

Ranking of hair dye substances according to predicted sensitization potency: quantitative structure–activity relationships

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Allergic contact dermatitis following the use of hair dyes is well known. Many chemicals are used in hair dyes and it is unlikely that all cases of hair dye allergy can be diagnosed by means of patch testing with *p*-phenylenediamine (PPD). The objectives of this study are to identify all hair dye substances registered in Europe and to provide their tonnage data. The sensitization potential of each substance was then estimated by using a quantitative structure–activity relationship (QSAR) model and the substances were ranked according to their predicted potency. A cluster analysis was performed in order to help select a number of chemically diverse hair dye substances that could be used in subsequent clinical work. Various information sources, including the Inventory of Cosmetics Ingredients, new regulations on cosmetics, data on total use and ChemId (the Chemical Search Input website provided by the National Library of Medicine), were used in order to identify the names and structures of the hair dyes. A QSAR model, developed with the help of experimental local lymph node assay data and topological sub-structural molecular descriptors (TOPS-MODE), was used in order to predict the likely sensitization potential. Predictions for sensitization potential were made for the 229 substances that could be identified by means of a chemical structure, the majority of these hair dyes (75%) being predicted to be strong/moderate sensitizers. Only 22% were predicted to be weak sensitizers and 3% were predicted to be extremely weak or non-sensitizing. Eight of the most widely used hair dye substances were predicted to be strong/moderate sensitizers, including PPD – which is the most commonly used hair dye allergy marker in patch testing. A cluster analysis by using TOPS-MODE descriptors as inputs helped us group the hair dye substances according to their chemical similarity. This would facilitate the selection of potential substances for clinical patch testing. A patch-test series with potent, frequently used, substances representing various chemical clusters is suggested. This may prove useful in diagnosing PPD-negative patients with symptoms of hair dye allergy and would provide some clinical validation of the QSAR predictions.

Key words: aminophenol; 4-amino-2-hydroxytoluene; 4-amino-3-nitrophenol; contact sensitization; hair dyes; PPD; *p*-phenylenediamine; QSAR; resorcinol; toluene-2,5-diamine. © Blackwell Munksgaard, 2004.

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Clinical experience shows that hair dyes can cause severe allergic contact reactions (1) and the marker and main patch-test substance in diagnosing hair dye allergy is *p*-phenylenediamine (PPD) (2–9). Patients with clinical hair dye dermatitis and negative patch test to PPD, but positive patch test to other hair dye ingredients, have been described (10, 11). Qualitative as well as quantitative information about the composition of hair dyes is critical of identifying possible allergens for risk assessment and provides a basis for selecting

appropriate patch-test materials for the individual patient. Despite ingredient labelling being a requirement for hair dyes (12), there is still no clear understanding of which substances are used in hair dyes nor any systematic assessment of their potential for contact sensitization.

Skin-sensitizing chemicals cause allergic contact dermatitis via a number of biochemical and physiological events. The induction of contact allergy involves the interaction of a hapten (a low-molecular-weight molecule) with a protein

to form an antigenic complex. Correlations between the readiness of chemicals to react with proteins in order to form covalently linked conjugates and their skin-sensitization ability form the basis for quantitative structure–activity relationship (QSAR) models (13). There are many approaches to developing QSARs for skin sensitization. One approach to establishing SARs is the relative alkylation index (RAI), a mathematical model derived by Roberts and Williams (14), based on electrophilicity and hydrophobicity parameters, as well as the dose of chemical. This RAI model has been used in order to evaluate data on various sets of skin-sensitizing chemicals, including sultones, *p*-nitrobenzyl halides, acrylates, sulfonate esters and substituted gamma butyrolactones (15, 16). Other approaches include the development of empirical QSARs by means of the application of statistical methods to sets of biological data and structural descriptors (17, 18). Despite the complexities and the still-limited understanding of some of the processes leading to skin sensitization, it is possible to describe some of the relationships between chemical structures and the ability to form covalent conjugates with proteins. This knowledge, which relates chemical structure to a specific end point, can be programmed into expert systems, such as Deductive Estimation of Risk from Existing Knowledge (DEREK) (19, 20).

For recent reviews on skin-sensitization QSAR models, the reader is directed to Rodford et al. (21) and Smith Pease et al. (16). The QSAR model applied in this work was developed by Estrada et al. in 2003 (13), relating topological sub-structural molecular descriptors (TOPS-MODE) to experimental data derived from the local lymph node assay (LLNA). This model was chosen, as it was a general model and not restricted to 1 chemical class. It was readily accessible and could provide an estimate of likely potency. The model was developed by using a selection of 93 diverse chemicals tested in the LLNA to the same protocol. The details of the LLNA protocol and the explanations of EC3 (Elicitation Concentration) derivations have been described in numerous publications and reviews (22–24). The model categorizes potency into 1 of 3 bands: strong/moderate, weak or extremely weak/non-sensitizing (13). These classification bands, although broad, are a consequence of the historical data used in order to develop the model and to reflect how relatively few strong sensitizers or extremely weak sensitizers were present in the data set. The classifications of strong, moderate and weak are based on studies where LLNA potency

data have been compared and have been correlated with human potency information (24). The aim of the present study is to collect information on all hair dye substances used in permanent or temporary hair dyes in Europe and then to rank these substances according to their estimated potency, the perspective being to supplement the current diagnostic work-up for hair dye allergy with new potential allergens.

