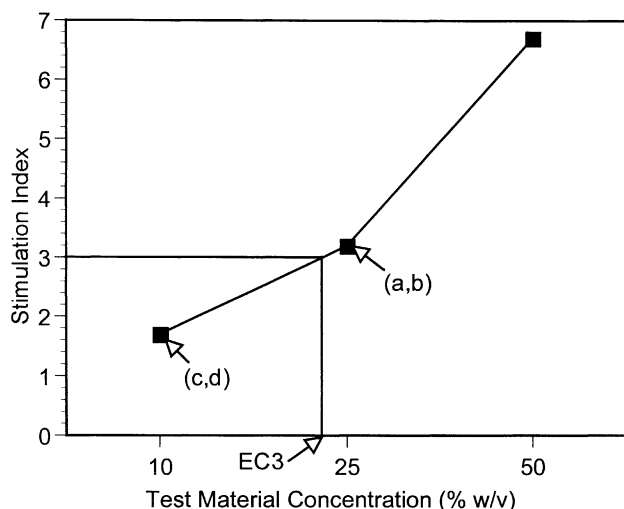


Fig. 1. Derivation of EC3 values from an LLNA dose–response using linear interpolation.

moderate, weak, extremely weak or nonsensitizers (Basketter et al., 2000). A lack of response in an HRIPT or other human patch study can also provide a NOAEL for the determination of potency. During the induction phase of the HRIPT, subjects are treated with three 24-h, patches per week, for three consecutive weeks for a total of nine induction patches on the same site on the upper back or outer, upper arm (Robinson et al., 1989). Following a 2-week rest period, subjects are challenged with a 24-h patch on the original patch site and an alternative, naïve site. The challenge sites are typically graded for reactions at 24- and 72-h after patch removal, but additional scoring can also be done at 48- and 96-h. This typically includes an erythema score, as well as an evaluation for the presence of edema, papules, and/or vesicles that could indicate an allergic response.

Because data are often insufficiently robust to identify a NOAEL with a high degree of precision, Gerberick et al. (2001a) developed a classification scheme to rank the potency of allergens based on a weight-of-evidence of available data from human and/or animal studies (EC3 values from LLNA). This scheme, comprised of six sensitization potency classes (including “nonsensitizers”), was used as a means for ranking the sensitiza-

tion potency of a number of perfume raw materials (Table 1). A weight-of-evidence approach using all of the available potency data from both animal models and human experience are then used to determine the appropriate potency category for the chemical being assessed. For each potency class, then, a “default NOAEL” has been assigned for use in QRAs. The main purpose for using a default NOAEL was to recognize that the NOAELs available in the literature for most allergenic compounds (e.g., perfume raw materials, preservatives) do not have a high degree of precision; in fact, they may be derived from a single human study utilizing a limited number of subjects. And, while LLNA data are useful for determining relative potency, we currently do not have sufficient experience to use these data quantitatively to determine a specific human NOAEL. As our experience with this assay grows, it is anticipated that the EC3 value from this test may be used directly in a QRA for sensitization. In the meantime, in the absence of good quantitative threshold data for sensitizers under evaluation, it is appropriate to use a conservative default NOAEL for conducting skin sensitization risk assessments. Of course, when robust data identifying a NOAEL are available, this value should be used, rather than a default value.

It is appropriate to include a discussion here on the appropriate dose metric for ACD, the importance of which has been recently highlighted by Robinson et al. (2000). While exposure for most systemic endpoints is expressed in units of mg/kg body weight, the relevant dose metric for skin sensitization potential is the amount of chemical per unit area of the allergen on the skin (i.e., $\mu\text{g}/\text{cm}^2$). While some of the historical literature in the field of contact sensitization has previously reported exposures as the concentration of a solution, we are now aware that it is not the percent (weight/volume) of material applied that is critical, but the total dose/area of exposed skin (Friedmann et al., 1983a,b; Rees et al., 1990; White et al., 1986). This was illustrated by Friedmann and his colleagues, who repeatedly exposed human subjects to dinitrochlorobenzene (DNCB), a potent contact allergen, and varied the doses per unit area of skin to observe the incidence of sensitization reaction upon challenge. When they kept the dose/unit

Table 1
Sensitization potency classification: default NOAELs for use in quantitative risk assessment (QRA)

