

Comparative sensitizing potencies of fragrances, preservatives, and hair dyes

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Summary

The local lymph node assay (LLNA) is used for assessing sensitizing potential in hazard identification and risk assessment for regulatory purposes. Sensitizing potency on the basis of the LLNA is categorized into extreme (EC3 value of $\leq 0.2\%$), strong ($> 0.2\%$ to $\leq 2\%$), and moderate ($> 2\%$). To compare the sensitizing potencies of fragrance substances, preservatives, and hair dye substances, which are skin sensitizers that frequently come into contact with the skin of consumers and workers, LLNA results and EC3 values for 72 fragrance substances, 25 preservatives and 107 hair dye substances were obtained from two published compilations of LLNA data and opinions by the Scientific Committee on Consumer Safety and its predecessors. The median EC3 values of fragrances ($n = 61$), preservatives ($n = 19$) and hair dyes ($n = 59$) were 5.9%, 0.9%, and 1.3%, respectively. The majority of sensitizing preservatives and hair dyes are thus strong or extreme sensitizers (EC3 value of $\leq 2\%$), and fragrances are mostly moderate sensitizers. Although fragrances are typically moderate sensitizers, they are among the most frequent causes of contact allergy. This indicates that factors other than potency need to be addressed more rigorously in risk assessment and risk management.

Key words: EC3 value; fragrances; hair dyes; hazard; preservatives; risk assessment; Scientific Committee on Consumer Safety.

Animal testing has been an important tool for assessing the potential of a chemical substance to cause skin sensitization. The three generally accepted animal test methods are the guinea-pig maximization test and the Buehler test in guinea-pigs, and the local lymph node assay (LLNA) in mice (1, 2). For registration of chemicals in Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the LLNA is the first-choice method (3). A European Commission expert working

group on skin sensitization proposed how animal data could be used to clarify sensitizing potency (4). The group proposed categorization of classified skin sensitizers into three groups: extreme, strong and moderate sensitizers.

Potency categorization based on the LLNA relies on the EC3 value, which is the concentration of a substance required to elicit a three-fold increase in lymph node cell proliferative activity in exposed animals as compared with vehicle control animals. Extreme potency was defined as an EC3 value of $\leq 0.2\%$, strong potency as an EC3 value of $> 0.2\%$ to $\leq 2\%$, and moderate potency as an EC3 value of $> 2\%$ (Table 1).

According to the EC regulation on the classification, labelling and packaging of substances and mixtures (CLP), positive human and/or animal data should be used for the classification and subcategorization (1A

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Table 1. Potency categorization of fragrance substances, preservatives, and hair dye substances, based on local lymph node assay EC3 values (% wt/vol)

EC3 value (category)	Fragrance substance (n = 72), n (%)	Preservative (n = 25), n (%)	Hair dye substance (n = 107), n (%)
≤0.2% (extreme)	2 (2.7)	7 (28)	10 (9.3)
>0.2% to ≤2% (strong)	3 (4.1)	6 (24)	25 (23)
>2% (moderate)	56 (77)	6 (24)	24 (22)
Insufficient testing ^a	10 (14)	0 (0)	39 (36)
Not classifiable ^b	1 (1.4)	6 (24)	9 (8.4)

Values gathered from compilations of data and opinions by the Scientific Committee on Consumer Safety and its predecessors (5–8). Categories are based on (4, 9).

^aThe highest concentration tested was too low.

^bThe substance did not, under the conditions of the test, elicit a three-fold increase in lymph node cell proliferative activity.

and 1B) of skin sensitizers (9). When human and animal data show conflicting results, a weight of evidence approach should be used; however, negative human data should not normally negate positive animal results. New animal testing for showing compliance with the EC Cosmetics Regulation has been banned since 2013; however, old data and data generated for other purpose can be used. No *in vitro* method has yet been validated by the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM; <https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam>, last accessed 9 September 2016) for determination of skin-sensitizing potency.

A risk assessment opinion by the EC Scientific Committee on Consumer Safety (SCCS) presented LLNA data concerning 70 fragrance substances and natural mixes, based on documents submitted by the fragrance industry and two additional publications (5–7). A review based on this opinion has also been published (10). The SCCS and its predecessors have presented LLNA data on substances in numerous opinions (available at the SCCS website http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm, last accessed 8 September 2016) and compilations of data on hair dye substances (11, 12).

The aim of the present study was to compare LLNA data on the skin-sensitizing potencies of fragrance substances, preservatives, and hair dye substances, three important groups of skin sensitizers that frequently come into contact with the skin of consumers and workers.

Materials and Methods

LLNA data concerning fragrances (n = 72) were obtained from risk assessment documents, and opinions by the SCCS, especially a recent opinion on fragrance allergens in cosmetic products (5). LLNA data concerning preservatives (n = 25) and hair dye substances (n = 107) were obtained from two published compilations of

EC3 values of various compounds and from opinions by the SCCS and its predecessors (available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm, last accessed 30 April 2016) (Table 1) (6, 7). Primary sources of data were searched for and assessed, based on the references in these compilations and opinions. It was not always possible to track the primary source. In the present article, preservative is defined in a broad sense as a compound used to inhibit the growth of microorganisms, including preservatives in a stricter sense, antimicrobials, disinfectants, and biocides.

