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Setting surface wipe limits for skin sensitizers

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Abstract

Guidance for managing potential dermal exposures has historically been qualitative in nature, for example, in the form of a DSEN notation. We propose a method that can provide quantitative guidance on how to establish and use surface wipe limits for skin sensitizers. The murine local lymph node assay (LLNA) is a validated test that not only identifies potential skin sensitizers but also provides an effective concentration (EC3) value. This provides quantitative dose–response information on induction of skin sensitization that permits estimates of sensitization thresholds and potency. Building upon the previously established correlation between LLNA EC3 values and human repeat insult patch testing no-effect levels, we present a quantitative method for setting surface wipe guidelines using the LLNA EC3. These limits can be used to assign compounds to occupational exposure bands and provide handling guidance for skin sensitizers of varying potency, supporting both exposure assessment and control strategies. A table is included that suggests a band of reasonable surface wipe limits (mg/100 cm^2) for potentially all chemical sensitizers. When used in conjunction with a comprehensive industrial hygiene program that includes hazard communication, engineering controls, and personal protective equipment, skin exposure and consequent skin sensitization risks in the workplace can be minimized.

Keywords

Exposure assessment, hazard identification, OEBs, risk assessment, sensitization potency

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Introduction

Industrial hygienists rely on occupational exposure limits (OELs) to define acceptable levels of exposure to chemicals in the workplace and to verify that control measures are adequate. Despite their important role in facilitating reduction of workplace contaminants and thus lowering worker exposures, OELs have been established for only a small percentage of chemicals in commerce (Deveau et al., 2015) and most of these apply to inhalation exposures. For dermal exposures, with few exceptions (e.g. lead, beryllium), guidance is limited to qualitative indicators. Chemicals for which evidence of skin sensitization is available are denoted using dermal sensitizer notations by some organizations to serve as an immediate reminder of this potential hazard. Examples include the DSEN notation used by the American Conference of Industrial Hygienists (ACGIH, 2019) and the DSEN designation used by the California Occupational Safety and Health Administration (Cal/OSHA, 2009). NIOSH (2017) also has an updated skin notation system that includes a SEN notation. While these notations are helpful in raising awareness of these hazards and thus contribute to some exposure reduction, quantitative guidance expressed as concentrations such as mass/surface area would be more helpful. There have been a number of challenges to setting wipe guidelines for sensitizers in the past including the historical focus on inhalation-based exposure assessments, the perception that there is no safe level for sensitizers, the paucity of standard guidelines for assessing dermal exposures, and the

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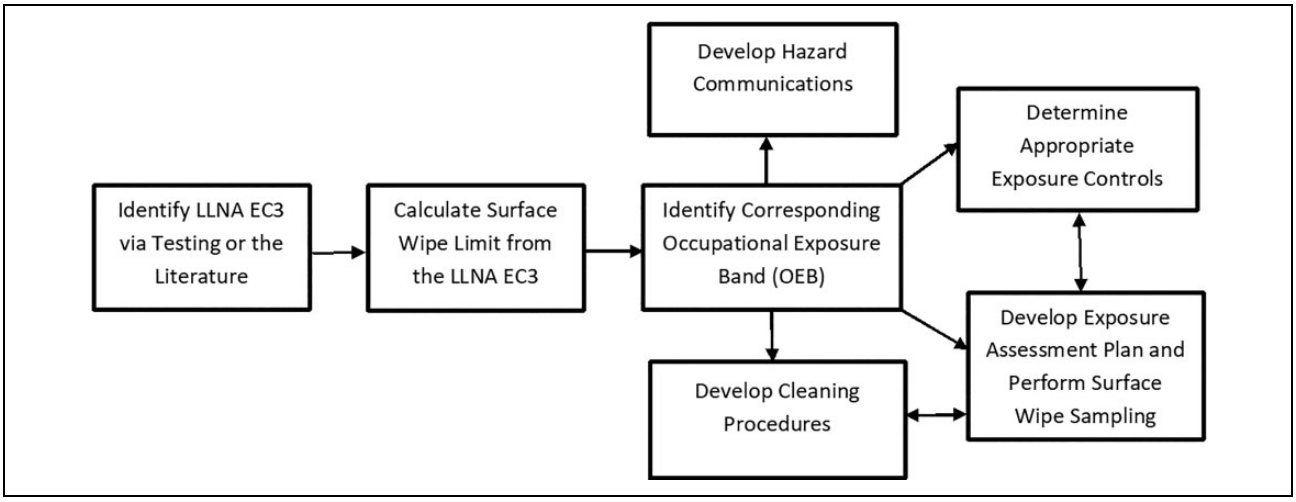

Figure 1. Process flow diagram for establishment and use of surface wipe limits.

limited experience in the field with respect to wipe sampling.