LLNA EC3	Sensitization potential	Experimental human NOAEL	Default NOAEL for use in QRA
NC ^a	Nonsensitizing	NC ^a	NA ^b
>10,000 $\mu\text{g}/\text{cm}^2$	Extremely weak	> 10,000 $\mu\text{g}/\text{cm}^2$	10,000 $\mu\text{g}/\text{cm}^2$
1000–10,000 $\mu\text{g}/\text{cm}^2$	Weak	1000–10,000 $\mu\text{g}/\text{cm}^2$	1000 $\mu\text{g}/\text{cm}^2$
100–1000 $\mu\text{g}/\text{cm}^2$	Moderate	100–1000 $\mu\text{g}/\text{cm}^2$	100 $\mu\text{g}/\text{cm}^2$
10–100 $\mu\text{g}/\text{cm}^2$	Strong	10–100 $\mu\text{g}/\text{cm}^2$	10 $\mu\text{g}/\text{cm}^2$
$\leq 10 \mu\text{g}/\text{cm}^2$	Potent	$\leq 10 \mu\text{g}/\text{cm}^2$	1 $\mu\text{g}/\text{cm}^2$

Table reprinted with permission, from Gerberick et al., 2001a.

^a NC, not calculated. No positive response is obtained at any concentration tested and, therefore, an EC3 value cannot be calculated.

^b Not applicable. The material is a nonsensitizer and, thus, a default NOAEL is not needed for risk assessment.

area the same by applying increasing total doses to proportionately increased skin surface areas, the reaction incidence was equal. When they kept the total dose constant, but varied the area of application, those subjects exposed within smaller areas of skin (and hence larger dose/unit area exposures) had the greater incidence of sensitization reactions.

Data extrapolation: sensitization uncertainty factors (SUFs)

After identifying the hazard and evaluating potency from experimental data, the next step in a risk assessment often involves the extrapolation of the experimental data to the actual human exposure scenarios. This is often done by the application of uncertainty factors (e.g., for inter- and intra-species extrapolation). While the areas of data extrapolation are somewhat unique for dermal sensitization, the principles are the same. Specifically, one evaluates the chemical database and the human exposures to be protected, and identifies areas in which assumptions and extrapolations are made. These are generally acknowledged by the application of “sensitization uncertainty factors” (SUFs), which are sufficiently conservative so as to provide confidence that the allowable human exposure will be unlikely to cause harm in a heterogeneous population. The SUFs have been broadly classified into three general areas (Gerberick et al., 2001a):

- Inter-individual response variability,
- Matrix differences between what was tested in the patch test versus the product formulation to which the consumer will be exposed, and
- Variations in product usage patterns that are not addressed in the exposure assessment (e.g., part of the body exposed and skin integrity, occluded or nonoccluded, etc.).

As a default approach, a factor in the range of 1–10 is suggested for each of these three categories. The scientific support for these factors is described in a recent publication (Felter et al., 2002). Using weighting factors of 1–10 for each of the three categories and multiplying the factors together provides an overall SUF that can range from 1 to 1000, unless there is justification for going beyond the factors identified. It is noted that a 4th category for inter-species extrapolation is not listed here, since LLNA data from the mouse are currently used to help assign a potency category, but are not used to identify an actual NOAEL for use in a QRA.

Calculation of the sensitization reference dose (S-RfD)

The next step in the risk assessment process is to calculate a “sensitization reference dose” (S-RfD),

which is based on the same principles as USEPA’s RfD. Specifically, an S-RfD is a conservative estimate, with associated uncertainty, of a dermal exposure (in units of $\mu\text{g}/\text{cm}^2$ skin) that would not be expected to result in the induction of sensitization in the general population, including more responsive subpopulations. It is calculated using the same equation as the RfD: essentially, the NOAEL (or default NOAEL) divided by appropriate SUFs. Accordingly, the S-RfD is expressed in units of $\mu\text{g}/\text{cm}^2$ skin. The S-RfD can be used to set an acceptable exposure limit, or it can be used to determine whether there is a sufficient degree of protection for a current or proposed exposure. For the latter, the next step is an exposure assessment.

Exposure assessment

To calculate exposures from the intended and reasonably foreseeable uses of a consumer product, one needs to know the concentration of the ingredient in the final product formulation, the amount of product applied, and the area of application. There will also be exposure differences based on the type of product. For example, for products that are applied to and left on the skin (leave-on products), the conservative assumption is that 100% of applied product (and all ingredients therein) is available for absorption. For chemicals that are used in treating fabrics (e.g., detergents, conditioners), calculations and assumptions are made regarding residual product (and ingredients) remaining on the fabric and its transfer and retention on skin.

