

Quantitative Risk Assessments for Dermal Sensitization to Fragrance Ingredients: The Utility of LLNA Data in the Weight of Evidence Approach to Identifying Thresholds

J. Lalko; A.M. Api; V.T. Politano, C. Letizia
Research Institute for Fragrance Materials, Inc. (RIFM)

ABSTRACT

Historical human data from human repeated insult patch tests (HRIPT) and/or maximization tests (HMT) are available for fragrance ingredients used in consumer products. When conducting a Quantitative Risk Assessment for the induction of dermal sensitization, these data add to the overall weight of evidence approach used to determine potency. Contemporary human studies are not conducted to determine hazard; rather they confirm the lack of dermal sensitization at an exposure level identified as a NOEL in an animal model. One model commonly employed is the murine Local Lymph Node Assay (LLNA). In addition to identifying dermal sensitization hazards, the LLNA provides a quantitative measure of relative skin sensitizing potency. These potency estimates are based on interpolation of the dose response data, yielding an estimated concentration (EC3) required to elicit a positive response. In the present study, the EC3 values of 57 fragrance materials were compared to those derived from human NOELs for induction determined by historic confirmatory HRIPT and/or HMAX. The human NOELs and EC3 values were converted to their dose per unit area ($\mu\text{g}/\text{cm}^2$) equivalents to allow for direct comparison. A good correlation existed between the EC3 values and the human NOELs for induction. The EC3 values were observed to predict and in some cases under predict the human NOELs for approximately ~80% of the materials tested. The results from this analysis demonstrate the utility of incorporating the EC3 value into the QRA approach. However, the lack of correlation for several materials highlights the importance of conducting a confirmatory HRIPT.

Introduction

When conducting a Quantitative Risk Assessment (QRA) for the induction of dermal sensitization, human data from repeated insult patch tests (HRIPT) and/or historical maximization tests (HMT) add to the overall weight of evidence approach used to determine potency (Api *et al.*, 2008). Current practice is to conduct an HRIPT to confirm a predicted dermal sensitization no-effect level (NOEL) from animal testing. The Murine Local Lymph Node Assay (LLNA) is currently the most commonly utilized animal model to determine hazard and potency. The utility of combining LLNA data with knowledge gained from human studies in a weight of evidence approach applied to the QRA for dermal sensitization is considered below.

Human Sensitization Test

A human sensitization test is not used to determine hazard. The test is not used as a predictive method nor is it used on substances with unknown dermal sensitization potential. It is a test to confirm the lack of dermal sensitization at an exposure level which was identified as a NOEL in an animal model or derived as a likely NOEL from quantitative structure-activity relationships.

Human patch testing methodology has evolved over more than 50 years. The test most typically conducted is the human repeated insult patch test (HRIPT) (McNamee *et al.*, 2008). The HRIPT is generally performed in 100 subjects by conducting a total of nine 24-hour occluded applications over 3-weeks with test material and appropriate controls followed by a 2-week rest period. A single 24-hour challenge application is then made to a naïve site with the same materials. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of potential contact allergens. The HMT is no longer used by RIFM. Historically, it was conducted on 25 human subjects by utilizing 5 alternate day 48-hour occluded induction applications of test material and appropriate controls. Following a ten to fourteen-day rest period 48-hour challenge applications are made to naïve sites. Patches may be made with and without pre-treatment of sodium lauryl sulfate depending upon the inherent irritancy of the test material.

The HRIPT was designed and refined to exaggerate normal, realistic use conditions. This focus makes the HRIPT more relevant, when compared to the HMT, to the identification of a threshold for induction under foreseeable usage scenarios.

Local Lymph Node Assay Data

In recent years, the LLNA has been increasingly used for hazard assessment (Kimber *et al.*, 1992; 1994). The LLNA measures events occurring after initial exposure to chemical sensitizers, specifically activity is measured as a function of lymphocyte proliferative responses induced in lymph nodes draining the site of topical exposure to the test chemical. In addition to its application as a method to identify potential contact allergens, the LLNA presents the opportunity for the objective and quantitative measure of relative skin sensitizing potency (Basketter *et al.*, 2000). This potency estimate takes the form of an EC3 value—the concentration required to stimulate a three fold increase in lymphocyte proliferative responses. LLNA potency data were gathered from both published and unpublished reports for each material.