It is not clear whether induction of sensitization results from one (or a few) exposures at relatively high doses/concentrations or from repeated low-level exposure. In some cases, workers may be exposed to a known allergen for years without incident, only to develop sensitization later in their careers. Once an individual is sensitized, the elicitation of symptoms may result from extremely low levels of exposure, thus requiring their removal from the work area and potentially, the workplace—an outcome that can be career-ending for that individual. Therefore, minimizing exposures to sensitizers is necessary and urgent. When exposure to a sensitizer is lowered, fewer workers will exhibit allergic symptoms. Studies have shown that the concentration of the solution placed at the site of contact (mg/cm^2) is more important than the total dose (mg) for induction of dermal sensitization (White et al., 1986). Therefore, the surface wipe limit should be expressed as the surface load (mg) divided by a surface area corresponding to that used as a standard for wipe sampling (i.e. 100 cm^2).

Dermal OELs expressed as surface wipe limits can potentially serve as important tools for verifying the adequacy of workplace hygiene practices. When used within a comprehensive program that includes hazard communication, engineering controls, and personal protective equipment, it is expected that the incidence of new cases of skin sensitization can be minimized. This article describes the rationale for establishing surface (wipe) limits for skin sensitizers using the local lymph node assay (LLNA) data. A key aspect was to determine the appropriate adjustment factor(s) to apply to the LLNA EC3 to provide a high level of confidence that the resulting surface wipe limit will be below the skin sensitization induction threshold in workers. These limits are intended to be used within a comprehensive exposure control program to manage the risk of skin sensitization in the workplace as shown in Figure 1.

This article will review the various approaches that have been recommended for collecting wipe samples and offers suggestions on which of these methods should be used based on the chemical properties of the specific sensitizer. It is important to ensure that the methods to determine the level of external surface contamination are sensitive, accurate, and appropriately validated to ensure adequate protection of workers.

To create quantitative surface wipe limits, one requires a basis for a quantitative point of departure (including an understanding of its variability), application of adjustment factors (interindividual and interspecies differences), how transfer and uptake influence dermal exposure, and the importance of the frequency and duration of exposure. In the following sections, we describe how each step was addressed in developing an LLNA-based surface wipe limit. We provide the background and scientific support for the terms used in the equation that we are proposing to establish surface wipe limits. The point of departure is the LLNA EC3 value, which is usually expressed as a concentration in the vehicle applied to the dorsal surface of the ears of mice (see below for description of the LLNA methodology). This surface loading is expressed as the mass in 25 μL applied to the ears (1 cm^2) using units of $\mu g/cm^2$ as follows:

$$\text{LLNA EC3} = \frac{\text{Concentration in vehicle } (\mu g/ml) \times \text{Volume applied } (0.025 \text{ ml/ear})}{\text{Application area } (cm^2/\text{ear})} = \mu g/cm^2$$

The surface wipe limit is then calculated by dividing the LLNA EC3 ($\mu g/cm^2$) value by a composite adjustment factor and expressing this adjusted surface loading value as the amount of material distributed over the standard area used for surface monitoring (100 cm^2) as follows:

$$\text{Surface wipe limit } (\mu g/100\ cm^2) = \frac{\text{LLNA EC3 } (\mu g/cm^2) \times 100}{\text{Composite adjustment factor}}$$

While there may be other chemicals that have important dermal pathways, as they are not eliciting immunological reactions, we are not attempting to capture those compounds with this methodology. Other methods that focus on dermal loading and rates of absorption, leading to systemic exposure demonstrated to result in adverse effects, would be required to address these chemicals.

The murine LLNA

The murine local lymph node assay (LLNA) (Gerberick et al., 2007) was developed in response to the need for improved methods for identifying potential skin sensitizers and has been extensively validated as a recommended alternative to the guinea pig maximization test (GPMT) (Magnusson and Kligman, 1969) and Buehler test (Buehler, 1965) in a series of interlaboratory studies. The LLNA identifies skin sensitizing chemicals by detecting an increase in lymphocyte proliferation in the draining auricular lymph nodes following dermal application of the test chemical to the ears of mice and, hence, induces less animal distress than in the guinea pig tests. As the LLNA provides a quantitative measure for the potential to cause sensitization, it is also used to classify chemicals based on potency.

The LLNA protocol involves repeated application of the chemical to the dorsal surface of the ears of mice at defined intervals and, following a 5-day induction period, quantitating the stimulation of lymphocyte proliferation (a measure of induction of sensitization) in the draining auricular lymph nodes using tritiated thymidine. A nonradioactive version of the LLNA using bromodeoxyuridine has also been developed (Reeder et al., 2007; Ulker et al., 2013). A chemical is regarded as a skin sensitizer in the LLNA if at least one of a series of increasing concentrations determined on a compound-specific basis results in a threefold increase in lymphocyte proliferation compared to controls. This concentration is denoted the EC3 and is considered the threshold for induction of skin sensitization.

Table 1. General criteria for the classification of skin sensitizers.[a]

Data source	Criteria
Human data	Case reports (industrial experience)
	Positive HRIPT results
	Positive data from diagnostic patch testing
	Epidemiologic evidence of allergic contact dermatitis
Animal data	Murine LLNA Stimulation Index >3
	Adjuvant tests (e.g. GPMT) $\geq$30% responders
	Nonadjuvant tests (e.g. Buehler) $\geq$15% responders
SAR	Close structural similarity to known skin sensitizers

GHS: Globally Harmonized System; GPMT: guinea pig maximization test; EU: European Union; LLNA: local lymph node assay; HRIPT: human repeat insult patch test; CLP: classification, labeling and Packaging; EC3: effective concentration; SAR: structural activity relationship.