Materials

The work undertaken can be described as follows. A list of hair dye substances that were used in Europe was compiled, together with potency and tonnage levels. Predictions for sensitization potency were made and a literature search was subsequently performed, which highlighted where the QSAR model performed particularly well or poorly.

Identification of hair dye substances

A list of hair dye ingredients was compiled from 3 main sources:

- The Inventory of Cosmetics Ingredients (INCI) found on The European Commissions homepage (25). This list covers 261 hair dye substances.
- European Commission Directive 26 on the approximation of the laws of the member states relating to cosmetic products (26). This is a list of 61 newly regulated hair dye substances.
- Provisional quantitative list of hair dyes used in hair colouring products on the European market year 2002, a list of 89 substances (27).

Inevitably there was considerable overlap between these sources. This was most particularly observed by differences in nomenclature, a single hair substance being described by several names, which needed to be resolved first in order to generate a single comprehensive list of all hair dye substances in Europe. The starting point was to identify each substance by means of a chemical structure and then to eliminate duplicates. Each substance name was identified by searching on several publicly available data sources, including ChemIDplus (28) and Chemfinder (29). Substances that were described by means of IUPAC chemical names were drawn with the help of Chemdraw Ultra (Version 6, Cambridgesoft, CA, USA) or were derived with the help of the Autonom program within Chemdraw Ultra; they were then cross-checked with one of these public sources. The resulting list contained 315 substances.

Methods

Prediction of sensitization potency

Structures identified were then imported into a molecular spreadsheet TSAR (Version 3.3, Accelrys Ltd., Cambridge, UK). Simplified Molecular Input Line Entry Specifications (SMILES, www.daylight.com), which are 1-dimensional representations of chemical structures, were generated. This list was filtered in order to remove any further duplicate structures, such as salt-containing ingredients (HCl and SO₄), leaving 229 hair dye substances.

Ranking the substances according to their sensitization potential

The TOPS-MODE QSAR model (13) was used in order to estimate the likely sensitization potency in 1 of 3 bands:

- strong/moderate sensitizers,
- weak sensitizers and
- extremely weak or non-sensitizers.

In addition to a qualitative potency, the predicted output provides a likelihood of a substance belonging to that group. The higher the number, the more likely a substance belongs to that category. The criteria for the ranking in the 3 categories of sensitization was described in more detail by Estrada et al. in 2003 (13). The predicted sensitization potency numbers represent an arbitrary relative ranking scheme, which makes it possible to compare substances within the same group.

Tonnage amount

The European Cosmetic Toiletry and Perfumery Association has generated tonnage data for use in the EU Commission for prioritizing hair dyes in risk assessment and risk management (27).

The tonnage data are given as an additional information.

Cluster analysis

The cluster analysis provided a means of grouping substances according to their chemical properties such that a representative diverse subset could be selected for further work. As little is understood about the chemical drivers or the specific mechanisms of hair dye substances allergy, the approach to calculating as much chemical information about these hair dye substances as possible should encompass those features important in sensitization. TOPS-MODE descriptors accounting for hydrophobi-

city, molar refractivity, polarizability, charges, polar surface area, molecular weight and van der Waals radii were calculated for the 229 substances. The algorithm applied was that of K-means, as implemented in STATISTICA (Version 6., Statsoft Inc., Tulsa, Oklahoma, USA). The algorithm was set to define 10 clusters, as 10 substances would be a reasonable number on which to focus any further work. Each cluster contained other 'similar' chemicals. In this case, similarity is in the context of the descriptors selected, rather than similarity in their likely sensitization response, although, if we have captured those descriptors that are relevant for sensitization; then, it is plausible that the sensitization mechanisms for chemicals within a class might be the same. K-means starts with k random clusters (in this case 10), and then moves substances between those clusters with the goal of minimizing variability within clusters and maximizing variability between clusters.

Literature review

LLNA data. A literature search for all the identified hair dye substances was performed with the help of the Medline data base (1966 to 21 July 2003) by using 'Local Lymph Node Assay' as the key phrase. 161 articles were retrieved.

Human evidence. A literature search was performed with the help of Medline data base from 1966 to 8 August 2003 by using 'contact dermatitis and hair dye(s)', 'contact allergy and hair dye(s)', 'sensitization and hair dye(s)' and 'sensitization' and hair dye(s)' as key phrases. 133 articles were retrieved. The human evidence was accepted, if a positive patch test to the specific allergen was found in persons with concrete or possible exposure to hair dyes.

Results

The predicted sensitization potentials of the 229 hair dye substances have been listed with their INCI names in Tables 1–3. Where substances were salts, predictions were made only for the non-salts. The substances predicted to belong to the strong/moderate sensitizer class have been listed in Table 1. About 75% of all the hair dyes were predicted to be strong/moderate sensitizers (172/229). PPD, which is included in the European standard patch-test series as a screening agent for hair dye allergy, was predicted to be a strong/moderate sensitizer with a predicted sensitization potency value of 1.8. Other substances were predicted to be far more potent. Direct Red 80 was predicted to be the most potent