Many assumptions are used to calculate the exposures. These relate to skin surface area for the application site and other exposed skin surfaces, the amount of product used and number of applications per day and, for rinse off products, the residual material left on the skin.

There are several government and trade association publications that serve as resource material for most of the assumptions used in these calculations—for example, the USEPA’s Exposure Factors Handbook (USEPA, 1997), the Cosmetics, Toiletry, and Fragrance Association (CTFA), and the EU Technical Guidance Document (currently being revised by European authorities). Additional sources of exposure data include “habits and practices” studies sponsored by individual companies.

As with the dose–response or potency determination for a sensitizer, the exposure assessment must be calculated and expressed in units of μg chemical per cm^2 skin. It is noted that *absolute* dermal bioavailability is typically not considered in an exposure-based risk assessment for sensitizers. Rather, as with assessments for other endpoints, the bioavailability must be considered relative to the bioavailability from the study (ies) from which the NOAEL or potency of the

sensitizer is determined. Since these are mostly dermal applications, the relative bioavailability should be assumed to be 1 unless data are available to support a different number.

Risk characterization

The final step of the risk assessment paradigm—Risk Characterization—brings the previous steps together to characterize the risk of a specific exposure. It is in this step that one calculates a MOS, which is the ratio of the S-RfD to the human exposure. As indicated previously, a MOS of <1 does not necessarily imply an adverse outcome, but clearly as the MOS increases, the concerns decrease. As with systemic toxicity endpoints, an acceptable exposure is generally represented by an $\text{MOS} \geq 1$ (i.e., the human exposure does not exceed the S-RfD).

An alternate approach to a sensitization risk assessment is to calculate a “Margin of Exposure” (MOE). This approach is analogous to the MOS approach, except that the areas of uncertainty and variability are considered at the end of the assessment. Specifically, one calculates an MOE as the NOAEL divided by the human exposure (so that, as with the MOS, the MOE is a unitless number). The risk assessor must then determine whether, taking the areas of uncertainty and variability into account, the MOE is sufficiently high. In the end, of course, these two approaches should lead to the same conclusions.

Case studies utilizing an exposure-based risk assessment approach for sensitization have been published in the dermatological literature. For example, Gerberick et al. (2001a) demonstrated the application of quantitative risk assessment methods for evaluating cinnamic aldehyde (a perfume raw material) in an eau de toilette and in a shampoo. While the cinnamic aldehyde was assumed to be present at a level of 0.1% in each product, the different exposure scenarios led to different conclusions about the acceptability of the exposure. Quantitative risk assessments have also been published recently for the chemical preservative methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), which has been associated with sensitization in the marketplace (Fewings and Menne, 1999; Robinson et al., 2000). These case studies highlight the need for a quantitative sensitization risk assessment method that integrates hazard, potency, and exposure, while also addressing areas of data extrapolation and uncertainty to ensure the protection of human health.

Following is a case study that describes the application of the NAS risk assessment paradigm to another category of personal care products not previously published. This case study illustrates the quantitative risk assessment for an allergenic perfume raw material in an

antiperspirant/deodorant (APDO). For this case study, the MOS approach is used.

Case study: hydroxycitronellal in an antiperspirant/deodorant

Background

Hydroxycitronellal is a perfume raw material that is commonly used at levels in perfumes ranging from $<0.001\%$ to levels as high as 2%. An evaluation of the physico-chemical properties of hydroxycitronellal indicates that it is likely to be efficiently absorbed through the stratum corneum, and it contains a structural alert for sensitization (i.e., the aldehyde) (Fig. 2). Although hydroxycitronellal is not considered to be a common human allergen, the International Fragrance Association (IFRA) has determined that it should not be used such that the level in a consumer product exceeds 1%, based on its sensitization potential (IFRA, 2000).

This case study evaluates two hypothetical APDOs and the consumer exposure to hydroxycitronellal that is associated with the use of these products. A MOS is calculated for each scenario to demonstrate how QRA principles can be used to determine if the levels of hydroxycitronellal in each case do, in fact, provide an adequate assurance of safety.