The LLNA should be conducted in accordance with Organization for Economic Co-operation and Development (OECD) guideline 429 (2). Studies with shortcomings that could substantially affect the results, typically too low test concentrations, were regarded as insufficient. Data on 10 fragrances and 39 hair dye substances were thus excluded from further analysis in the present study (Table 1). When more than one EC3 value had been reported, only the lowest EC3 value was included. EC3 values are generally expressed as a percentage value (wt/vol). In addition, we calculated the EC3 values in mol/l (M) (conversion is explained in File S1).

Results

The median EC3 values of fragrance substances, preservatives and hair dye substances were 5.9%, 0.87%, and 1.3%, respectively (Fig. 1a, Tables 2–4). The corresponding median molar EC3 values were 0.38, 0.035 and 0.081 M, respectively (Fig. 1b, Tables 2–4). The individual EC3 values (% and M) for all compounds, and the distribution of EC3 values and molecular weights (MWs), are shown in Tables 2–4 and Fig. 1.

Five of the 72 fragrance substances (8.2% of those with an EC3 value) had an EC3 value of ≤2%, and were therefore categorized as strong or extreme sensitizers, and 56 (92% of EC3 values) had an EC3 value of >2%, and were

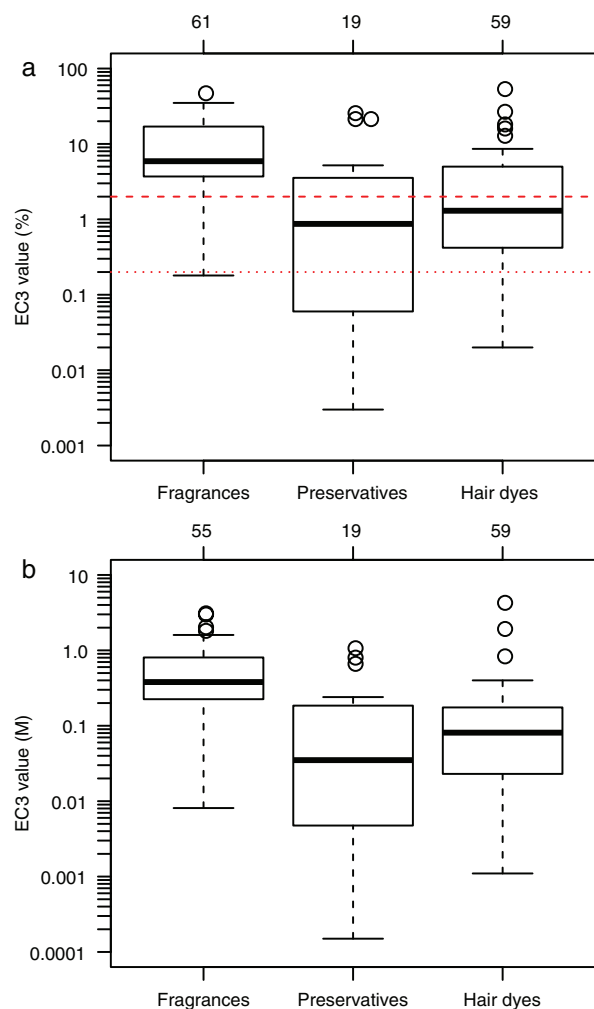


Fig. 1. Distribution of EC3 values of fragrances, preservatives, and hair dyes (log scale). The bottom of the box denotes the 25% percentile, the thick line the median, and the top of the box the 75% percentile. Whiskers extend to extreme data points that are not considered to be outliers. Outliers, more than 1.5 box widths from edge of the box, are shown as circles. (a) EC3 values in % (generally wt/vol). The red reference lines indicate the thresholds for categorization as an extreme and a strong skin sensitizer (4). (b) EC3 values in M (mol/l). It was not possible to calculate the EC3 values in M for six natural products (mixtures), explaining the lower number of fragrances in (b) than in (a).

therefore categorized as moderate sensitizers. In contrast, 13 of the 25 preservatives (68% of EC3 values) and 35 of the 107 hair dyes (59% of EC3 values) were categorized as strong or extreme sensitizers (Table 1).

Expressing the EC3 values as M instead of % did not notably affect the relative potencies when the three groups of substances were compared, as their average MWs are similar (Fig. 1, Tables 2–4). The median EC3 values of preservatives and hair dyes were rather similar,

irrespective of the chosen unit, whereas the median EC3 values of fragrances were higher by a factor of four to six than those of hair dyes and preservatives (Fig. 1, Tables 2–4).