[a]The GHS and EU CLP systems include specific criteria to distinguish between strong (Cat 1A—EC3 < 2%) and mild/moderate (Cat 1B—EC3 > 2%) skin sensitizers based on the induction concentrations. Refer to the GHS and CLP guidelines for detailed classification criteria. Several *in vitro* alternatives are now required prior to *in vivo* testing, if needed, to classify compounds for skin sensitization.

Use of the LLNA for hazard identification and risk assessment

Since its original development, the LLNA has been the subject of extensive comparisons with guinea pig tests (Basketter and Scholes, 1992; Scholes et al., 1992) and/or with human data (Edwards et al., 1994; Basketter et al., 1994; Wahlkvist et al., 1999). As a result of these validation studies, the LLNA has gained regulatory acceptance in the European Union and the United States as a stand-alone replacement for the guinea pig assays (Balls and Hellsten, 2000; ICCVAM, 1999) Recent updates to the globally harmonized system (GHS) and EU Classification, labeling and packaging (CLP) classification criteria allow for the differentiation between mild–moderate and strong skin sensitizers based on the EC3 value (Table 1). During the validation processes, it was concluded that

the LLNA provided an equivalent prediction of the risk for human contact, as weak sensitizers in humans yielded negative or equivocal results in the LLNA. Further comparisons between LLNA results and human sensitization data have shown that the LLNA was able to discriminate between skin sensitizers and chemicals, which do not possess a significant skin sensitization potential in humans (Ryan et al., 2000). The quantitative measure of allergen potency, the EC3 value obtained in the LLNA, correlated well with human data/clinical evidence (Basketter et al., 2001; Griem et al., 2003; Kimber et al., 2001) and with human potency classes (Gerberick et al., 2001). As discussed below, in addition to being the preferred *in vivo* test when required to identify potential skin sensitizers, the LLNA is best suited to provide an estimation of the relative sensitization potency in the context of risk assessment (Kimber et al., 2001). In fact, the LLNA has demonstrated its value in providing quantitative estimates of potency and utility in performing risk assessments for cosmetic products (Gerberick et al., 2001; Robinson et al., 2000). While there are no validated *in vivo* or *in vitro* assays to identify respiratory sensitizers, the LLNA protocol can be used to evaluate which cytokines are elevated following dermal exposure that help determine whether the immune response is dominated by Th1 (skin sensitization) or Th2 (respiratory sensitization) T-helper cells (Dearman et al., 2000).

Surface wipe limit method development

The use of the LLNA for risk assessment is premised on the knowledge that skin sensitization (type IV: delayed T-cell–mediated allergic response; allergic contact dermatitis) is a toxicological effect that is characterized by a dose–response relationship and a threshold below which no response is expected (Gerberick et al., 2001). This premise is supported by an understanding of the underlying immunological mechanisms of contact sensitization and empirical evidence from studies in animals and humans.

The human sensitization data shown in Table 2 demonstrate that, even for a potent skin sensitizer like dinitrochlorobenzene (DNCB), which was used as a positive control in the GPMT, there is a concentration that is not likely to induce an allergic response in exposed individuals. At higher concentrations, the incidence can reach 100%. In the case of DNCB, the median effective concentration was 16.4 μg/cm^2 and the no-effect concentration was estimated to be 2 μg/cm^2 (Friedmann et al., 1983). This is equivalent to a surface load of 200 μg spread over a surface area of 100 cm^2, the standard surface area for industrial hygiene wipe sampling. It also coincides closely with the visual threshold for surface residues, that is, when they become detectible by the unaided eye.

Table 2. Human dose–response data for DNCB.[a]

Induction dose (μg) (single application)	Concentration (μg/cm^2)	Percent responding (no. sensitized/total)
62.5	8.8	8% (2/24)
125	17.7	63% (25/40)
250	35.4	83% (25/30)
500	71.0	100% (30/30)
1000	142.0	100% (8/8)

DNCB: dinitrochlorobenzene.
[a]From Friedmann et al. (1983); ED50 = 116 μg (16.4 μg/cm^2); ED08 = 62.5 (8.8 μg/cm^2); Estimated no-effect level = 2 μg/cm^2 (200 μg/100 cm^2).

To identify the appropriate use of the LLNA in deriving surface wipe limits, LLNA EC3 and human NOEL data from published studies were reviewed. The goal was to identify previous assessments that focused on comparing the potency of chemicals in the LLNA to the potency in human repeat insult patch tests to determine whether there was a consistent pattern. Specifically, the stability/variability of the LLNA EC3/human repeat insult patch testing (HRIPT) no-observed-effect level (NOEL) ratio was evaluated across multiple compounds representing a wide range of skin sensitization potencies. The idea was that, if we understood the relationship between the LLNA EC3 and the HRIPT NOEL, we could establish an adjustment factor to apply to the LLNA EC3 to estimate a no-effect level in humans. This value, when combined with other exposure-related considerations and adjustment factors, could serve as the basis for a surface wipe test.

