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Patch testing with the European baseline series and 10 added allergens: Single-centre study of 748 patients

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Abstract

Background: The European baseline series (EBS) of contact allergens is subject to change. An allergen is considered for inclusion when routine patch testing of patients with suspected contact dermatitis results in ≥0.5% prevalence rate.

Objectives: We aimed to determine the frequency of sensitizations to 30 EBS allergens and 10 locally added allergens. Additionally, we assessed the strength and evolution of reactions to all tested allergens and co-reactivity of additional allergens.

Methods: Patch testing with our baseline series of 40 allergens was done in 748 consecutive adults. Tests were applied to the upper back and removed by patients after 48 h. Readings were done on Day 3 (D3) and D6 or D7 (D6/7). Positive reactions fulfilled the criteria of at least one plus (+) reaction. A retrospective analysis was done.

Results: Eight allergens not listed in the EBS had ≥0.5% prevalence rate (i.e., cocamidopropyl betaine, thiomersal, disperse blue mix 106/124, 2-bromo-2-nitropropane-1,3-diol, diazolidinyl urea, propylene glycol, Compositae mix II and dexamethasone-21-phosphate), and 16.6% of positive reactions would have been missed without D6/7 readings.

Conclusion: We propose further studies to evaluate whether cocamidopropyl betaine, disperse blue mix 106/124, 2-bromo-2-nitropropane-1,3-diol, diazolidinyl urea and Compositae mix II need to be added to the EBS.

KEYWORDS
baseline series, clinical epidemiology, contact sensitization, patch testing, simultaneous reactivity

1 | INTRODUCTION

The European baseline series (EBS) of contact allergens is used as a diagnostic screening tool in patients with suspected allergic contact dermatitis and it is subject to change to capture changes in exposure

Abbreviations: Aq., water; CI, confidence interval; Conc., concentration; D3, Day 3; D6/7, Day 6 or 7; EBS, European baseline series; ESSCA, European Surveillance System of Contact Allergies; FR, formaldehyde releaser; ICDRG, International Contact Dermatitis Research Group; IQR, interquartile range; NACDG, North American Contact Dermatitis Group; Neg., negative; Pos., positive.

to environmental allergens.[1] The ESCD recommends patch test readings at: (a) Day 2 (D2), D3 or D4 and around D7 as optimal, and (b) D3 or D4 and around D7 as a fair alternative.[2] An allergen is considered for inclusion in the EBS when routine ('consecutive') patch testing of patients with suspected contact dermatitis results in at least 0.5% prevalence rate,[1,3,4] and when this particular allergen is ubiquitous and/or clinically highly relevant.[2,5]

We aimed to determine the frequency of sensitizations to 10 contact allergens not included in the 2015[2] and 2019[6] versions of the EBS (i.e., cocamidopropyl betaine, thiomersal, disperse blue mix 106/124, 2-bromo-2-nitropropane-1,3-diol, diazolidinyl urea, imidazolidinyl urea, Compositae mix II, propylene glycol, dexamethasone-21-phosphate and cetearyl alcohol) in consecutive patients with suspected contact dermatitis. Additionally, we aimed to determine: (a) the usefulness of late readings on D6 or D7, the strength of patch test reactions and the evolution of reactions between both readings (i.e., 'decrescendo', 'plateau', 'crescendo') for all 40 allergens, and (b) patterns of simultaneous patch test reactions (co-reactivity) to 10 additional allergens.

Exposure to *cocamidopropyl betaine* occurs via rinse-off products (e.g., liquid soaps, shampoos) and via leave-on products (e.g., deodorants).[7] The inorganic mercurial *thiomersal* has a broad spectrum of antimicrobial properties and may still be used as a preservative in vaccines and topical products for eyes and ears.[8,9] For years, it has been on the list of the most common contact sensitizers, but with great difficulties in finding clinical relevance of positive tests.[4,9–11] It was taken out of the German baseline series in 2001.[12] *Disperse blue mix 106/124* contains two commonest textile dye allergens in concentrations of 0.5%. It is included in the British baseline series[13] and North American Contact Dermatitis Group (NACDG) baseline series,[14] but not in the EBS. Textile dye mix 6.6% pet. from the EBS contains disperse blue 106 and 142 in lower concentrations of 0.3%. *Quaternium-15*, *2-bromo-2-nitropropane-1,3-diol*, *diazolidinyl urea* and *imidazolidinyl urea* are formaldehyde releasers (FRs).[1] Contact allergic reactions to FRs can be directed against the released formaldehyde, against the substance itself, or both.[1] The current EBS 2019 includes only formaldehyde 2% aq. and quaternium-15 1% pet.[6] Whitehouse et al. (2020) reported that patch testing with formaldehyde 2% aq. is an inadequate screen to identify independent contact sensitization to FRs and suggested to add FRs currently used in cosmetics to the EBS.[1] Sensitization to *Compositae mix II* allergens may occur in private or in occupational settings (e.g., cooks and florists).[15] They may also be present in emollients, even for atopic children. Plants of the Compositae (Asteraceae) family are responsible for the majority of diagnosed type IV sensitization to phytochemicals in Europe.[15,16] *Propylene glycol* is used as a solvent, a vehicle for topical medicaments such as corticosteroids or acyclovir, and an emulsifier and humectant in food and cosmetics. It is included in the NACDG baseline series.[14] Patch tests to propylene glycol are sometimes irritant.[7] *Dexamethasone* may be used to test steroid sensitivity. *Cetearyl alcohol* 20% pet. evoked positive patch test reactions in ≤1% of consecutively tested patients and was thus described as a borderline component of baseline series.[17]