All substances with EC3 values ($n = 133$) were compiled in a list with their respective EC3 values (% and M) and MWs. The ranking according to sensitizing potency varied according to whether EC3 (%) or EC3 (M) was used. Twenty-seven substances changed position by >10 ranks and seven changed position by >20 ranks in the potency ranking based on EC3 (%) as compared with EC3 (M). All changes to a higher potency ranking of this magnitude with EC3 (M) were for substances with relatively high MWs, and similar changes to a lower potency ranking were for substances with relatively low MWs. Acid Black 1 (MW 616.49), HC Yellow No. 17 (MW 903), HC Violet No. 2 (MW 299.32) and iso-cyclocitral (MW 304) received a higher potency ranking (lower EC3 value) with the EC3 (M) value. Glyoxal (MW 58.04), formaldehyde (MW 30.0258) and trans-2-hexenal (MW 98.144) received a lower potency ranking (higher EC3 value) with the EC3 (M) value.

Discussion

The median EC3 value, calculated in terms of % and M, was considerably higher for fragrances than that for preservatives and hair dyes. Correspondingly, few fragrance substances were categorized as strong or extreme skin sensitizers as compared with preservatives and hair dyes. Although fragrances are usually moderate sensitizers, according to the LLNA, they are probably responsible for at least as many cases of contact allergy resulting from the use of consumer products as the much more potent preservatives and hair dyes. EC3 values for all fragrance substances in the European baseline series, except coumarin, were available in the SCCS opinion (5, 13, 29). The vast majority (11 of 14) were categorized as moderate sensitizers, with EC3 values of >2%.

Frequency of contact allergy

The frequency of fragrance contact allergy in patients, defined as a reaction to at least one of the five screening allergens/mixtures in the German baseline series for diagnostic patch testing, has been estimated to be ~16% (5). In a study of the general population in five European countries, 4.1% were allergic to at least one fragrance substance (30). The overall burden of preservative contact allergy in dermatitis patients has been increasing during recent decades, and was 12% in 2013 in Denmark (31). Although hair dye substances that are strong or extreme

Table 2. Local lymph node assay data of 61 fragrances (55 substances^a and six natural products). Data are based on compilations of data and original publications listed below and in Table S1 in File S1. EC3 values are in %, µg/cm² and M (mol/l) when applicable. Usage in the European baseline series for patch testing (13), functions and use according to the Cosmetics Regulation and harmonized classification as a skin sensitizer (H317) according to the classification, labelling and packaging of substances and mixtures (CLP) regulation^b are indicated

Substance (INCI or other name)	CAS no.	EC3 value ^c			Baseline patch test	Cosmetics Regulation, conditions for use	CLP, concentration limit for H317
		%	M	References			
Hexyl salicylate	6259-76-3	0.18	0.0081	(5, 7)		–	–
Cinnamal	104-55-2	0.20	0.015	(5)	FM I	List	–
Methyl 2-octynoate	111-12-6	0.50	0.032	(5, 7)		0.01% list	–
Isoeugenol	97-54-1	0.54	0.033	(5)	FM I	0.02% list	0.01% ^d
Citral	5392-40-5	1.2	0.079	(5)	FM II	List	1%
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(5)		0.06%	–
Methyl octine carbonate	111-80-8	2.5	0.15	(5, 6, 14)		0.002%	–
Balsam of Peru absolute	8007-00-9	2.5	NA	(5)		0.4%	–
<i>trans</i> -2-Hexenal	6728-26-3	2.6	0.26	(5)		0.002%	–
Benzyl salicylate	118-58-1	2.9	0.13	(5, 7)		List	–
Butylphenyl methylpropional	80-54-6	2.9	0.14	(5)		List	–
Phenylacetaldehyde	122-78-1	3.0	0.25	(5, 6, 15)		–	–
DLimonene (oxidized for 10 weeks) ^a	5989-27-5	3.0	0.22	(5, 16)		List	1%
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(5)		^e	–
Linalyl acetate (oxidized for 10 weeks) ^a	115-95-7	3.6	0.18	(5, 17)		–	–
3-Propylenephthalide	17369-59-4	3.7	0.21	(5, 6, 14)		0.01%	–
Benzylideneacetone	122-57-6	3.7	0.25	(5, 6, 14)		Prohibited	–
<i>Evernia prunastri</i> (oak moss extract)	90028-68-5	3.9	NA	(5)	FM I	List ^f	–
<i>Myroxylon pereirae</i>	8007-00-9	4.0	NA	(5)	x	0.4%	–
Farnesol	4602-84-0	4.1	0.18	(5)	FM II	List	–
<i>p</i> -tert-Butyl-dihydrocinnamal	18127-01-0	4.3	0.23	(5)		0.6%	–
Geraniol (oxidized for 10 weeks) ^a	106-24-1	4.4	0.29	(5, 18)		List	–
α-Methyl cinnamal	101-39-3	4.5	0.31	(5, 6, 19)		–	–
Linalool (oxidized for 10 weeks) ^a	78-70-6	9.4	0.61	(5, 20)		List	1% ^d
Eugenol	97-53-0	5.3	0.32	(5)	FM I	List	–
Hexyl cinnamal	101-86-0	5.3	0.25	(5)	FM II	List	–
Dihydrocoumarin	119-84-6	5.6	0.38	(5, 6)		Prohibited	–
Geraniol	106-24-1	5.6	0.36	(5)	FM I	List	–
Carvone	6485-40-1	5.7	0.38	(5)		–	–
Diethyl maleate	141-05-9	5.8	0.34	(5, 6, 14)		Prohibited	–
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(5, 6)		–	–
Anise alcohol	105-13-5	5.9	0.43	(5, 7)		List	–
Jasmine absolute (<i>J. grandiflorum</i>)	8022-96-6	5.9	NA	(5)		–	–
Dibenzyl ether	103-50-4	6.3	0.32	(5)		–	–
<i>Cananga odorata</i> leaf/flower oil (Ylang Ylang 'extra')	8006-81-3	6.8	NA	(5)		–	–
Iso-cyclocitral	1335-66-6	7.3	0.24	(5)		–	–
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(5, 6, 21)		–	–
Amyl cinnamal	122-40-7	7.6	0.38	(5)	FM I	–	–
Perillaldehyde	2111-75-3	8.1	0.54	(5, 6, 21)		0.1%	–
Vetiveryl acetate	62563-80-8	9.3	0.35	(8)		–	–
<i>p</i> -Isobutyl-α-methyl hydrocinnamal	6658-48-6	9.5	0.46	(5)		–	–
DLimonene (low level of oxidation)	5989-27-5	10	0.73	(5)		List	1%
Methylundecanal	110-41-8	10	0.54	(5, 6, 21)		–	–
Acetylcedrene	32388-55-9	14	0.56	(5, 7)		–	–
Methylenedioxyphenyl methylpropanal	1205-17-0	16	0.85	(5, 7)		–	–
Benzyl benzoate	120-51-4	17	0.80	(5, 6)		–	–
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)	31906-04-4; 51414-25-6	17	0.81	(5, 6, 21)	x; FM II	List ^f	0.1% ^d
Benzyl cinnamate	103-41-3	18	0.77	(5, 7)		List	–
Hydroxycitronellal	107-75-5	19	1.1	(5)	FM I	1.0% list	–
Cinnamyl alcohol	104-54-1	21	1.6	(5, 6)	FM I	List	–