Several approaches were used to understand the variability around the LLNA EC3/HRIPT NOEL ratio. An initial attempt was made to visualize the distribution of ratios by plotting them as a cumulative relative frequency distribution. The LLNA EC3/HRIPT NOEL ratios from Gerberick et al. (2001) were calculated for the 10 compounds listed in Table 3 and the cumulative relative frequency distribution was plotted (Figure 2). The upper and lower confidence limits on the fitted line are also shown. About 70% of the ratios were greater than 1.0, and all of the ratios were less than 10.

Table 3. Comparison of LLNA EC3 and HRIPT NOEL values.[a]

Compound	LLNA EC3 ($\mu g/cm^2$)	HRIPT NOEL ($\mu g/cm^2$)	LLNA EC3/ HRIPT NOEL ratio
Methylchloroisothiazolinone/ methylisothiazolinone	2.5	1	2.5
2,4-Dinitrochlorobenzene	20	8.8	2.3
p-Phenylenediamine	15	10	1.5
Formaldehyde	162	37	4.4
Isoeugenol	450	69	6.5
Glutaraldehyde	23	100	0.2
Cinnamic aldehyde	500	591	0.8
Citral	3250	775	4.2
Eugenol	2225	1938	1.1
Hydroxycitronellal	5000	2953	1.7

LLNA: local lymph node assay; HRIPT: human repeat insult patch test; EC3: effective concentration; NOEL: no-observed-effect level.

[a] Adapted from Gerberick et al. (2001).

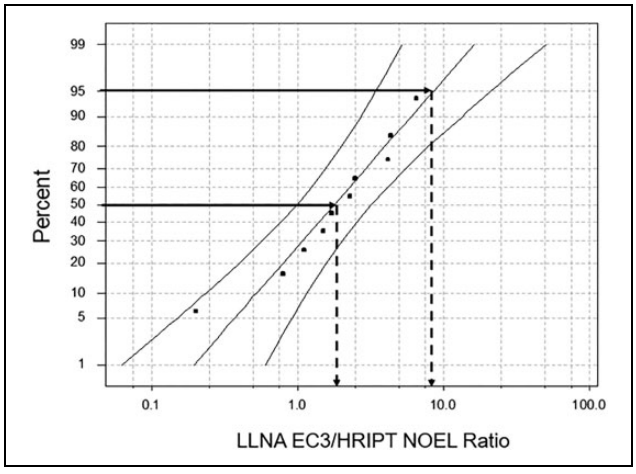

Figure 2. Cumulative relative frequency distribution for LLNA EC3/HRIPT NOEL ratios. EC3: effective concentration; LLNA: local lymph node assay; HRIPT: human repeat insult patch test.

The mean ratio using this same data set was determined to be 2.52 with a standard deviation (SD) of 1.95. The value 2 SDs above the mean is 6.4, which corresponds to the 95th percentile point estimate of the distribution. In other words, this is the upper bound value for the ratio predicting human outcomes based on the LLNA EC3 value. For newly tested sensitizers, this value can be interpreted to indicate that the human no-effect level is assumed to be, with a high degree of confidence, at most a factor of 6 below the LLNA EC3 when converted to $\mu g/cm^2$.

The same calculations were performed on LLNA and HRIPT data for 95 compounds: 20 assembled by Griem et al. (2003), 23 compounds summarized by Basketter et al. (2005), and 42 compounds reviewed by Api et al. (2015). The mean (SD) ratios were 1.76 (1.88), 4.26 (7.83), and 3.09 (6.81), respectively. The upper 95th percentile point estimates were 5.5, 19.9, and 16.7, respectively. The latter two analyses included several chemicals (two each) with very high ratios, illustrating how the LLNA can occasionally underestimate the human NOEL by a significant margin. Conversely, the LLNA can also overestimate the actual potency of the sensitizing agent in humans. The assay can err on either side while it generally identifies safe doses within a fairly narrow band when reasonable or small safety factors are applied. Api et al. (2015) noted the correlation between LLNA EC3 values and HRIPT NOELs and grouped them according to whether the LLNA EC was less than 3.2-fold higher (72%) or greater than 3.2-fold higher (12%) than the HRIPT NOEL. The remainder (12%) had LLNA EC3 values less than the HRIPT NOEL (i.e. overestimated the human potency).

By focusing on the LLNA EC3/HRIPT NOEL ratio, determination of how much of a margin of safety is required when extrapolating an EC3 value to an anticipated no-effect level in humans can be made. These values were used to define the range of adjustment (safety) factors to address various uncertainties and the severity of outcome when setting surface (wipe) limits. These limits are intended to be used within a comprehensive exposure control system, which should include a medical surveillance program designed to identify potential dermal sensitization cases that may occur in exquisitely sensitive workers.