2 | METHODS

Patch testing with our baseline series of 40 allergens was done in 748 consecutive (unselected) adult patients with suspected allergic contact dermatitis who were evaluated at our tertiary referral centre from 23 January 2019 to 30 October 2021. Their median age was 45 years (IQR 32–59), 61.5% were aged ≥40 years and 73.5% were female. Our series of allergens in this time period contained: (a) 29 allergens listed in the 2015 EBS panel[2] marked with † in Table 1 (clioquinol 5% pet. was not tested due to low, 0.6% [4/691] positivity in past tests); (b) 28 allergens listed in the 2019 EBS panel[5] marked with ‡ in Table 1 (2-hydroxyethyl methacrylate 2% pet. and caine mix 10% pet. were not tested); and (c) 10 allergens not included in the EBS but expected to be widely distributed in the patients' environment: (a) seven cosmetics allergens (i.e., four preservatives: 2-bromo-2-nitropropane-1,3-diol 0.5% pet. [bronopol; CAS no. 52-51-7], diazolidinyl urea 2% pet. [CAS no. 78491-02-8], imidazolidinyl urea 2% pet. [CAS no. 39236-46-9], thiomersal 0.1% pet. [thimerosal; CAS no. 54-64-8]; cocamidopropyl betaine 1% aq. [CAS no. 61789-40-0], propylene glycol 30% aq. [CAS no. 57-55-6], cetearyl alcohol 20% pet. [CAS no. 8005-44-5]); (b) dye/colourant disperse blue mix 106/124 1% pet. (CAS no. 12223-01-7, 15 141-18-1); (c) plant allergens of the Compositae mix 5% pet.; and (d) a corticosteroid dexamethasone-21-phosphate 1% pet. (CAS no. 2392-39-4).

The substances were provided by Chemotechnique and allergEAZE (SmartPractice) (Table 1). Patch tests with square patch test allergEAZE chambers were prepared at our department and applied to the upper back for 48 h. Patients were asked to remove the patches after that time. They were also instructed to re-mark the borders of patches before removing them. Readings were done by physicians on Day 3 (D3) and D6 or D7 (D6/7). Procedures were in accordance with the current Helsinki Declaration. All patients gave written informed consent.

Results were evaluated according to recommendations of the International Contact Dermatitis Research Group (ICDRG) as doubtful (?+), weak positive (+), strong positive (++), extreme positive (+++) and irritant (IR).[2] Positive reactions fulfilled the criteria of at least one plus (+) reaction on D3 and/or D6/7. The term 'patch test reactivity' was also used for such reactions. We also analysed evolution of positive reactions from D3 to D6/7. 'Decrescendo' was defined as a decrease in their strength (+++, ++, +, ?+ or 0), 'plateau' as unaltered morphology (+++, +++; ++, ++; +, +), and 'crescendo' as an increase in the strength of reactions (0 or ?+, +, ++, +++).

Data were routinely collected in an electronic databank. IBM SPSS software version 25 was used for analysis. Descriptive measures included frequencies, proportions and medians with the first and third quartile range. The Pearson chi-square test was used to detect statistically significant co-reactivity of 10 additional allergens, differences in the frequency of contact sensitizations to these allergens based on gender and differences in the frequency of contact sensitizations to these allergens based on age groups (i.e., ≥40 or <40 years). The Mann–Whitney *U* test was used to detect differences in the frequency of contact sensitizations to these allergens based on age

TABLE 1 Results of patch testing with our extended European baseline series (EBS) of contact allergens