Table 2. Continued

Substance (INCI or other name)	CAS no.	EC3 value ^c			Baseline patch test	Cosmetics Regulation, conditions for use	CLP, concentration limit for H317
		%	M	References			
α -iso-Methylionone	127-51-5	22	1.1	(5, 7)		List	—
Cyclamen aldehyde	103-95-7	22	1.2	(5, 6, 15)		—	—
4-Methoxy- α -methyl benzenopropanal	5462-06-06	24	1.3	(5)		Not in regulation	—
Linalyl acetate (pure)	115-95-7	25	1.3	(5, 17)		—	—
Amyl cinnamyl alcohol	101-85-9	25	1.2	(5, 7)		List	—
Tetramethyl acetyloctahydronaphthalenes	54464-57-2	25	1.1	(5)		—	—
Ethyl acrylate	140-88-5	29	2.9	(5, 6, 22)		Prohibited	1%
Trimethylbenzenepropanol	103694-68-4	30	1.7	(5)		—	—
Linalool	78-70-6	30	1.9	(5, 6, 14)		List	1% ^d
<i>Jasminum sambac</i> flower extract/water	91770-14-8	35	NA	(5)		—	—
Citronellol	106-22-9	44	2.8	(5, 7)	FM II	List	—
Distribution of MW and EC3 values		MW		%		M	
Minimum		98		0.18		0.0081	
Q1		150		3.7		0.22	
Median		172		5.9		0.38	
Mean		178		10		0.61	
Q3		204		17		0.81	
Maximum		304		44		2.9	

—, no restriction by the Cosmetics Regulation or no harmonized classification by CLP; FMI, fragrance mix I ingredient; FMII, fragrance mix II ingredient; list, presence of the substance above certain concentrations must be indicated in the list of ingredients; MW, molecular weight; NA, not applicable; x, substance in the European baseline series.

^aIncluding four oxidized substances and the corresponding non-oxidized substances. Molarity for the oxidized substances was calculated by using the MWs of the non-oxidized substances.

^bClassification and Labelling Inventory (<http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database>, last accessed 30 April 2016).

^cThe dose/area ($\mu\text{g}/\text{cm}^2$) can be calculated by multiplying the concentration (%) by 250 (23).

^dClassification according to opinion by the Risk Assessment Committee and decided by the European Commission; not yet (by 14 June 2016) legally binding (<http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling>, last accessed 14 June 2016).

^eLevel of free allyl alcohol in the ester restricted.