Lessons learned from using the LLNA EC3 to assess cosmetic products

The use of safety factors to address various uncertainties when extrapolating animal data to support the establishment of safe exposure levels in humans has become a foundation for risk assessment since their introduction 65 years ago and adopted by regulatory agencies (Dourson and Stara, 1983; Lehman and Fitzhugh, 1954). In a more recent update and review of the literature, Dankovic et al. (2015) summarized the scientific basis for the uncertainty factors used to establish OELs. Similar to the study by Dourson and

Stara (1983), our method relies on upper statistical bounds (e.g. 2 SD above the mean) to demonstrate how the uncertainties (or variability) affect the margin of safety captured by the surface wipe limits. The addition of additional adjustment factors beyond the traditional ones to address factors unique to skin sensitizers helps to further extend the safety margins for the most potent compounds in this category.

Various proposals for using the LLNA EC3 for risk assessment for cosmetic products suggested the use of adjustment factors to address various sources of variability and uncertainty, including inter-individual (intra-species) differences, interspecies differences, matrix considerations (e.g. testing vehicle vs. product formulation), and exposure considerations. Felter et al. (2002) reviewed the scientific basis for uncertainty factors to address each of these and concluded that a range of 1–10 would be appropriate for each area. They acknowledged that these individual factors were not independent and that professional judgment was required to apply them in a sensitization risk assessment. The composite adjustment factors ranged from 1 to 1000 depending on the specific circumstances of the risk assessment, however, in practical use spanned a narrower range (i.e. 3–300). Gerberick et al. (2001) proposed a similar range but justified a sensitization assessment factor (SAF) of 100 for cinnamic aldehyde used in a fragrance: 10X for inter-individual variability, 3X for product matrix (surfactant matrix), and 3X for use exposure patterns (lifetime use; compromised skin). Api et al. (2008) derived no-expected-sensitization-induction levels, also using SAFs to account of inter-individual variability (10X), vehicle/product matrix effects (1, 3, 10X), and use considerations (1, 3, 10X) with a range of 10–1000. These risk assessments are for consumer use of cosmetics, fragrances, and so on and not for worker protection. Basketter and Safford (2016) provide an excellent review of the state-of-the-art with respect to the underlying assumptions and adjustment factor considerations when performing quantitative risk assessments for skin sensitizers.

It can be argued that the LLNA EC3/HRIPT NOEL ratio reflects both intra-species and inter-species differences. However, additional adjustment factors may be appropriate to address vehicle/matrix and exposure considerations. For each compound and worker exposure situation, these factors should be scenario dependent and considered in relation to actual testing conditions. Worker exposures to the pure substance on contaminated surfaces would not be expected to be absorbed as readily as under testing conditions when dissolved in a vehicle designed to enhance dermal penetration. However, there may be some opportunity for creation of occlusive conditions or contact with broken or sensitive skin areas (e.g. face and neck) and chronic exposures may also occur, so additional adjustment factors beyond the factor addressing the variability in the EC3/HRIPT ratio are recommended to address these possibilities.

Taking all of the above into consideration, it is proposed that the LLNA EC3 can be used as the point-of-departure, with application of appropriate adjustment factors, for deriving surface wipe limits for skin sensitizers. These adjustment factors should not be viewed as inflexible values. Depending on the compound's physicochemical characteristics, testing data, and exposure circumstances, a composite adjustment factor could easily range from 10 to over 100. A default composite adjustment factor (AF_C) of 50 is recommended based on the following assumptions: EC3/HRIPT ratio variability ($AF_R = 6$), matrix considerations ($AF_M = 3$), and exposure considerations ($AF_E = 3$) reflecting the middle of the range of values recommended by Felter et al. (2002), Gerberick et al. (2001), and Api et al. (2008) for cosmetics. The factor for the EC3/HRIPT ratio ($AF_R = 6$) is based on the analysis presented earlier. The factor for matrix effects ($AF_M = 3$) reflects that absorption following dermal exposure in the workplace to the pure material is likely to be different from the mixture containing absorption enhancers used in the LLNA or HRIPT testing. The factor for exposure considerations ($AF_E = 3$) also reflects the fact that accidental intermittent exposure in the workplace differs from the deliberate dermal application with cosmetics use.

It should be emphasized that $AF_C = 50$ is considered a default value, that is, a value that can be used in the absence of compound-specific data. This default value can and should be adjusted further, either upward or downward, based on the exposure scenario, test results, molecular class, and physicochemical considerations (e.g. MW, Log P_{OW}). This can be accomplished by choosing higher or lower values for AF_M and/or AF_E or, alternatively, through the use of a modifying factor (MF) to address the residual uncertainties that may not be adequately covered by the other factors. The choice of factors is a matter of judgment but can be informed by the characteristics of the compound and exposure scenario (Basketter and Safford, 2016). When considering the physical form and composition in which exposures may occur (i.e. the differences between worker exposures and testing conditions), an AF_M as low as 1 could

Table 4. LLNA EC3 values and estimated surface (wipe) limits for selected chemicals.[a]