Table 1. Strong/moderate sensitizers

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount used in oxidative hair-colouring products†	Tonnage amount used in direct hair-colouring products†	Cluster number‡
1	1,2,4-Benzenetriacetate	613-03-6	1.8			2
2	1,2,4-Trihydroxybenzene	533-73-3	2.8	0.1		6
3	1,3-Bis-(2,4-diaminophenoxy)propane	74918-21-1	1.1	2		8
4	1,5-Naphthalenediol	83-56-7	1.9	1		6
5	1,7-Naphthalenediol	575-38-2	1.7	0.1		6
6	1-b-hydroxyethyl-2,4-diaminobenzene	14572-93-1	1.3			8
7	1-Hydroxy-3-amino-2,4-dichlorobenzene	61693-43-4	2.0			5
8	1-Hydroxyethyl-4,5-diaminopyrazole sulfate	155601-30-2	1.7	9		8
9	1-Methyl-2,6-diaminobenzene	823-40-5	0.5			8
10	1-Naphthol	90-15-3	1.4	6		6
11	2,3-Naphthalenediol	92-44-4	1.6	1		6
12	2,4,5,6-Tetraaminopyridine	1004-74-6	3.2	5		8
13	2,4-Diamino-5-methylphenoxylethanol HCl	113715-27-8; 141614-05-3 615-05-4	0.3			8
14	2,4-Diaminoanisole		0.3			8
15	2,4-Diaminodiphenylamine		0.4			8
16	2,4-Diaminophenol§	95-86-3	2.3			8
17	2,4-Diaminophenoxylethanol HCl	66422-95-5	1.8	9		8
18	2,4-Diaminotoluene	95-80-7	0.3			8
19	2,5,6-Triamino-4-pyrimidinol sulfate	1603-02-7	4.8	1		8
20	2,5-Diaminoanisole	5307-02-08	0.3			8
21	2,6-Bis(2-hydroxyethoxy)-3,5-pyridinediamine HCl	117907-42-3	2.9			8
22	2,6-Diaminopyridine	141-86-6	2.6	0.1		8
23	2,6-Dihydroxy-3,4-dimethylpyridine	84540-47-6	0.3	1		6
24	2,6-Dihydroxy-4-methylpyridine		1.6			6
25	2,7-Naphthalenediol§	582-17-2	1.4	3		6
26	2-Amino-3-hydroxypyridine	16867-03-1	2.4	4		5
27	2-Amino-3-nitrophenol	603-85-0	3.6			2
28	2-Amino-4-nitrophenol	99-57-0	3.1			2
29	2-Amino-5-nitrophenol	121-88-0	3.1			2
30	2-Amino-6-chloro-4-nitrophenol	6358-09-04	3.1	4	1	2
31	2-Aminomethyl- <i>p</i> -aminophenol HCl§	79352-72-0	1.6	1		8
32	2-Chloro-5-nitro-N-hydroxyethyl- <i>p</i> -phenylenediamine	50610-28-1	2.7			2
33	2-Chloro-6-ethylamino-4-nitrophenol	131657-78-8	1.2			6
34	2-Chloro- <i>p</i> -phenylenediamine§	615-66-7	1.6	0.1		8
35	2-Hydroxyethylamino-5-nitroanisole	66095-81-6	2.6			6
36	2-Methoxymethyl- <i>p</i> -aminophenol HCl		0.2			5
37	2-Methyl-5-hydroxyethylaminophenol	55302-96-0	0.3	30		6
38	2-Methylresorcinol	608-25-3	1.1	30		6
39	2-Nitro-5-glyceryl methylaniline		0.2			5
40	2-Nitro-N-hydroxyethyl- <i>p</i> -anisidine		1.1			6
41	2-Nitro- <i>p</i> -phenylenediamine§	5307-14-2	3.1	1	0.1	8
42	3,4-Diaminobenzoic acid	619-05-6	2.8			8
43	3,4-Methylenedioxyaniline		0.5			5
44	3,4-Methylenedioxyphenol		0.7			6
45	3-Methylamino-4-nitrophenoxylethanol	59820-63-2	1.1			6
46	3-Nitro-4-aminophenoxylethanol	50982-74-6	2.8			2