^fThe European Commission has, owing to skin sensitization risk, proposed a ban on the use of chloroatranol and atranol in the natural extracts *Evernia prunastri* and *Evernia furfuracea*, and of hydroxyisohexyl 3-cyclohexene carboxaldehyde; not yet (by 14 June 2016) decided.

sensitizers are very common in consumer hair dye products on the European market, dermatitis patients are generally patch tested with one substance only, namely *p*-phenylenediamine (32–34). Therefore, it is at present not possible to accurately estimate the overall burden of hair dye allergy in dermatitis patients. Information on the overall burden in the general population of allergy to preservatives and hair dyes is similarly limited.

Potency, exposure, and effects

The above results underscore that factors other than the sensitizing potency derived from the LLNA strongly determine the sensitization risk. Factors that appear to be relevant in this context are as follows:

Repeated skin exposure. A large proportion of the population is repeatedly and frequently exposed to products such as cosmetics and household products that generally contain fragrances and preservatives (35–38). Repeated low-dose

exposure has been acknowledged as an important factor for both induction and elicitation of contact allergy (39–42).

Combination effects. Combined exposures to several allergens and also to allergens and irritants may lead to augmented danger signals, and the latter also to increased skin penetration. Consumers are exposed to a multitude of fragrances, preservatives and hair dye substances in different combinations, as shown in assessments of cosmetics on the market (32, 33, 37, 38). Exposure to a mixture of common fragrance allergens in mice augmented both induction and elicitation of contact allergy, as compared with exposure to single allergens (43). In addition, a number of substances that are used frequently in consumer products, such as terpenes and surfactants, are efficient skin penetration enhancers (44).

Prehaptens and prohaptens. Some compounds that are non-sensitizing or that have low sensitizing potency

Table 3. Local lymph node assay data of 19 preservatives (including biocides and antimicrobials). Data are based on compilations of data and original publications listed below and in Table S1 in File S1. EC3 values are in %, µg/cm² and M (mol/l) when applicable. Usage in the European baseline series for patch testing (13), functions and use according to the Cosmetics Regulation and harmonized classification as a skin sensitizer (H317) according to the classification, labelling and packaging of substances and mixtures (CLP) regulation^a are indicated

Substance (INCI or other name) (abbreviation)	CAS no.	EC3 value ^b			Baseline patch test	Cosmetics Regulation, conditions for use	CLP, concentration limit for H317
		%	M	References			
Methylchloroisothiazolinone/methylisothiazolinone 3:1 (MCI/MI, CMIT/MIT)	55965-84-9	0.0030	0.00021	(8)	x	Rinse-off 0.0015%	0.0015%
Chlorothalonil	1897-45-6	0.0039	0.00015	(7, 24)		Prohibited	1%
Methylchloroisothiazolinone (MCI, CMIT)	26172-55-4	0.0090	0.00060	(6)		0.0015% rinse-off	–
3,3',4',5-Tetrachlorosalicylanilide	1154-59-2	0.040	0.0011	(6)		–	–
1-Chloro-2,4-dinitrobenzene (DNCB)	97-00-7	0.050	0.0025	(6)		–	–
Glutaral	111-30-8	0.070	0.0070	(6, 25)		0.1%	Current 0.5%; decided 0.1% ^c
Propiolactone	57-57-8	0.15	0.021	(6)		Prohibited	–
Formaldehyde	50-00-0	0.29	0.10	(8, 26)	x	Oral 0.1%; other 0.2%; nail hardening 5%	0.2%
Methylisothiazolinone (MI, MIT)	2682-20-4	0.40	0.035	(8, 25)	x	0.01% ^d	Current –; decided 0.0015% ^c
Iodopropynyl butylcarbamate (IPBC)	55406-53-6	0.87	0.031	(7, 27)		Rinse-off 0.02%; leave-on 0.01%; deodorants 0.0075%	1%
Methyldibromo glutaronitrile (MDBGN)	35691-65-7	0.90	0.034	(6)	x	– ^e	–
Glyoxal	107-22-2	1.4	0.24	(6, 21)		Antimicrobial 100 mg/kg	1%
Mercaptobenzothiazole (MBT)	149-30-4	1.7	0.10	(6)	x; Mmix	–	1%
Benzisothiazolinone (BIT)	2634-33-5	2.3	0.15	(6)		Antimicrobial	0.05%
Clotrimazole	23593-75-1	4.8	0.14	(6)		Antidandruff; antimicrobial	–
Thiram (disulfiram)	137-26-8	5.2	0.22	(6)	Tmix	Antimicrobial; prohibited	1%
Phenyl benzoate	93-99-2	20	1.0	(6)		0.5%	–
Pentachlorophenol	87-86-5	20	0.75	(6)		Prohibited	–
Imidazolidinyl urea	39236-46-9	24	0.62	(6)		0.6%	–
Distribution of MW and EC3 values		MW		%		M	
Minimum		30		0.0030		0.00015	
Q1		128		0.060		0.0047	
Median		198		0.87		0.035	
Mean		199		4.3		0.18	
Q3		266		3.6		0.18	
Maximum		388		24		1.0	

–, no restriction by the Cosmetics Regulation or no harmonized classification by CLP; Mmix, mercapto mix ingredient; MW, molecular weight; Tmix, thiuram mix ingredient; x, substance in the European baseline series.