Analysis

The available data for 57 fragrance materials is presented in Table 1. The data show that for 40/57 of the fragrance ingredients reviewed, there is a very good correlation between the EC3 value from the LLNA and the NOEL in confirmatory human dermal sensitization tests. It should be pointed out that for 12 of the 40 materials, a true no effect level has not been determined in humans. The no effect level reported is the maximum tested concentration.

For the remaining 17 materials, the correlation is less predictive. Of these 17 materials, the data for 5 (α -amylcinnamaldehyde, benzyl salicylate, α -hexylcinnamaldehyde, hexyl salicylate, and α -iso-methylionone) reveal that the LLNA EC3 value is an order of magnitude greater than the maximum tested NOEL in humans (the NOEL reported reflects the highest concentration tested, not the highest achievable). The absence of significant clinically relevant positive reactions in dermatology clinics provides support for these data (see Table 2).

For 12 materials (α -amylcinnamyl alcohol, benzaldehyde, benzyl alcohol, cinnamyl nitrile, coumarin, t-2-hexenal, isocyclohexanone menthadiene-7-methyl formate, 6-methyl-3, 5-heptadien-2-one, methyl 2-nonynoate, 1-octen-3-yl acetate and treemoss) the data show that the EC3 value overestimates the NOEL in confirmatory human tests. Of these 12 materials only 4 (benzaldehyde, t-2-hexenal, and methyl 2-nonynoate) showed that the human no effect level was an order of magnitude lower than the LLNA EC3 value.

The data for the remaining 8 materials showed that the human no effect level was lower than the LLNA EC value but the data are equivocal. None of the LLNAs were conducted up to 100%. Further there is no lowest observed effect level for α -amylcinnamyl alcohol data so it may be possible that the LLNA EC3 value and the human no effect level could be similar. These data illustrate the importance of conducting a confirmatory human sensitization test.

There are seven essential oils that were reviewed and with the exception of treemoss the correlation between the LLNA EC3 value and the human data is good. These data are similar to the results found with other essential oils (Lalko and Api, 2006).

Table 1: No Expected Sensitization Induction Level (NESIL) for Fragrance Ingredients Derived by Application of Weight of Evidence Guidelines