Compound	CAS number	LLNA potency classification	LLNA EC3 (% w/v)	Surface wipe limit (mg/100 cm^2)
Oxazolone	15646-46-5	Extreme	0.01	0.001
Dinitrochlorobenzene	97-00-7	Extreme	0.08	0.01
p-Phenylenediamine	106-50-3	Extreme	0.09	0.01
Glutaraldehyde	111-30-8	Strong	0.20	0.05
Trimellitic anhydride	552-30-7	Strong	0.22	0.06
Phthalic anhydride	85-44-9	Strong	0.36	0.09
Formaldehyde	50-00-0	Strong	0.40	0.10
Diethylmaleate	141-05-9	Moderate	2.1	1.05
Phenylacetaldehyde	122-78-1	Moderate	4.7	2.35
Citral	5392-40-5	Weak	13	6.50
Diethanolamine	111-42-4	Weak	40	20.0

LLNA: local lymph node assay.
[a]Adapted from Kimber et al. (2003).

be reserved for exposures to the solid material while an AF_M up to 10 could be reserved for situations where the compound is handled in a solution containing a solvent (e.g. dimethylformamide or dimethylsulfoxide) that may increase the rate of dermal absorption. Similarly, compounds with a higher MW or a low Log P_{OW} are less likely to penetrate into the skin, supporting a lower AF_M value. Low MW compounds with high Log P_{OW} values should receive a higher AF_M value.

The MF can also be used to capture other aspects such as the severity of the effect (e.g. for strong or extreme sensitizers) or the molecular class the compound belongs to. If the test results, matrix effects and exposure considerations are straightforward for a compound, then a default modifying factor (MF = 1) can be used; MF = 2 could be used for strong sensitizers, and MF = 3 could be used for extreme sensitizers. Likewise, if the compound is from a molecular class that is known to be associated with strong or extreme sensitizers, perhaps due to their reactivity, a higher MF could be considered.

Example surface wipe limit calculation

The following example illustrates how a surface wipe limit can be derived using the LLNA EC3 value of 0.051% determined by Anderson et al. (2010) for *o*-phthalaldehyde:

Compound: *o*-Phthalaldehyde (0.051%) (25 µl applied to 1 cm^2 surface area on both ears in the LLNA)
Convert EC3 from volume percentage to surface area concentration
EC3: 0.051% = 510 µg/ml × 0.025 ml/ear × 2 ears ÷ 2 cm^2 = 13 µg/cm^2
Calculate wipe limit
Surface wipe limit = (EC3 (µg/cm^2) ÷ composite adjustment factor) × 100
Surface wipe limit = 13 µg/cm^2 ÷ 50 = 0.25 µg/cm^2 × 100 = 25 µg/100 cm^2

Occupational exposure guidance using surface wipe limits

Example surface (wipe) limits derived for a range of chemicals using the LLNA EC3 and applying a 50-fold adjustment factor for weak-to-moderate sensitizers, a factor of 100 for strong sensitizers and a factor of 150 for extreme sensitizers, are summarized in Table 4. As expected, there was a range of recommended limits consistent with the range of skin sensitization potencies reflected by the EC3 values. As discussed above, compound-specific considerations could result in different composite adjustment factors and alter these estimates, but it is expected the final values would be in a similar range (e.g. within an order of magnitude). Note that the surface (wipe) limits for the moderate and weak skin sensitizers are in the visible dust range for solids and may not be needed given general cleanliness requirements in some industries. The use of surface limits is especially useful in heavy manufacturing areas with low levels of cleanliness.

Practical application of surface wipe limits

Given that skin sensitization potency can span many orders of magnitude based on EC3 values, acceptance

Table 5. Proposed alignment of the sensitization classification scheme and OEB assignment based on the LLNA EC3 value[a].

			Surface wipe limit	
LLNA EC3	Sensitization classification	OEB	(mg/100 cm^2)	Examples
<0.1%	Extreme	5	<0.1	Dinitrochlorobenzene and *p*-phenylenediamine
≥0.1 to <1%	Strong	4	0.1–1	Phthalic anhydride and trimellitic anhydride
≥1 to <10%	Moderate	3	1–10	Diethylmaleate and phenylacetaldehyde
≥10 to ≤100%	Weak	2	10–100	Citral and diethanolamine
No Response	Not a sensitizer	1	Not required	Glycerol and diethylphthalate

LLNA: local lymph node assay; OEB: occupational exposure band; HRIPT: human repeat insult patch test; EC3: effective concentration.
[a]Adapted from Kimber et al. (2003).

limits calculated using the approach presented here using proposed adjustment factors can provide meaningful information to guide recommended handling practices. The resulting numerical surface wipe limits based on the LLNA EC3 value, and their alignment with the ranges of surface wipe limits that correspond to specific OEB categories within existing performance-based exposure control systems (OEBs), can also provide valuable guidance and leverage previously verified control strategies (see Table 6). Ongoing verification studies will be required to firmly entrench the use of surface wipe limits for skin sensitizers within these exposure control systems. One of the main strengths of the proposed method for setting surface wipe limits is that it is data based and builds on the successful use of the LLNA EC in skin product risk assessments. The main limitation is that it is a new method and has not been extensively verified. This should not discourage users from realizing the significant benefits it may provide. Conventional control methods and good workplace housekeeping remain important for any sensitizer. When a compound is shown to be a moderate-to-strong skin sensitizer in the LLNA, a number of additional actions should be taken, beyond the standard practices, in the following areas: (1) hazard communication, (2) engineering controls, (3) personal protective equipment, (4) cleaning procedures, and (5) surface monitoring. These are often grouped in a programmatic fashion as an "exposure control plan" that covers these aspects in a systematic way: risk assessment, risk communications, controls (using the hierarchy of controls), on-going monitoring, and health monitoring (if needed).