^aClassification and Labelling Inventory (<http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database>, last accessed 30 April 2016).

^bThe dose/area (µg/cm²) can be calculated by multiplying the concentration (%) by 250 (23).

^cClassification according to opinion by the Risk Assessment Committee and decided by the European Commission; not yet (by 14 June 2016) legally binding (<http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling>, last accessed 14 June 2016).

^dCurrent restriction of MI 0.01%. The European Commission has proposed 0.0015% for rinse-off products and a ban for leave-on products, owing to skin sensitization risk; not yet (by 14 June 2016) decided.

^ePhased out as a preservative in cosmetic products, owing to skin sensitization risk.

can be transformed into more potent sensitizers by air oxidation (prehaptens) or by metabolic activation in the skin (prohaptens) (45). Many fragrance substances have these properties, sometimes (as in the case of geraniol) acting by both pathways (5, 46). If only non-oxidized forms of these compounds are assessed in the LLNA, their sensitizing potency – after transformation – will be severely underestimated.

Dose. Few fragrance substances are restricted in terms of allowed use concentrations in cosmetics. This is in contrast to preservatives and hair dyes, many of which have use concentration limits in the Cosmetics Regulation (47). Moreover, aggregate exposure may occur via products of different categories. For instance, the hands are exposed not only to hand creams, but also to face creams, body lotions, hair products, liquid soaps,

Table 4. Local lymph node assay data of 59 hair dye substances. Data are based on compilations of data and original publications listed below and in Table S1. EC3 values are in %, µg/cm² and M (mol/l) when applicable. Usage in the European baseline series for patch testing (13), functions and use according to the Cosmetics Regulation and harmonized classification as a skin sensitizer (H317) according to the classification, labelling and packaging of substances and mixtures (CLP) regulation^a are indicated

Substance (INCI or other name) (abbreviation)	CAS no.	EC3 value ^b			Cosmetics Regulation, conditions for use	CLP, concentration limit for H317
		%	M	References		
<i>N</i> -phenyl- <i>p</i> -phenylenediamine	101-54-2	0.020	0.0011	(8)	Oxidative HD 2%	–
4-Nitro- <i>o</i> -phenylenediamine	99-56-9	0.050	0.0033	(7, 8)	Oxidative HD 0.5%	–
<i>p</i> -Phenylenediamine (PPD) ^c	106-50-3	0.060	0.0055	(8)	Oxidative HD 2.0%	1%
3-Nitro- <i>p</i> -hydroxyethylaminophenol	65235-31-6	0.070	0.0035	(7, 8)	Oxidative HD 3.0%; Non-oxidative HD 1.85%	–
1,2,4-Trihydroxybenzene	533-73-3	0.080	0.0063	(8)	Hair dye	–
2,6-Diaminopyridine	141-86-6	0.090	0.0082	(8)	Oxidative HD 0.15%	–
6-Hydroxyindole	2380-86-1	0.13	0.010	(8)	Oxidative HD 0.5%	1%
Dihydroxyindole	3131-52-0	0.17	0.011	(8)	Oxidative HD 0.5%	–
4-Amino-3-nitrophenol	610-81-1	0.20	0.013	(7, 8)	Oxidative HD 1.5%; Non-oxidative HD 1%	–
Toluene-2,5-diamine (PTD; TDA; 2,5-TDA)	95-70-5	0.20	0.016	(6)	Oxidative HD 4.0%	1%
<i>m</i> -Aminophenol	591-27-5	0.24	0.022	(8)	Oxidative HD 1.2%	–
<i>N,N</i> -bis(2-hydroxyethyl)- <i>p</i> -phenylenediamine sulfate	54381-16-7	0.25	0.0080	(8)	Hair dye 2.5%	–
Toluene-2,5-diamine sulfate	615-50-9	0.31	0.014	(8, 28)	Oxidative HD 4.0%	1%
2-Methyl-5-hydroxyethylaminophenol	55302-96-0	0.40	0.024	(6)	Oxidative HD 1.5%	–
<i>o</i> -Aminophenol	95-55-6	0.40	0.037	(6)	Prohibited	–
4-Amino-2-hydroxytoluene	2835-95-2	0.44	0.036	(8)	Oxidative HD 1.5%	–
Hydroxyethyl-3,4-methylenedioxyaniline HCl	94158-14-2	0.50	0.023	(8)	Oxidative HD 1.5%	–
Hydroxyethyl- <i>p</i> -phenylenediamine sulfate	93841-25-9	0.57	0.023	(8)	Oxidative HD 2.0%	–
2-Amino-6-chloro-4-nitrophenol	6358-09-4	0.68	0.036	(8)	Oxidative HD 2.0%; Non-oxidative HD 2.0%	–
HC Yellow No. 17	1450801-55-4	0.70	0.0078	(8)	–	–
<i>p</i> -Methylaminophenol sulfate	55-55-0	0.80	0.036	(6)	Oxidative HD 0.68%	1%
HC Violet No. 1	82576-75-8	0.90	0.043	(8)	Oxidative HD 0.25%; Non-oxidative HD 0.28%	–
Phenyl methyl pyrazolone	89-25-8	1.0	0.057	(8)	Oxidative HD 0.25%	–
Isatin	91-56-5	1.0	0.068	(8)	Non-oxidative HD 1.6%	–
Basic Blue 124	67846-56-4	1.1	0.036	(8)	–	–
HC Orange No. 2	85765-48-6	1.1	0.046	(8)	Non-oxidative HD 1.0%	–
HC Red No. 7	24905-87-1	1.2	0.061	(8)	Non-oxidative HD 1.0%	–
2,6-Dimethoxy-3,5-pyridinediamine HCl	56216-28-5	1.3	0.052	(8)	Oxidative HD 0.25%	–
2-Methyl-1-naphthol	7469-77-4	1.3	0.081	(8)	Oxidative HD 2.0%	–
1-Naphthol	90-15-3	1.3	0.090	(6, 8)	Oxidative HD 2.0%	–
Resorcinol	108-46-3	1.4	0.13	(8)	Oxidative HD 1.25%	–
4-Amino- <i>m</i> -cresol	2835-99-6	1.5	0.12	(7, 8)	Oxidative HD 1.5%	–
6-Amino- <i>m</i> -cresol	2835-98-5	1.6	0.13	(8)	–	–
Hydroxyethoxy aminopyrazolopyridine HCl	1079221-49-0	1.9	0.083	(8)	–	–
HC Red No. 1	2784-89-6	2.0	0.087	(8)	Non-oxidative HD 1.0%	–
Acid Black 1	1064-48-8	2.1	0.034	(8)	Non-oxidative HD 0.5%	–
HC Red No. 3	2871-01-4	2.2	0.11	(7, 8)	Oxidative HD 0.45%; Non-oxidative HD 3.0%	–
1,5-Naphthalenediol	83-56-7	2.5	0.16	(8)	Oxidative HD 1.0%; Non-oxidative HD 1.0%	–
2-Chloro-6-ethylamino-4-nitrophenol	131657-78-8	2.8	0.13	(8)	Oxidative HD 3.0%; Non-oxidative HD 3.0%	1%
2,7-Naphthalenediol	582-17-2	2.8	0.17	(8)	Oxidative HD 1.0%; Non-oxidative HD 1.0%	–