Fragrance Ingredient	CAS No.	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Potency	Human Data			WoE NESIL ⁴ ($\mu\text{g}/\text{cm}^2$)
				NOEL HRIPT ²	NOEL HMT	LOEL ³	
Allyl phenoxyacetate	7493-74-5	775 [1] ⁵	Moderate	709 ⁴	690	NA	700
α -Amylcinnamaldehyde	122-40-7	2420 [4]	Weak	23,622 ⁴	NA	NA	23,600
α -Amylcinnamyl alcohol	101-85-9	>6250 [1] ⁵	Weak	3543 ⁴	NA	NA	3500
Anisyl Alcohol	105-43-5	1475 [1] ⁵	Moderate	NA	3448 ⁵	NA	1500
Benzaldehyde	100-52-7	> 6250 [1] ⁵	Weak	590		2760 ⁵	590
Benzyl Alcohol	100-51-6	>12,500 [1] ⁵	Weak	5906	6897	8858	5900
Benzyl Benzoate	120-51-4	>12,500 [1] ⁵	Weak	59,050 ⁴	20,690 ⁵	NA	59,000
Benzyl Cinnamate	103-41-3	4600 [1] ⁵	Weak	4720 ⁴	5517 ⁵	NA	4700
Benzyl Salicylate	118-58-1	725 [1] ⁵	Moderate	17,717 ⁴	20,690 ⁵	NA	17,700
p-t-Butyl-dihydrocinnamaldehyde (Bourgenol)	18127-01-0	1075[1] ⁵	Moderate	1181	4138 ⁵	7087	1100
p-t-Butyl- α -methylhydro-cinnamic aldehyde (BMHCA)	80-54-6	2372 [6]	Weak	4125	NA	29,528	4100
Carvone	99-49-0 2244-16-8 6485-40-1	2650 [3]	Weak	2657	1376	18,898	2650
Cinnamaldehyde	104-55-2	262 [22]	Moderate	591	NA	775	590
Cinnamyl Alcohol	104-54-1	5250[1] ⁵	Weak	3000	2759	4724	3000
Cinnamyl nitrile	1885-38-7	>2500 [1] ⁵	Weak	1063	3448	1938	1060
Citral	5392-40-5	1414 [11]	Moderate	1400	NA	3876	1400
dl-Citronellol	106-22-9	10,875 [1] ⁵	Weak	29,528 ⁴	4138 ⁵	NA	29,500
Coumarin	91-64-5	>12,500	Weak	3543	5517	8858	3,500
Dibenzyl ether	103-50-4	1575 [1] ⁵	Weak	2362 ⁴	2760	NA	2300
Eugenol	97-53-0	2703 [6]	Weak	5906 ⁴	5517 ⁵	NA	5900
Farnesol	4602-84-0	1200 [2]	Moderate	2755	NA	6897 ⁵	2700
Geraniol	106-24-1	4120 [6]	Weak	11,811 ⁴	4138 ⁵	NA	11,800
trans-2-Hexenal	6728-26-3	1012 [2]	Moderate	24	NA	236	24
α -Hexyl-cinnamaldehyde	101-86-0	2372 [2-5]	Weak	23,622 ⁴	NA	NA	23,600
2-Hexylidene cyclopentanone	17373-89-6	600[1] ⁵	Weak	300	NA	500	300
Hexyl salicylate	6259-76-3	45 [1] ⁵	Weak	35,433 ⁴	2069 ⁵	NA	35,400
Hydroxycitronellal	107-75-5	5612 [9]	Weak	5000	NA	5906	5000
3 & 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC)	31906-04-4	4275 [1] ⁵	Weak	4000 ⁴	NA	NA	4000
Isocyclocitral	1335-66-6	1825 [1] ⁵	Moderate	7087 ⁴	2759 ⁵	NA	7000
Isocyclohexanone	68527-77-5	>6250 [1] ⁵	Weak	3898	NA	5000	3800
Isoeugenol	97-54-1	498 [18]	Moderate	250	NA	775	250
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	<2500 [1] ⁵ Estimated 2375	Weak	2362 ⁴	5520	NA	2300
Jasmine Absolute (Grandiflorum)	8022-96-6 8024-43-9 90045-94-6 84776-64-7	1475[1] ⁵	Weak	1475	NA	2069	1470
Jasmine Absolute (Sambac)	91770-14-8	9100 [1] ⁵	Weak	8858 ⁴	NA	NA	8850
Majantol	103694-68-4	>7500 [1] ⁵	Weak	9900 ⁴	NA	NA	9900
p-Mentha-1,8-dien-7-al	2111-75-3	2175 [2]	Moderate	709	690	2760	700
Menthadiene-7-methyl formate	68683-20-5	>2500 [1] ⁵	Weak	1063	690	6900	1060
4-Methoxy- α -methyl benzene propanal	5462-06-6	5900 [1] ⁵	Weak	5905 ⁴	1380	NA	5900
α -Methyl-1,3-benzodioxole-5-propionaldehyde	1205-17-0	4100 [1] ⁵	Weak	4016	13,800	15,000	4000
α -Methyl cinnamic aldehyde	101-39-3	1125[1] ⁵	Moderate	3543 ⁴	5517 ⁵	NA	3500
6-Methyl-3,5-heptadien-2-one	1604-28-0	>1250 [1] ⁵	Weak	118		1299	110
α -iso-Methylionone	127-51-5	5450 [1] ⁵	Weak	70,866 ⁴	NA	NA	70,000
Methyl 2-octynoate (Methyl heptene carbonate)	111-12-6	<125 [1] ⁵	Strong	118	NA	194	110
Methyl 2-nonynoate (Methyl octene carbonate)	111-80-8	<1250 Estimated 625 [1] ⁵	Moderate	24	NA	118	24
2-Methoxy-4-methylphenol	93-51-6	1450	Moderate	118 ⁴	NA	NA	118
1-Octen-3-yl acetate	2442-10-6	>7500 [1] ⁵	Weak	3543	NA	6900	3500
OTNE	54464-57-2	6825 [1] ⁵	Weak	47,244 ⁴	NA	NA	47,200
Balsam oil, Peru (Myroxylon pereiarae Klotzsch)	8007-00-9	987 [1] ⁵	Moderate	950 ⁴	5517 ⁵	NA	950
Peru balsam absolute	8007-00-9	625 [1] ⁵	Moderate	NA	NA	5517	950
Peru balsam anhydrol	8007-00-9	NA	Moderate	NA	5517 ⁵	NA	950
Phenylacetalddehyde	122-78-1	962 [2]	Moderate	591	NA	1181	590
3-Propylidene-phthalide	17369-59-4	925 [1] ⁵	Moderate	945	345	2760	920
Oakmoss	90028-68-5	970 [1] ⁵	Moderate	700	1724	1414	700
Treemoss	90028-67-4	>5000 [1] ⁵	Moderate	700	6896	1417	700
Tea Leaf Absolute	84650-60-2	>1250 [1] ⁵	Moderate	480 ⁴	NA	NA	480
Vanillin	121-33-5	>12,500 [1] ⁵	Weak	1181 ⁴	3450	NA	1100
Ylang Ylang	8006-81-3 68606-83-7 83863-30-3	1700	Weak	1772	6897	7752	1770
d-Limonene ⁷	5989-27-5	10,075 [5]	Weak	10,000 ⁴	5517 ⁵	NA	10,000
Linalool ⁷	78-70-6	12,650 [2]	Weak	15,000 ⁴	13,793 ⁵	NA	15,000