Table 1. Continued

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount used in oxidative hair-colouring products†	Tonnage amount used in direct hair-colouring products†	Cluster number‡
47	3-Nitro- <i>p</i> -cresol	2042-14-0	1.6			6
48	3-Nitro- <i>p</i> -hydroxyethylaminophenol	65235-31-6	3.2	1	3	5
49	Hydroxyanthraquinoneaminopropyl methyl morpholinium methosulfate	38866-20-5	0.3			7
50	4,4'-Diaminodiphenylamine		0.1			8
51	4,6-Bis(2-hydroxyethoxy)- <i>m</i> -phenylenediamine HCl	94082-85-6	1.8			8
52	4-Amino-2-hydroxytoluene	2835-95-2	0.6	70		5
53	4-Amino-2-nitrodiphenylamine-2'-carboxylic acid		2.7	1		2
54	4-Amino-2-nitrophenol	119-34-6	3.4			2
55	4-Amino-3-nitrophenol§	610-81-1	3.4	2	1	2
56	4-Amino- <i>m</i> -cresol	2835-99-6	0.5	20		5
57	4-Chlororesorcinol	95-88-5	2.2	7		6
58	4-Ethoxy- <i>m</i> -phenylenediamine	5862-77-1	0.1			8
59	4-Hydroxyindole		0.8			6
60	4-Hydroxypropylamino-3-nitrophenol	92952-81-3	0.7	2	3	5
61	4-Nitro- <i>m</i> -phenylenediamine	5131-58-8	3.1			8
62	4-Nitro- <i>o</i> -phenylenediamine	99-56-9	2.8	1		8
63	4-Nitrophenyl aminoethylurea	27080-42-8	2.0	1	1	2
64	5-Amino-2,6-dimethoxy-3-hydroxypyridine		0.2			5
65	5-Amino-6-chloro- <i>o</i> -cresol		0.7	1		5
66	6-Amino- <i>m</i> -cresol	2835-98-5	0.6	1		5
67	6-Amino- <i>o</i> -cresol	17672-22-9	0.8			5
68	6-Hydroxyindole		0.5	1		6
69	6-Methoxy-2,3-pyridinediamine HCl		1.2			8
70	6-Nitro-2,5-pyridinediamine	69825-83-8	4.0			8
71	6-Nitro- <i>o</i> -toluidine	570-24-1	1.6			2
72	Acid Black 1	1064-48-8	9.6			1
73	Acid Blue 62	4368-56-3	1.1			1
74	Acid Blue 74	860-22-0	3.5			1
75	Acid Brown 13	6373-79-1	4.6			9
76	Acid Orange 24	1320-07-6	3.5			4
77	Acid Orange 3	6373-74-6	3.1		0.1	1
78	Acid Orange 6	547-57-9	5.2	0.1		4
79	Acid Orange 7	633-96-5	4.4			4
80	Acid Red 14	3567-69-9	6.6			1
81	Acid Red 18	2611-82-7	8.7			9
82	Acid Red 27	915-67-3	8.6			9
83	Acid Red 33 (CI 17200)	3567-66-6	7.3			1
84	Acid Red 35	6441-93-6	3.5			1
85	Acid Red 73	5413-75-2	7.6			1
86	Acid Red 87	17372-87-1	4.7			7
87	Acid Violet 43	4430-18-6	0.9	0.1	2	1
88	Acid Yellow 1	846-70-8	6.2			1
89	Acid Yellow 23	1934-21-0	6.6			1
90	Acid Yellow 3	8004-92-0	2.9			1
91	Acid Yellow 36	587-98-4	2.8			4
92	Basic Blue 41	12270-13-2	0.5			4

Table 1. Continued

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount used in oxidative hair-colouring products†	Tonnage amount used in direct hair-colouring products†	Cluster number‡
93	Basic Blue 99§	68123-13-7	2.0			7
94	Basic Brown 16	26381-41-9	1.0			4
95	Basic Brown 17	68391-32-2	2.3			7
96	Basic Violet 14	632-99-5	0.5	0.1	0.1	2
97	Brilliant Black 1	2519-30-4	9.6			9
98	C.I. Mordant Black 1	3618-58-4	5.1			1
99	Curry Red	25956-17-6	3.6			1
100	Dihydroxyindole	5638-85-7	2.2	0.1		6
101	Direct Black 51	3442-21-5	6.9			1
102	Direct Red 23	3441-14-3	5.9			9
103	Direct Red 80	2610-10-08	16.3			9
104	Direct Red 81	2610-11-09	6.4			1
105	Direct Yellow 12	2870-32-8	3.7			1
106	Disperse Black 9	20721-50-0	2.3			2
107	Disperse Blue 1	2475-45-8	2.2			8
108	Disperse Blue 7	3179-90-6	2.4			4
109	Disperse Brown 1§	23355-64-8	3.0			4
110	Disperse Orange 3§	730-40-5	3.2			2
111	Disperse Red 11	2872-48-2	0.1			2
112	Disperse Red 15	116-85-8	1.8			4
113	Disperse Violet 1	128-95-0	1.5	0.1	2	2
114	HC Blue 2	33229-34-4	2.8	1	9	2
115	HC Blue 10	173994-75-7	0.3			2
116	HC Blue 11	23920-15-2	1.2			2
117	HC Brown 1	83803-98-9	3.1			7
118	HC Brown 2	83803-99-0	1.9			7
119	HC Green 1	52136-25-1	0.8			4
120	HC Orange 1	54381-08-7	1.7			6
121	HC Orange 2	85765-48-6	2.6			2
122	HC Red 1	2784-89-6	1.5			2
123	HC Red 3	2871-01-04	2.9	1	4	2
124	HC Red 7	24905-87-1	2.8			2
125	HC Red 13	94158-13-1	3.0			2
126	HC Violet 1	82576-75-8	1.4			2
127	HC Yellow 10	109023-83-8	2.5			2
128	HC Yellow 2	4926-55-0	2.9	1	1	6
129	HC Yellow 4	59820-43-8	2.4	0.1	0.1	5
130	HC Yellow 5	56932-44-6	2.6	1	1	2
131	HC Yellow 6	104333-00-8	3.7	0.1	1	4
132	Hydroquinone§	123-31-9	2.3	1		6
133	Hydroxybenzomorpholine	977067-94-9;	0.4	1	6	
		26021-57-8				
134	Hydroxyethyl-2,6-dinitro- <i>p</i> -anisidine	12252-11-3	2.7			2
135	Hydroxyethyl-2-nitro- <i>p</i> -toluidine	100418-33-5	1.2	1	1	6
136	Hydroxyethyl-3,4-methylenedioxylaniline HCl	94158-14-2	0.2		1	6
137	Hydroxyethyl- <i>p</i> -phenylenediamine sulfate	93841-25-9	1.3	2		8
138	Lawson	83-72-7	2.1			6