Table 4. Continued

Substance (INCI or other name) (abbreviation)	CAS no.	EC3 value ^b			Cosmetics Regulation, conditions for use	CLP, concentration limit for H317
		%	M	References		
Basic Orange 31	97404-02-9	3.1	0.12	(8)	Oxidative HD	–
					Non-oxidative HD 1.0%	
2,4-Diaminophenoxyethanol HCl	66422-95-5	3.2	0.13	(8)	Oxidative HD 2.0%	–
Disperse Violet 1	128-95-0	3.8	0.16	(8)	Non-oxidative HD 0.5%	–
HC Blue No. 12	132885-85-9	5.0	0.16	(8)	Oxidative HD 0.75%	1%
					Non-oxidative HD 1.5%	
HC Blue No. 2	33229-34-4	5.0	0.18	(8)	Non-oxidative HD 2.8%	–
HC Red No. 16	160219-76-1	5.0	0.26	(8)	Prohibited	–
6-Methoxy-2-methylamino-3-aminopyridine HCl	90817-34-8	5.6	0.37	(8)	Oxidative HD 1.0%	–
					Non-oxidative HD 1.0%	
4-Chlororesorcinol	95-88-5	5.8	0.40	(8)	Oxidative HD 2.5%	–
HC Violet No. 2	104226-19-9	5.9	0.20	(8)	Non-oxidative HD 2.0%	1%
2-Amino-5-ethylphenol HCl	149861-22-3	6.6	0.38	(8)	–	–
Sodium picramate	831-52-7	6.7	0.30	(8)	Oxidative HD 0.6%	–
					Non-oxidative HD 0.6%	
2-Methoxy-methyl- <i>p</i> -phenylenediamine sulfate	337906-37-3	7.1	0.28	(8)	Oxidative HD 1.8%	–
HC Red No. 13	94158-13-1	8.2	0.30	(8)	Oxidative HD 1.25%	–
					Non-oxidative HD 2.5%	
1-Hexyl-4,5-diaminopyrazole dihydrochloride	1361000-06-7	8.6	0.34	(8)	–	–
Basic Brown 16	26381-41-9	12	0.34	(8)	–	–
1,3-Bis-(2,4-diaminophenoxy)propane HCl	74918-21-1	15	0.34	(7, 8)	Oxidative HD 1.2%	–
3-Amino-2,4-dichlorophenol HCl	61693-43-4	17	0.78	(8)	Oxidative HD 1.5%	–
					Non-oxidative HD 1.5%	
2,6-Dihydroxy-3,4-dimethylpyridine	84540-47-6	25	1.8	(8)	Oxidative HD 1.0%	–
2-Methylresorcinol	608-25-3	50	4.0	(8)	Oxidative HD 1.8%	–
					Non-oxidative HD 1.8%	
Distribution of MW and EC3 values		MW		%		M
Minimum		108		0.020		0.0011
Q1		146		0.42		0.023
Median		197		1.3		0.081
Mean		217		4.0		0.22
Q3		242		5		0.18
Maximum		903		50		4.0