No.	Patch test allergen	Conc. (%)	Pos. reactions[a] *n* (% of tested; 95% CI)	Reading criteria of the ICDRG[2,b] Doubt. (?+) *n* (% of pos.)	Weak pos. (+) *n* (% of pos.)	Strong pos. (++) *n* (% of pos.)	Extreme pos. (+++) *n* (% of pos.)	Irritant (IR) *n* (% of tested)	Only late (D6/7) pos. reactions[c] *n* (% of pos.)	Evolution of pos. reactions[d] Decrescendo (↓) *n* (% of pos.)	Plateau (↔) *n* (% of pos.)	Crescendo (↑) *n* (% of pos.)
1	Nickel sulfate[f]	5.0[†‡]	142 (19.0; 16.2–22.0)	0	47 (33.1)	49 (34.5)	46 (32.4)	4 (0.5)	25 (17.6)	35 (24.6)	62 (43.7)	45 (31.7)
2	Potassium dichromate[f]	0.5[†‡]	72 (9.6; 7.6–12.0)	3 (0.4)	42 (58.3)	17 (23.6)	13 (18.1)	4 (0.5)	11 (15.2)	28 (38.9)	26 (36.1)	18 (25.0)
3	Methyldibromo glutaronitrile[f]	0.5[†‡]	69 (9.2; 7.2–11.5)	10 (1.3)	55 (79.7)	11 (15.9)	3 (4.3)	10 (1.3)	4 (5.8)	48 (69.6)	13 (18.8)	8 (11.6)
4	*Myroxylon pereirae* (balsam of Peru)[f]	25.0[†‡]	61 (8.2; 6.3–10.3)	23 (3.1)	44 (72.1)	13 (21.3)	4 (6.6)	2 (0.3)	6 (9.8)	54 (88.5)	1 (1.6)	6 (9.8)
5	Fragrance mix I (FMI)[e,Mix1]	8.0[†‡]	53 (7.1; 5.4–9.2)	1 (0.1)	29 (54.7)	16 (30.2)	8 (15.1)	1 (0.1)	8 (15.1)	22 (41.5)	19 (35.8)	12 (22.6)
6	**Cocamidopropyl betaine[e]**	**1.0 aq.**	**50 (6.7; 5.0–8.7)**	**39 (5.2)**	**48 (96.0)**	**2 (4.0)**	**0**	**8 (1.1)**	**2 (4.0)**	**46 (92.0)**	**2 (4.0)**	**2 (4.0)**
7	Methylchloroisothiazolinone (MCI, 150 ppm) and methylisothiazolinone (MI, 50 ppm)[f]	0.02 aq.[†‡]	44 (5.9; 4.3–7.8)	14 (1.9)	20 (45.5)	8 (18.2)	16 (36.4)	4 (0.5)	9 (20.5)	17 (38.6)	11 (25.0)	16 (36.4)
8	Textile dye mix[e,Mix2]	6.6[†‡]	43 (5.7; 4.2–7.7)	26 (3.5)	27 (62.8)	7 (16.3)	9 (20.9)	5 (0.7)	9 (20.9)	21 (48.8)	7 (16.3)	15 (34.9)
9	Formaldehyde[e]	2.0 aq.[†‡]	42 (5.6; 4.1–7.5)	0	26 (61.9)	11 (26.2)	5 (11.9)	4 (0.5)	5 (20.5)	18 (42.9)	12 (28.6)	12 (28.6)
10	Colophony (colophonium)[f]	20.0[†‡]	38 (5.1; 3.6–6.9)	0	8 (21.1)	14 (36.8)	16 (42.1)	2 (0.3)	3 (7.9)	6 (15.8)	23 (60.5)	9 (23.7)
11	Fragrance mix II (FMII)[f,Mix3]	14.0[†‡]	32 (4.3; 2.9–6.0)	2 (0.3)	18 (56.3)	9 (28.1)	5 (15.6)	3 (0.4)	1 (3.1)	12 (37.5)	17 (53.1)	3 (9.4)
12	Methylisothiazolinone (MI)[f]	0.2 aq.[†‡]	30 (4.0; 2.7–5.7)	2 (0.3)	11 (36.7)	8 (26.7)	11 (36.7)	2 (0.3)	6 (20.0)	7 (23.3)	11 (36.7)	12 (40.0)
13	Propolis[e]	10.0[‡]	30 (4.0; 2.7–5.7)	0	22 (73.3)	6 (20.0)	2 (6.7)	2 (0.3)	7 (23.3)	17 (56.7)	3 (10.0)	10 (33.3)