Table 1. Continued

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount used in oxidative hair-colouring products†	Tonnage amount used in direct hair-colouring products‡	Cluster number‡
139	<i>m</i> -Aminophenol§	591-27-5	2.1	30		5
140	<i>m</i> -Phenylenediamine§	108-45-2	1.8	2		8
141	<i>N,N'</i> -Bis(2-hydroxyethyl)-2-nitro- <i>p</i> -phenylenediamine	84041-77-0	2.6	0.1	1	5
142	<i>N,N</i> -bis(2-hydroxyethyl)- <i>p</i> -phenylenediamine	54381-16-7	1.6	5		5
143	Nitromethane	75-52-5	1.6			6
144	<i>N</i> -Methyl-3-nitro- <i>p</i> -phenylenediamine		1.3			2
145	<i>N</i> -Phenyl- <i>p</i> -phenylenediamine§	101-54-2	0.1	1		5
146	<i>o</i> -Aminophenol§	95-55-6	2.3	6		5
147	<i>o</i> -Phenylenediamine§	95-54-5	2.0			8
148	<i>p</i> -Aminophenol§	123-30-8	2.0	50		5
149	<i>p</i> -Phenylenediamine§	106-50-3; 624-18-0; 16245-77-5	1.8	80		8
150	Phloroglucinol	108-73-6	2.7			6
151	Picramic acid	96-91-3	4.6	2	0.1	2
152	Pigment Orange 5	3468-63-1	4.8			4
153	Pigment Red 4	2814-77-9	3.2			4
154	Pigment Red 48	3564-21-4	4.4			1
155	Pigment Red 57	5858-81-1	4.6			1
156	Pigment Red 64:1	6371-76-2	6.1			7
157	<i>p</i> -Methylaminophenol	150-75-4	0.2	4		6
158	Ponceau SX (CI 14700)	4548-53-2	4.0			1
159	Resorcinol§	108-46-3	2.3	90		6
160	Sodium picramate	831-52-7	4.5			8
161	Solvent Green 7	6358-69-6	7.0			9
162	Solvent Orange 1	2051-85-6	3.3			6
163	Solvent Red 1§	1229-55-6	1.1			4
164	Solvent Red 23	85-86-9	3.2			4
165	Solvent Red 3	6535-42-8	0.5			4
166	Solvent Red 43	15086-94-9	7.4			3
167	Solvent Red 48	13473-26-2	7.5			3
168	Solvent Red 72	596-03-2	5.6			3
169	Sunset Yellow	2783-94-0	6.0			1
170	Toluene-2,5-diamine§	95-70-5	0.3	100		8
171	Toluene-3,4-diamine	496-72-0	0.3	1		8
172	Vat Red 1	2379-74-0	0.9			7

*Substances in this table are predicted as strong or moderate sensitizers. The higher the number the stronger the predicted sensitization potency. The numbers are arbitrary and express the substances in terms of potential to sensitize within the group.

†The tonnage amount is data from the hair dye industry, showing the amount of a given substance used in the year 2002. Blank means no data.

‡The cluster number exists from 1 to 10 and represents arbitrary chemical spaces, in which substances with similar physical-chemical properties can be grouped in.

§See Table 4 for clinical reports on human contact dermatitis or local lymph node assay (LLNA) data on mice.

INCI, Inventory of Cosmetics Ingredients.

Table 2. Weak sensitizers

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount /number used in oxidative hair-colouring products†	Tonnage amount /number used in direct hair-colouring products†	Cluster number‡
173	2,4-Diamino-5-methylphenetol HCl	113715-25-6	5.1			8
174	2-Amino-4-hydroxyethyl-aminoaniso	83763-47-7	6.6	5		5
175	4,5-Diamino-1-methylpyrazole HCl		0.3			8
176	4-Methoxytoluene-2,5-diamine HCl		3.9			8
177	Acid Blue 1	129-17-9	36.4			1
178	Acid Blue 9	2650-18-2	61.7			9
179	Acid Green 25	4403-90-1	43.5			1
180	Acid Green 50	3087-16-9	41.3			1
181	Acid Red 51	16423-68-0	11.3			10
182	Acid Red 52	3520-42-1	32.8			1
183	Acid Violet 9	6252-76-2	37.5			1
184	Basic Blue 26 (CI 44045)	2580-56-5	18.4			7
185	Basic Blue 6	966-62-1	6.8			4
186	Basic Blue 7	2390-60-5	15.9	0.1	1	7
187	Basic Blue 9	61-73-4	6.9	0.1	0.1	4
188	Basic Brown 4	4482-25-1	19.7			8
189	Basic Green 1	633-03-4	15.1			4
190	Basic Red 2	477-73-6	19.1	1	0.1	4
191	Basic Red 76	68391-30-0	9.6	0.1	2	4
192	Basic Violet 2		23.6			2
193	Basic Violet 3	548-62-9 (467-63-0-64070-98-2)	10.4			4
194	Basic Yellow 57	68391-31-1	12.7			4
195	CI 42640	1694-09-3	49.4			1
196	Dihydroxyindoline		13.0	1		6
197	Disperse Blue 3	2475-46-9	12.5			4
198	Disperse Violet 4	1220-94-6	14.0	1		4
199	Fast Green FCF	2353-45-9	65.1			9
200	HC Blue 8	22366-99-0	12.60			4
201	HC Red 9	56330-88-2	7.6			2
202	HC Yellow 8	66612-11-1	9.1			4
203	Hydroxyethylaminomethyl- <i>p</i> -aminophenol HCl	110952-46-0	12.6			5
204	N,N-diethyl- <i>m</i> -aminophenol		2.5			6
205	N,N'-Dimethyl-N-hydroxyethyl-3-nitro- <i>p</i> -phenylenediamine		7.1			6
206	N,N-Dimethyl- <i>p</i> -phenylenediamine		1.2	0.1		5
207	Phenyl methylpyrazolone	89-25-8	4.8	20		9
208	Pigment Blue 15	147-14-8	11.7			7
209	Pigment Red 112	6535-46-2	21.1			7
210	Pigment Red 5	6410-41-9	16.6			7
211	Pigment Violet 19	1047-16-1	17.8			4
212	Pigment Violet 23	6358-30-1	15.9			7
213	Pigment Yellow 12	6358-85-6	27.2			7
214	Pigment Yellow 13	5102-83-0	26.2			7
215	Pigment Yellow 73	13515-40-7	17.9			4
216	Solvent Black 3	4197-25-5	3.0			7
217	Solvent Blue 35	12769-17-4	13.9			4
218	Solvent Green 3	128-80-3	19.8			7
219	Solvent Violet 13	81-48-1	21.9			4
220	Solvent Yellow 29	6706-82-7	25.9			7
221	Solvent Yellow 33	8003-22-3	27.3			4
222	Solvent Yellow 44	2478-20-8	14.3			4
223	Thymol	89-83-8	4.2			6