Hazard communication

The dose–response information provided with the LLNA and the estimate of potency indicated by the EC3 value provide industrial hygienists a scientific basis for several risk management approaches. The first, and one that should not be overlooked, is the opportunity to provide perspective through use of appropriate hazard statements on labels and safety data sheets (SDSs). Training could reinforce why internal labeling with "EXTREME SKIN SENSITIZER" is used on occasion to supplement the standard GHS/CLP Hazard Statement "May cause an allergic skin reaction." Just increasing the awareness of the potential hazards can lower the incidence of workplace illnesses.

Engineering controls and personal protective equipment

The LLNA EC3 provides an estimate of potency that permits a more quantitative evaluation that can support risk management decisions that are more closely aligned with actual risks. Depending on how potent a compound is, and the corresponding surface wipe limit, recommendations could range from installation of closed equipment allowing no open handling for extreme sensitizers, to reinforcing the use of standard personal protective equipment (e.g. proper use of gloves) and cleanup procedures for mild-to-moderate sensitizers.

Assignment of a skin sensitizer into one of several performance-based exposure control categories, commonly referred to as occupational exposure bands (OEBs) or control bands, should be based on the compound's inherent toxicological properties (Naumann et al., 1996). In this case, the key properties are skin sensitization potential and potency. The range of EC3 values and corresponding ranges of surface wipe limits for mild, moderate, strong, and extreme sensitizers shown in Table 5, which was adapted by the scheme proposed by Kimber et al. (2003). Minor

Table 6. Classification and labeling criteria for skin sensitizers using LLNA data.[a]

Potency category	Not a sensitizer	Weak sensitizer	Moderate sensitizer	Strong sensitizer	Extreme sensitizer
EC3 (%)	NA	>10	1–10	0.1–1	<0.1
OEB	1	2	3	4	5
Surface limit (mg/100 cm^2)	None	≥10–100	1–10[b]	0.1–1	<0.1
DSEN notation	None	Yes	Yes	Yes	Yes
SDSs	No	Sections 2, 3, 11	Sections 2, 3, 11	Sections 2, 3, 11	Sections 2, 3, 11
Labels[c]	No	Yes	Yes	Yes	Yes

LLNA: local lymph node assay; OEB: occupational exposure band; HRIPT: human repeat insult patch test; SDSs: safety data sheets; GHS: Globally Harmonized System; CLP: classification, labeling and Packaging; EC3: effective concentration.

[a] Classifications also reflect information on structural similarity to known sensitizers and any human data available such as anecdotal case reports and results of HRIPT or clinical trials.

[b] May not be needed given general cleanliness requirements.

[c] GHS/CLP Hazard Statement—H317: May cause an allergic skin reaction.

Skin Sensitizer Cat 1A (EC3≤2%); Cat 1B (EC3>2%).

variations on this scheme have also been offered (Gerberick et al., 2001).

The GHS/CLP criteria for classifying skin sensitizers also use potency information, although they consider compounds with EC3 values ≤2% as strong skin sensitizers and those with EC3 values >2% as mild-to-moderate skin sensitizers. Considering the multiple orders-of-magnitude difference in sensitization potency between compounds, the recommendations for controlling exposures to skin sensitizers probably fall into three categories at most since the least potent skin sensitizers probably do not require more than good chemical handling practices. General recommendations for mild, moderate/strong, and extreme skin sensitizers are also given in Table 6.

While it is always advisable to consider engineering controls as the primary means of protection, the unique nature of the skin sensitization response and the importance of dermal contact typically require a hybrid approach. Keeping dust out of the workplace air will certainly result in lower levels of contamination on external equipment surfaces; however, additional measures to prevent contact through the use of gloves and additional protective clothing (e.g. sleevelets), and more frequent cleaning, may be required to prevent sensitization in the workplace. It may also require a new way of approaching design for engineering controls. An example is a glove box with a slot opening designed for charging a process intermediate into a vessel. The vendor's design was based on the airborne levels associated with the performance-based exposure control limit (OEB or control band) assigned to the intermediate without consideration of its skin sensitization potential. Rather than having the operator insert his/her arms into the box through the slot, the glove box should have been outfitted with glove ports and gloves to prevent direct contact with the operator's protective clothing. The latter would also have precluded the need for special decontamination and de-gowning procedures. Another example is a product with low levels of a dermal sensitizer that was reformulated as a foam and a liquid formulation instead of a solid that had a greater potential for dispersion and contamination of surfaces at a distance from the area of product use. Product stewardship information on product use also included specific recommendations on the type of glove and other protective clothing to minimize skin contact. Finally, even application to nail salons could help identify ways of limiting dermal exposure such as requiring use of gloves for certain tasks.