Hazard identification

Animal studies have been conducted to assess the skin sensitization hazard of hydroxycitronellal. Under the stringent conditions of the GPMT, hydroxycitronellal has been reported to be a sensitizer (Basketter and Scholes, 1992; Marzulli and Maguire, 1982), with positive responses observed in 27–60% of the test animals. Testing of hydroxycitronellal by the Buehler method resulted in a 25% incidence of positive responses (Basketter and Gerberick, 1996).

The sensitization hazard of hydroxycitronellal has also been confirmed in the LLNA. When tested at concentrations of 10%, 25%, and 50% in an acetone:olive oil (4:1) vehicle, hydroxycitronellal induced SIs of 1.7, 3.2, and 6.7, respectively (Basketter et al., 1994), thereby meeting the criteria for classification as a sensitizer, with $\text{SI} \geq 3$ obtained at both 25% and 50%.

While hazard identification studies are typically conducted with animal test methods, the published literature also reveals studies in humans that have evaluated the

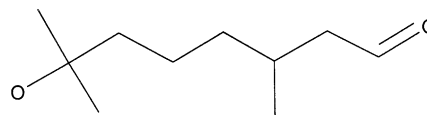


Fig. 2. Structure of hydroxycitronellal.

sensitization potential of hydroxycitronellal (Ford et al., 1988). A series of 15 human maximization tests (HMT) (Kligman, 1966) was conducted for the Research Institute for Fragrance Materials (RIFM) using hydroxycitronellal from four different sources. Subjects were induced with a test chemical volume of 1.0 ml on a 1.5-in. square Webril patch (area = 14.5 cm²). Induction concentrations ranged from 5% to 12%, with either petrolatum or diethyl phthalate as the vehicle. No positive responses were observed in the HMTs where 5% hydroxycitronellal in petrolatum (3448 µg/cm²) was used. In two separate HMTs conducted using 10% hydroxycitronellal, 0/25 subjects reacted in one study, and 2/25 reacted in the other. These apparently disparate results may just reflect the small sample sizes in these studies. From the results of these HMTs, RIFM concluded that hydroxycitronellal may induce allergic contact sensitization when tested at concentrations of 10% or greater (Ford et al., 1988).

Dose–response assessment

Using the dose–response information obtained from the LLNA described above, an EC3 value was mathematically derived by linear interpolation. The estimated concentration required to induce a threshold positive response (SI = 3) was calculated to be 23%. Using a dose volume of 25 µl, and, assuming the area of a mouse's ear to be 1 cm², conversion of this concentration to the dose per unit area metric results in 5750 µg/cm². According to the classification scheme presented in Table 1, these LLNA data rank the sensitization potential of hydroxycitronellal as 'weak' with a corresponding conservative default NOAEL of 1000 µg/cm².

As described in the Hazard Identification section, a series of HMT studies were conducted on hydroxycitronellal. The NOAEL and LOAEL from these studies, taken as a whole, was estimated to be 5% and 10%, respectively, in petrolatum. These studies employed a dose volume of 1 ml over 14.5 cm². Therefore, the NOAEL and LOAEL from the HMTs can be estimated to be 3448 and 6900 µg/cm², respectively.

In order to determine a NOAEL for the induction of sensitization to hydroxycitronellal in humans, RIFM conducted two HRIPTs (Ford et al., 1988). A panel of 197 subjects was divided into three groups. Each group was induced and challenged with a different concentration of hydroxycitronellal in an ethanol:diethyl phthalate (75:25) vehicle using a 0.3-ml dose volume and 25 mm Hill Top Chamber occluded patches (area = 2.54 cm² based on the 18 mm diameter pad in the chamber). Following challenge, positive responses indicative of allergic contact sensitization were observed in 1/65 and 1/66 exposed to 7.5% and 5% hydroxycitronellal, respectively. No positive responses were seen in the 65 subjects patched with 2.5% hydroxycitronellal.

From this study the LOAEL can be taken as 5%, which is ~5900 µg/cm² and the experimental NOAEL was 2.5% or ~3000 µg/cm². The second HRIPT was conducted 6 months after the first study and involved 100 of the original 197 subjects. In this second study, 29/100 responded with allergic reactions during the induction phase to concentrations as low as 2.5%. Of the subjects who had been exposed only to 2.5% hydroxycitronellal during both HRIPTs, 22% (4/18) responded to challenge with 2.5% hydroxycitronellal during the second study. Contrary to the first HRIPT, the second study did not identify a NOAEL.