51 substances are classified as weak sensitizers. See Table 4 for clinical reports on human contact dermatitis or local lymph node assay (LLNA) data on mice.

*Substances in this table are predicted as strong or moderate sensitizers. The higher the number the stronger the predicted sensitization potency. The numbers are arbitrary and express the substances in terms of potential to sensitize within the group.

†The tonnage amount is data from the hair dye industry, showing the amount of a given substance used in the year 2002. Blank means no data.

‡The cluster number exists from 1 to 10 and represents arbitrary chemical spaces, in which substances with similar physical-chemical properties can be grouped in.

Table 3. Extremely weak sensitizers or non-sensitizers

Number	INCI name	Case number	Predicted sensitization potency*	Tonnage amount /number used in oxidative hair-colouring products†	Tonnage amount /number used in direct hair-colouring products†	Cluster number‡
224	2,6-Dimethoxy-3,5-pyridinediamine	56216-28-5	-3.9	1		8
225	2-Hydroxyethyl picramic acid	99610-72-7	-3.9	1	1	8
226	Basic Red 22§	12221-52-2	-8.6	0.1	0.1	6
227	Basic Yellow 11	4208-80-4	-10.7	0.1		7
228	N,N-Dimethyl 2,6-pyridinediamine HCl		-4.2			5
229	N-Methoxyethyl-p-phenylenediamine HCl		-2.4			5

6 substances are classified as extremely weak sensitizers or nonsensitizers.

*Substances in this table are predicted as strong or moderate sensitizers. The higher the number the stronger the predicted sensitization potency. The numbers are arbitrary and express the substances in terms of potential to sensitize within the group.

†The tonnage amount is data from the hair dye industry, showing the amount of a given substance used in the year 2002. Blank means no data.

‡The cluster number exists from 1 to 10 and represents arbitrary chemical spaces, in which substances with similar physical-chemical properties can be grouped in.

§See Table 4 for clinical reports on human contact dermatitis or local lymph node assay (LLNA) data on mice.

INCI, Inventory of Cosmetics Ingredients.

sensitizer (predicted sensitization potency = 16.3), but it is not listed as a substance that was frequently used in hair dyes in 2002. Table 2 contains the substances that were predicted to be weak sensitizers, which included 22% (51/229) of all the substances. Table 3 shows that 3% (6/229) of the hair dye substances were predicted to be extremely weak or non-sensitizing. The 229 substances were divided into 10 clusters, each covering between 1 and 40 substances (Tables 1–3). The cluster number is given in the right-hand column. Chemicals with similar structural information are in the same cluster.

Many substances had little available data; of the 229 substances investigated, only 21 substances could be identified as allergens based on human evidence and/or LLNA data (Table 4).

Table 5 depicts 28 substances that were predicted to be strong/moderate sensitizers and which are used in excess of 2 tonnes per annum. These substances are listed according to their cluster numbers, such that substances from various clusters could be chosen in the development of a new patch-test series. Substances from only 4 clusters are used over 2 tonnes and are predicted to be strong/moderate sensitizers; 8 have been reported as clinical contact allergens. Despite an extensive search to identify commercial suppliers for these 28 substances, only 5 substances were found to be commercially available as patch-test materials.

Discussion

A list of all hair dye substances and their tonnage per annum has been collected for the first time.

Chemical structures were identified for 229 substances and predictions were made by means of a published QSAR model (13). While the model had not been developed specifically for hair dye substances, it provides a means of estimating the likely sensitization potential. In total, 75% (172/229) of the substances were predicted to be strong or moderate sensitizers.

The most well-known hair dye allergen is PPD. This was first described as a strong allergen in 1939 (2), is included in the standard patch-test series and is considered the best indicator of hair dye allergy (30). PPD was predicted to be a strong/moderate allergen with the help of the TOPS-MODE QSAR model (13). This is in accordance with clinical experience (1,3–9,30–49), results from the LLNA (22–24,50–57) and predictive testing in both humans (58) and guinea pigs (51). 80 tons of PPD is used annually and it is allowed at up to 6% in hair dyes (12). In Tables 1–3, the predicted sensitization potency is expressed by a number, the higher the number the greater the likelihood of belonging to that sensitization category. PPD is more likely to be a strong/moderate sensitizer than toluene-2,5-diamine, because it has a higher number. Both are in the same cluster (no. 8), and cross-reactivity is expected (9).