Cleaning procedures and surface monitoring

It is important to clean facility and external equipment surfaces to prevent induction of skin sensitization in workers. Depending on how potent the skin sensitizer is, the cleaning procedure may require extra effort to achieve levels that are below visual thresholds. Surface sampling would be needed in these situations with supporting analytical methods that permit detection and quantification below the recommended surface limit. This will be challenging for some compounds which will require more work to pursue greater method sensitivity and may require use of surrogates with low detection limits. The sampling strategy should consider sampling locations and the frequency of sampling to ensure that the surface contamination levels in the workplace remain

consistently below the recommended surface wipe limits. The surface wipe limits for the chemicals included in Table 4 provide estimates of acceptance criteria for evaluation and verification of the effectiveness of the cleaning procedures.

The replacement of the GPMT with the LLNA in the current battery of tests enhances our ability to identify and control potential skin sensitization hazards for employees. The more accurate definition of skin sensitizers and their relative potency provides greater confidence that we are providing adequate protection to employees while benefiting from reduced costs associated with less stringent workplace controls than would be triggered by an all-or-none approach.

For the occupational toxicologist and industrial hygienist, the LLNA provides a scientific basis for tailoring guidance on how to handle skin sensitizers safely. It provides quantitative information on sensitization thresholds and no-effect levels, and the stimulation index (EC3) from the assay corresponds well to human NOELs when the same compounds were tested in both assays. When used to set surface limits, it has the potential to support a better assessment of sensitization risk; however, the use of the LLNA for risk assessment should be done with caution. Skin sensitization is a complex biological response to foreign chemicals and there can be significant inter-individual differences in susceptibility. Due to the underlying uncertainties, the surface (wipe) limit should not be considered a bright line between safe and unsafe surface contamination levels. Use of the upper 95% confidence limit on the mean LLNA EC3/HRIPT NOEL ratio as one of the factors used to derive surface wipe limits is considered a conservative approach. Surface limits derived by applying adjustment factors to the LLNA EC3 are believed to be health protective. Similar risk assessment approaches used in the cosmetics and fragrance industries have been quite successful (Basketter and Safford, 2016; Gerberick et al., 2001; Robinson et al., 2000).

For new compounds with information in SDSs, the use of occupational exposure bands (OEBs) can be useful. Categorical levels of guidance are provided for certain types of control strategies, including training, engineering controls, and use of personal protective equipment. Where an OEB is available, users can approximate a surface wipe limit from the ranges given in Table 5.

The current recommendations rely on the literature values for LLNA EC3 s and HRIPT NOELs. Industrial experience indicates that when the skin sensitization potential of the compounds evaluated by Durand et al. (2003) became known, and appropriate control measures were implemented, no new cases were reported. The effectiveness of using the LLNA to establish surface wipe limits and potency-based control strategies, with possible future adjustments resulting from further research, should be adequately investigated over time. The reader is referred to an excellent example of comprehensive strategy for assessing of actual and potential dermal exposures to an antineoplastic agent in a hospital setting, which could easily be adopted for skin sensitizers in any number of industries (Fransman et al., 2005).

Meanwhile, *in vitro* alternatives to the LLNA are being developed (e.g. KeratinoSens, DPRA, In Vitro Skin Sensitization assay) (DeGeorge and Troese, 2014; ECVAM, 2014; Gibbs et al., 2013). Gene expression assays have also recently become available (Johansson et al., 2014; Zeller et al., 2017). Eventually, the *in vivo* LLNA, and its quantitative interpretation that permits establishment of surface wipe limits, may be replaced by one or more of these alternatives. Comparative studies will be needed to validate their ability to provide potency estimates required to derive surface wipe limits. Hopefully, parallel *in vivo/in vitro/in silico* models and a similar strategy will also emerge to provide similar guidance for compounds that can induce respiratory sensitization or other immune-mediated diseases following dermal exposure.

Summary and conclusions

Surface wipe limits can be derived using data from the murine lymph node assay describing the threshold concentration associated with induction of skin sensitization and application of appropriate adjustment factors. Worker skin exposure at the resulting surface load should help to reduce the number of new cases of skin sensitization. There is growing evidence that induction of respiratory sensitization can result from dermal exposure, so using surface wipe limits to manage potential skin exposures to these compounds (e.g. phthalic anhydride) may also reduce the number of new cases of occupational asthma. As a key part of a comprehensive exposure control program, these limits can improve workplace hygiene through more effective surveillance. Thus, they represent an important advancement toward reducing exposures to sensitizers in the workplace.

Recommendations

The range of handling practices required to limit surface contamination should be commensurate with the level of skin sensitization potency. The exposure control program should be multifaceted and performance-based, encompassing hazard communication, engineering controls, personal protective equipment, cleaning procedures, and surface monitoring. Verification studies using surface sampling can be used to ensure that facility surfaces remain sufficiently clean.

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