These potency data for hydroxycitronellal are summarized in Table 2. Overall, the weight-of-evidence from the LLNA, HMT, and HRIPT studies support the classification of hydroxycitronellal as a weak sensitizer with a default induction NOAEL of 1000 µg/cm².

Sensitization uncertainty factors (SUFs)

As described previously, three areas have been identified that represent areas of data extrapolation for which SUFs can be applied. For the APDO case study, the following SUFs have been determined to be appropriate:

- *Inter-individual variability* (10×): Use default of 10-fold to account for inherent differences between individuals. Data do not exist to suggest a different value.
- *Product matrix* (3×): The human data for hydroxycitronellal came from patch studies where it was dissolved in a simple matrix of ethanol:diethyl phthalate (75:25). Antiperspirant matrices are clearly more complex and can have varying degrees of irritation associated with their use. The matrix may also affect the penetration rate of hydroxycitronellal, although there are no data to support a specific value. While a more irritating AP matrix might warrant the use of a 10-fold factor, a reduced factor of 3 can be justified for a mild formulation. For this case study, we will assume a mild formulation, and thus choose a factor of 3.
- *Skin exposure considerations* (10×): The axillae represent a part of the body that is typically semi-occluded, warm, and moist such that the skin is kept at a high level of hydration. Data on regional dermal permeability of various body sites indicates that the

Table 2
Data supporting potency classification of hydroxycitronellol (CASNO 107-75-5)

Dose–response data	NOAEL or LOAEL
EC3 from LLNA	5750 µg/cm ²
NOAEL from human RIPT	3000 µg/cm ²
LOAEL from human RIPT	5900 µg/cm ²
NOAEL from HMT	3448 µg/cm ²
LOAEL from HMT	6900 µg/cm ²

skin of the axillae is likely more penetrable than the skin of the upper back or arms, where patch test data are most often generated. It is also recognized that some people apply an antiperspirant more than one time/day and the exposure assessment (below) is based on one high-end application per day. These considerations suggest that a full 10-fold factor is appropriate to account for extrapolation across different exposure scenarios.

For the AP case study, then, the total SUF is $10 \times 3 \times 10 = 300$. Finally, the S-RfD is calculated by dividing the default NOAEL by the composite uncertainty factor:

$$\text{S-RfD} = 1000 \mu\text{g}/\text{cm}^2 / 300 = 3.3 \mu\text{g}/\text{cm}^2.$$

Exposure assessment

Some of the publicly available data on consumer exposure to APDO products are provided in Table 3. Some sources are specific as to the form of product (e.g., solid stick, aerosol, roll-on) and whether the data are for men or women. Other sources of data provide a single value without specifying the basis for the data. These differences notwithstanding, the data are fairly consistent and can be used to support an exposure assessment for ingredients in an APDO product. Rounded values that represent estimated high-end exposure values are used for this case study as follows:

- Amount of APDO applied (AP): 1 g/day.
 - Surface area of two axillae (SA): 180 cm^2 .
- For Case A, we will assume:
- Perfume level in APDO ([Perf]) = 1%.
 - Level of hydroxycitronellal in perfume ([HC]) = 0.01%.

For Case B, we will assume:

- Perfume level in APDO ([Perf]) = 3.25%.
- Level of hydroxycitronellal in perfume ([HC]) = 2%.

Exposure to hydroxycitronellal, in the desired dose metric of $\mu\text{g}/\text{cm}^2$, is calculated as follows:

- Exposure = (AP)[Perf][HC]/SA.
- Case A: $(1 \text{ g}/\text{day} \times 1,000,000 \mu\text{g}/\text{g} \times 0.01 \times 0.0001) / 180 \text{ cm}^2 = 0.0056 \mu\text{g}/\text{cm}^2$.
- Case B: $(1 \text{ g}/\text{day} \times 1,000,000 \mu\text{g}/\text{g} \times 0.0325 \times 0.02) / 180 \text{ cm}^2 = 3.6 \mu\text{g}/\text{cm}^2$.