All data in this Table are available from RIFM and are listed in the RIFM Database.

NOEL= No observed effect level; HRIPT= Human Repeat Insult Patch Test; HMT= Human Maximization Test; LOEL= lowest observed effect level; NA = Not Available

¹Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003

²All HRIPT use a solvent either consisting of all ethanol or a combination of ethanol and diethyl phthalate with the exception of those noted. The vehicle is given in parentheses after the dose. All HRIPTs were conducted with at least 100 subjects.

³Data derived from HRIPT or HMT

⁴WoE NESIL limited to three significant figures

⁵EC3 value from one LLNA, not the mean.

⁶MT-NOEL = Maximum Tested No Effect Level. No sensitization was observed in human predictive studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

⁷LOEL from human maximization test, not a human repeated insult patch test.

⁷d-Limonene and linalool are not contact allergens, but some hydroperoxides formed by autoxidation are known to be dermal sensitizers. In addition, d-limonene and linalool are known human irritants. The irritancy profile of d-limonene and linalool is being further investigated by RIFM

Table 2. Frequency of positive clinical patch test reactions (representative references)

Fragrance Ingredient	Frequency of positive patch test reactions	Reference
1% α -amylcinnamaldehyde	4 / 2062 (0.2%)	Schnuch <i>et al.</i> 2007
1% benzyl salicylate	2 / 2041 (0.1%)	Schnuch <i>et al.</i> 2007
10% α -hexylcinnamaldehyde	3 / 2019 (0.1%)	Schnuch <i>et al.</i> 2007
5% hexyl salicylate	0 / 218 (no reactions)	Larsen <i>et al.</i> 2002
1% α -iso-methylionone	1 / 2004 (<0.1%)	Schnuch <i>et al.</i> 2007

References

- Api, A.M. *et al.*, 2008. Regulatory Toxicology and Pharmacology, 52(1):3-23.
- Basketter, D.A., *et al.*, 2000. Contact Dermatitis 42, 344-348.
- Kimber, I., Basketter, D., 1992. Food and Chemical Toxicology 30, 165-169.
- Kimber, I., *et al.*, 1994. Toxicology 93, 13-31.
- Lalko, J. and Api, A.M. 2006. Food and Chemical Toxicology, 44:739-746.
- Larsen, W., *et al.*, 2002. Contact Dermatitis, 46(3), 141-144
- McNamee, P.M., *et al.*, 2008. Regulatory Toxicology and Pharmacology, 52(1):24-3
- Organization for Economic Co-Operation and Development (OECD) 2002. Guideline reference 429.
- Schnuch, A., *et al.*, 2007. Contact Dermatitis, 57(1), 1-10.

Summary and Conclusion

- A human dermal sensitization test is not used to determine hazard, rather it is a test to confirm the lack of sensitization at an exposure level which was identified as a NOEL in an animal model or derived as a likely NOEL from quantitative structure-activity relationships.
- The induction of dermal sensitization from confirmatory human tests is rare because the assay is used to confirm a NOEL.
- The confirmatory HRIPT methodology is robust in design in terms of number of individuals, exposure conditions and evaluation parameters. The test conditions in the HRIPT are exaggerated compared to real life scenarios and are relevant to the generation of data that are very important to the application of a QRA approach.
- The EC3 value has recently been demonstrated to closely correlate with the NOEL from confirmatory human sensitization tests designed to confirm lack of induction.
- A detailed analysis of the dermal sensitization data in the RIFM database for 57 fragrance ingredients that have exhibited dermal sensitization potential revealed that for the majority of these fragrance ingredients, there is a very good correlation between the predicted NOEL from the murine local lymph node assay and the NOEL in confirmatory human tests.