A literature study was undertaken in order to determine whether data were available that would either validate predictions or highlight areas of improvement required for the QSAR model. LLNA has been validated with human induction data published elsewhere (22, 53, 55). The LLNA data found in the literature supported the

Table 4. Literature overview of human evidence and local lymph node assay (LLNA) data on contact sensitization and hair dyes

Number	INCI name	LLNA	Human evidence based on patch-test data
Strong or moderate sensitizers			
16	2,4-Diaminophenol		1 case clinical relevant hair dye reaction (1).
25	2,7-Naphthalenediol		1 case clinical relevant hair dye reaction (64).
31	2-Aminomethyl- <i>p</i> -aminophenol HCL		1 case clinical relevant hair dye reaction (65).
34	2-Chloro- <i>p</i> -phenylenediamine		1 case clinical relevant eyelashes eyebrows dye reaction (35).
41	2-Nitro- <i>p</i> -phenylenediamine		1 case clinical relevant (66). 1 case clinical relevant eyelashes eyebrows dye reaction (35). A dentist has reactions on dyed hair from dental patients' hair (67). 2 hairdressers (68). 2 persons clinical relevant hair dye reactions (3). 4 hairdressers out of 103 tested (45). 5 cases clinical relevant hair dye reactions (1). 6 hairdressers out of 40 (9). 12 hairdressers' clients (5). 24 hairdressers (6). 93 patients out of 5202 (7).
55	4-Amino-3-nitrophenol		1 case clinical relevant hair dye reaction (10).
93	Basic Blue 99		1 case clinical relevant hair dye reaction (10,11).
109	Disperse Brown 1		8 patients in a trial (30).
110	Disperse Orange 3		1 case clinical relevant hair dye reaction (1,48,69). 3 persons clinical relevant hair dye reactions (3). 29 patients in a trial (30).
132	Hydroquinone	Positive (51–53,56,70,71)	1 case clinical relevant eyelashes eyebrows dye reaction (72).
139	<i>m</i> -Aminophenol	Positive (23,50–53,56)	2 cases of clinical relevant hair dye reactions at hair dressers' clients (34). 3 cases clinical relevant hair dye reactions (1).
140	<i>m</i> -Phenylenediamine	Positive (23,50,53,56)	
145	N-Phenyl- <i>p</i> -phenylenediamine		4 patients in a trial (30).
146	<i>o</i> -Aminophenol	Positive (23,50,53,56)	1 beautician out of four tested (39).
147	<i>o</i> -Phenylenediamine	Positive (23)	
148	<i>p</i> -Aminophenol		1 case occupational (39). 2 cases of clinical relevant hair dye reactions at hair dressers' clients (34). 3 cases (1). 5 patients in a trial (30).
149	<i>p</i> -Phenylenediamine	LLNA potency class Strong (22–24,50–57)	1 patient clinical relevant contact dermatitis (CD) to hair dye (32,33,37,43,46,48). A baby gets CD played with mother's dyed hair (40). 1 case CD and depigmentation to a 16% PPD hair dye (31). 1 case CD to partner's hair dye (47). 1 case clinical relevant eyelashes eyebrows dye reaction (35). 1 case report occupational allergic to hair dye (38). 2 cases of clinical relevant hair dye reactions at hair dressers' clients (34). 4 cases of clinical relevant hair dye reactions (42). 5 patients in a trial (30). 6 hairdressers out of 103 tested (45). 8 hairdressers with CD (68). 8 cases of beard dermatitis (36). 8 patients telogen effluvium induced by CD to hair dyes (44). 12 occupational CD beauticians (39). 13 occupational dermatitis in hairdressers (41). 15 patients consecutive adult patients (73). 16 persons (1). Seventeen CD patients out of 107 (49). 19 hairdressers' clients (5). 28 patients out of 363 tested (8). 30 people clinical relevant hair dye reactions (3). 38 hairdressers out of 40 (9). 667 occupational patients (4). 73 hairdressers (6). 374 patients out of 5202 (7).
159	Resorcinol	Negative (22,23,52,53,56)	1 clinical relevant reaction to hair dye (59). 1 patient telogen effluvium induced by CD to hair dyes (44). 1 hairdresser client with CD (5). 4 hairdressers (6).
163	Solvent Red 1		11 patients out of 5202 (7). 1 case clinical relevant reaction to brilliantine (74).
170	Toluene-2,5-diamine		1 case clinical relevant hair dye dermatitis (37). 1 case clinical relevant eyelashes eyebrows dye reaction (35). 1 patient clinical relevant CD to hair dye (43). 1 patient telogen effluvium induced by CD to hair dyes (44). 1 case report occupational allergic to hair dye (38). 2 hairdressers with CD (68). 2 cases of clinical relevant hair dye reactions at hair dressers' clients (34).

Extremely weak or-non-sensitizing
226 Basic Red 22

2 hairdressers out of 103 tested (45).
3 occupational dermatitis in hairdressers (41).
7 persons with allergic CD to hair dyes (1).
7 cases occupational (39).
8 men with beard dermatitis (36). 12 hair dressers' clients with CD (5). 12 patients open test (3).
15 hairdressers out of 40 (9). 15 cases (30).
40 hairdressers (6). 81 patients out of 5202 (7).
1 patient clinical relevant CD to hair dye (69).