Risk characterization

The last step in the assessment is to characterize the risk. This can be done by calculating the MOS, which is a comparison of the S-RfD and the human exposure:

$$\text{Case A: MOS} = 3.3 \mu\text{g}/\text{cm}^2 / 0.0056 \mu\text{g}/\text{cm}^2 = 590.$$

$$\text{Case B: MOS} = 3.3 \mu\text{g}/\text{cm}^2 / 3.6 \mu\text{g}/\text{cm}^2 = 0.9.$$

For Case A, the consumer exposure is well below the S-RfD as indicated by an MOS of 590. This suggests that additional testing would not be needed to confirm a lack of sensitization potential for this product on the basis of the hydroxycitronellal content. For Case B, the consumer exposure is calculated to be slightly higher than the S-RfD, such that the MOS is slightly lower than 1. Clearly there are sufficient data to allow small-scale consumer evaluations involving short-term exposures to this formulation (e.g., consumer evaluation for sensory endpoints); however, the outcome of the risk assessment suggests that confirmatory HRIP-testing might be appropriate before broad marketing of this product. Before making this determination, the risk assessor should re-evaluate and refine as appropriate (based on the available data) the assumptions made in the potency estimation (and the use of a conservative default NOAEL), determination of SUFs, and exposure assessment. If testing is initiated to provide confirmatory data, one would anticipate that results of the HRIPT will confirm a lack of sensitization potential based on the results of the risk assessment.

Conclusions

QRA has been used for decades to establish a consistent method for characterizing risk and/or determin-

Table 3
Consumer exposure data for antiperspirant/deodorants

SA of two axillae	Product application	Application frequency (per day)	Source
NP ^a	0.52 g	NP	USEPA (1997) ^b
NP	1.01 g	1.29 (90th percentile)	CTFA (as reported in USEPA, 1997)
NP	0.80	1.29 (90th percentile)	Cosmetic Company (as reported in USEPA, 1997)
NP	1.10	2.0 (90th percentile)	Market Research Bureau (as reported in USEPA, 1997)
Male: 240 cm^2	Male: 1.29 g	Male: 1.01	Dow Corning Cyclomethicone
Female: 122 cm^2	Female: 0.65 g	Female: 0.99	Exposure Assessment (1996) ^c
M or F: 180 cm^2		(average values)	

^a Not provided.

^b These data are reported for "Underarm Deodorants," which do not appear to be distinguished from antiperspirants; no data are given on the form of the product (e.g., stick, roll-on, aerosol).

^c Data provided are for a solid (stick) APDO formulation; additional data are provided for roll-on and aerosol technologies which are not listed in the table above.

ing acceptable exposure levels. For toxicants thought to have a threshold, the most common risk assessment method involves determination of the hazard and potency (this can be reflected by a NOAEL, LOAEL, or other point of departure), and the areas of extrapolation which are typically handled by the application of uncertainty factors. This method has traditionally been used for noncancer systemic endpoints to determine an acceptable human exposure level.

This paper describes the extension of the NAS risk assessment paradigm and QRA methodology to the induction of skin sensitization. The principles are the same—after identifying a sensitization hazard, the potency is determined. Because we often do not have very precise data on a NOAEL, and cannot generate these data in humans for ethical reasons, the potency determination often defaults to an assignment into one of the six categories: nonsensitizing, extremely weak, weak, moderate, strong, and potent. For each potency category, then, a conservative “default NOAEL” is assigned. For example, for a sensitizer determined to be of moderate potency, a default NOAEL of 100 µg/cm² is used in a quantitative risk assessment. Appropriate sensitization uncertainty factors are then determined to account for areas of data extrapolation (e.g., to protect more sensitive subgroups). A “Sensitization Reference Dose” is calculated as the NOAEL divided by the composite SUF. This can be used to establish an acceptable exposure level, or can be used to calculate a Margin-of-Safety compared to an actual human exposure.

This paper also presents a case study for a QRA for the induction of skin sensitization using a hypothetical APDO containing two different levels of an allergenic perfume raw material. The risk assessment process reveals that an APDO containing 0.01% hydroxycitronellal in a perfume used at 1% in the product has a robust MOS of close to 600, whereas an APDO containing 2% hydroxycitronellal in a perfume used at 3.25% in the product has an MOS = 0.9, and therefore might be a case for which confirmatory HRIP-testing under exaggerated exposure conditions (e.g., occluded patch) is appropriate prior to large-scale marketing. The consistent application of the risk assessment approach discussed in this paper can be used to establish improved guidelines for specific perfume raw materials or other raw materials known to cause sensitization and, equally important, be used for setting limits for newly developed raw materials that might pose a sensitization hazard.

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