–, no restriction by the Cosmetics Regulation or no harmonized classification by CLP; HD, hair dye product; MW, molecular weight; x, substance in the European baseline series.

^aClassification and Labelling Inventory (<http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database>, last accessed 30 April 2016).

^bThe dose/area ($\mu\text{g}/\text{cm}^2$) can be calculated by multiplying the concentration (%) by 250 (23).

^cUsed in the European Baseline Series.

household products, etc., through application of these with the hands. Although some fragrances are subject to self-regulation in terms of International Fragrance Association standards (48), the high contact allergy prevalence suggests that some of these standards are not sufficiently protective, and that there may be significant limitations in the risk assessment approach presently used. The quantitative risk assessment (QRA) approach used by industry has been critically reviewed by the SCCP (49). Prompted by the issues raised, attempts are ongoing to improve the QRA, for example by incorporating cocktail and aggregate exposures (37).

EC3 values and their significance

A molar EC3 value (M) is generally more accurate than a percentage value when the sensitizing potencies of

substances are compared. The sensitizing potencies of large molecules will be underestimated as compared with low-MW compounds with the EC3 (%). Expressing the EC3 values in M instead of % did not notably affect the relative potencies when the three groups of substances in this review were compared, as their overall MWs are similar. However, the current OECD guideline and EC regulations are based on EC3 (%), and this is also the case regarding potency categorization of sensitizers (2). Therefore, it is of relevance to report both EC3 (M) and EC3 (%).

The EC3 values in this and many other compilations of data are based on the lowest value from studies following the OECD guideline (5–8). EC3 values are often based on results from single studies only, whereas some are based on several studies; the latter may be considered to be more

reliable. However, because of animal welfare considerations, only one experiment is usually conducted, beyond substances that have been subjected to initial validation studies with the LLNA or are used as recommended positive controls. It is also well known that LLNA results for a substance may vary significantly between experiments with different vehicles (5, 10, 25). EC3 values should thus be regarded as indicative of the sensitizing potency, rather than providing an absolute measure.

It should also be remembered that the LLNA studies the induction of skin sensitization, and not the elicitation that results in disease, that is, allergic contact dermatitis. It is well known that individuals with contact allergy may develop allergic contact dermatitis after exposure to very low doses of skin sensitizers, regardless of their sensitizing potency. This has been shown repeatedly by dose–response studies, and may be expressed as the dose level required to elicit allergic contact dermatitis in 10% of sensitized individuals (ED_{10}) (50).

All substances in this study generated an EC3 value. Thus, they fulfil the CLP criteria for classification as skin sensitizer (H317). Only few of these sensitizers currently have a legally binding harmonized classification, although several are restricted and some are prohibited in cosmetic products. Classification and labelling of health hazards, including skin sensitization, may provide important information to industry, workers, and consumers, and could contribute to safe use concentrations in chemical products. However, this requires the concentration limits triggering classification of mixtures to be low enough to generate information on the presence of skin-sensitizing substances in products, which is often not the case.

The preservatives methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) and MI may serve as examples of the possible impact on actual use concentrations of concentration limits for classification. MCI/MI has, since 2001, been classified as skin sensitizer

at 0.0015%. MI does not yet have a legally binding classification, but 0.0015% was decided on in 2016 (Table 3). In a recent study on isothiazolinones in paints, the maximum concentration of MCI was 0.0011%, and that of MI was 0.018% (51). It will be of interest to monitor how rapidly the concentration of MI will fall to just below the recently decided concentration limit for classification.

Conclusions

Potency information based on EC3 values in the LLNA is valuable for the prevention of contact allergy. The results presented in this review, however, clearly show that one cannot focus solely on the strong and extreme skin sensitizers. Many compounds categorized as moderate sensitizers (EC3 value of >2%) are also well-recognized contact allergens causing significant clinical problems. Factors coupled to exposure need to be addressed more thoroughly when the sensitization risks of fragrances and other skin sensitizers are assessed. Currently, large gaps between identified sensitization hazards, risks and measures for effective risk management can be noted.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Conversion of EC3 values.

Table S1. Opinions by the SCCS and its predecessors. Detailed information on the publication numbers of the most recent opinions that mention the EC3 values in Tables 2–4, and other SCCS opinions and memoranda.

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