Blank means No data.

predicted potency for 6 of the substances, and for 19 substances, evidence of sensitization in humans was found. Only resorcinol was identified as a strong/moderate sensitizer in TOPS-MODE, whereas it was a non-allergen in the LLNA (22, 23, 52, 53, 56). Clinical experience showed that resorcinol occasionally sensitizes, and clinical reactions could be strong when they were found (5–7, 44, 59). In the prediction, resorcinol was a strong/moderate sensitizer and ranked higher

than PPD. The differences in the frequency of sensitization between PPD and resorcinol might be explained by means of concentration differences in the residue after the hair dye coupling reaction. Another parameter that at least theoretically may play a role is skin metabolism, which might either detoxify potential allergens or create allergens (60, 61). However, resorcinol seems to be substantially less potent than PPD in humans. There may be interspecies differences that could

Table 5. Proposed list of commonly used hair dye ingredients for patch testing of hairdressers and patients with suspected hair dye dermatitis.

INCI name	Cluster number of chemical properties	Tonnage amount used in hair-colouring products
Acid Violet 43	1	2.1
2-Amino-6-chloro-4-nitrophenol	2	5
4-Amino-3-nitrophenol*	2	3
Disperse Violet 1	2	2.1
HC Red 3	2	5.0
HC Blue 2	2	10
Picramic acid	2	2.1
2-Amino-3-hydroxypyridine	5	4
3-Nitro- <i>p</i> -hydroxyethylaminophenol	5	4
4-Amino-2-hydroxytoluene	5	70
4-Amino- <i>m</i> -cresol	5	20
4-Hydroxypropylamino-3-nitrophenol	5	5
<i>m</i> -Aminophenol*†	5	30
N,N-bis(2-hydroxyethyl)- <i>p</i> -phenylenediamine	5	5
<i>o</i> -Aminophenol*	5	6
<i>p</i> -Aminophenol*†	5	50
1-Naphthol	6	6
2,7-Naphthalenediol*	6	3
2-Methyl-5-hydroxyethylaminophenol	6	30
2-Methylresorcinol	6	30
4-Chlororesorcinol	6	7
<i>p</i> -Methylaminophenol	6	4
Resorcinol*†	6	90
1-Hydroxyethyl-4,5-diaminopyrazole sulfate	8	9
2,4,5,6-Tetraaminopyridine	8	5
2,4-Diaminophenoxyethanol HCl	8	9
<i>p</i> -Phenylenediamine*†	8	80
Toluene-2,5-diamine*†	8	100

The substances are selected because of their predicted sensitizing potential in the QSAR model and usage of more than two tonnes in hair dye products in 2002.

10 clusters are available; only 5 clusters are represented in the table, because the substances in cluster 3, 4, 7 and 9 are highly potent, but they are not used. Cluster 10 does not contain any strong/moderate sensitizers.

*Reported as clinical contact allergens (see Table 4).

†Commercially available from Chemotechnique Diagnostics, Malmö, Sweden.

also explain the differences between predictions based on animal data and clinical experience in special cases. Genetic polymorphism in skin metabolism of PPD has been found (62), and it may be that some people are at higher risk of skin sensitization simply because of interindividual variations in skin enzyme activity. This is an area for future investigation (63).

A cluster analysis was performed grouping the substances according to chemical similarity based on TOPS-MODE descriptors. This enabled a diverse selection of substances to be considered for clinical work. Testing patients with substances selected as representatives of each cluster could provide clinical validation of the QSAR analysis.

The current analysis has elucidated that a number of hair dye substances are predicted to be strong/moderate sensitizers, even stronger than PPD. They are also used in relatively high quantities, which means that the population has the highest total exposure to these, but they are not currently used for diagnosing contact allergy to hair dyes (Table 5). Toluene-2,5-diamine, *p*-aminophenol and *m*-aminophenol are all available as patch-test preparations. While the following commonly used substances, 4-amino-2-hydroxytoluene, 2-methyl-5-hydroxyethylaminophenol and 2-methylresorcinol, are not standard allergens, they might be relevant for patch testing in the context of suspected hair dye allergy. The commonly used 4-amino-3-nitrophenol has been described as patch-test-positive in a PPD-negative patient (10), but it is not routinely used for patch testing in patients, who has been exposed to hair dyes.

These new insights may form the basis for developing a new hair dye screening series, which, in turn, may improve the diagnosis of contact allergy to hair dyes. Correct and early diagnosis is an important tool in secondary and tertiary prevention. Future work could include patch testing with all 28 substances in Table 5 in order to investigate whether substances within a cluster cross-react and/or whether their mechanisms of action are the same. In conclusion, an overview of the substances used in hair dyes and a systematic assessment of their potential for contact sensitization by using a QSAR model have been given. About 229 hair dye substances were identified, the majority of these (75%) being predicted to be strong/moderate sensitizers. Among these, 28 substances were used in high tonnage and had a high predicted sensitization potential, some of them even higher than PPD. Only PPD is already routine for patch-testing patients with suspected hair dye allergy. Based on the results, a list of commonly used substances with high

predicted potency is proposed for consideration in relation to improving the diagnostic work-up of hair dye allergy cases.

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