14	**Thiomersal (thimerosal)[f]**	**0.1**	**27 (3.6; 2.4–5.2)**	**0**	**15 (55.6)**	**11 (40.7)**	**1 (3.7)**	**0**	**12 (44.4)**	**3 (11.1)**	**7 (25.9)**	**17 (63.0)**
15	Cobalt chloride[e]	1.0[†‡]	27 (3.6; 2.4–5.2)	7 (0.9)	14 (51.9)	10 (37.0)	3 (11.1)	1 (0.1)	1 (3.7)	9 (33.3)	13 (48.1)	5 (18.5)
16	**Disperse blue mix 106/124[e,Mix4]**	**1.0**	**27 (3.6; 2.4–5.2)**	**24 (3.2)**	**19 (70.4)**	**6 (22.2)**	**2 (7.4)**	**5 (0.7)**	**9 (33.3)**	**12 (44.4)**	**4 (14.8)**	**11 (40.7)**
17	Lanolin alcohols (wool alcohols)[f]	30.0[†‡]	23 (3.1; 2.0–4.6)	12 (1.6)	21 (91.3)	1 (4.3)	1 (4.3)	0	3 (13.0)	16 (69.6)	3 (13.0)	4 (17.4)
18	p-Phenylenediamine (free base)[f]	1.0[†‡]	22 (2.9; 1.9–4.4)	4 (0.5)	8 (36.4)	3 (13.6)	11 (50.0)	0	3 (13.6)	8 (36.4)	10 (45.5)	4 (18.2)
19	Neomycin sulfate[e]	20.0[†‡]	21 (2.8; 1.8–4.3)	6 (0.8)	13 (61.9)	7 (33.3)	1 (4.8)	0	11 (52.4)	2 (9.5)	5 (23.8)	14 (66.7)
20	**2-bromo-2-nitropropane-1,3-diol (bronopol)[e,g]**	**0.5**	**20 (2.7; 1.6–4.1)**	**5 (0.7)**	**14 (70.0)**	**5 (25.0)**	**1 (5.0)**	**1 (0.1)**	**1 (5.0)**	**11 (55.0)**	**5 (25.0)**	**4 (20.0)**
21	**Propylene glycol[e]**	**30.0 aq.**	**19 (2.5; 1.5–3.9)**	**19 (2.5)**	**16 (84.2)**	**3 (15.8)**	**0**	**1 (0.1)**	**1 (5.3)**	**16 (84.2)**	**1 (5.3)**	**2 (10.5)**
22	Epoxy resin[f]	1.0[†‡]	12 (1.6; 0.8–2.8)	7 (0.9)	4 (33.3)	7 (58.3)	1 (8.3)	1 (0.1)	3 (25.0)	2 (16.7)	3 (25.0)	7 (58.3)
23	Budesonide[f]	0.01[†‡]	12 (1.6; 0.8–2.8)	12 (1.6)	8 (66.7)	1 (8.3)	3 (25.0)	2 (0.3)	3 (25.0)	8 (66.7)	1 (8.3)	3 (25.0)
24	Benzocaine[e]	5.0[†]	11 (1.5; 0.7–2.6)	4 (0.5)	7 (63.6)	4 (36.4)	0	1 (0.1)	1 (9.1)	9 (81.8)	1 (9.1)	1 (9.1)
24	Thiuram mix[f,Mix5]	1.0[†‡]	11 (1.5; 0.7–2.6)	1 (0.1)	6 (54.5)	2 (18.2)	3 (27.3)	0	2 (18.2)	2 (18.2)	5 (45.5)	4 (36.4)
26	Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICCI)[f]	5.0[†‡]	9 (1.2; 0.5–2.2)	2 (0.3)	4 (44.4)	2 (22.2)	3 (33.3)	1 (0.1)	1 (11.1)	3 (33.3)	3 (33.3)	3 (33.3)

(Continues)

TABLE 1 (Continued)

No.	Patch test allergen	Conc. (%)	Pos. reactions[a] *n* (% of tested; 95% CI)	Reading criteria of the ICDRG[2,b] Doubt. (?+) *n* (% of pos.)	Weak pos. (+) *n* (% of pos.)	Strong pos. (++) *n* (% of pos.)	Extreme pos. (+++) *n* (% of pos.)	Irritant (IR) *n* (% of tested)	Only late (D6/7) pos. reactions[c] *n* (% of pos.)	Evolution of pos. reactions[d] Decrescendo (↓) *n* (% of pos.)	Plateau (↔) *n* (% of pos.)	Crescendo (↑) *n* (% of pos.)
27	Primin (2-Methoxy-6-n-pentyl-4-benzoquinone)[e]	0.01†	8 (1.1; 0.5–2.1)	11 (1.5)	4 (50.0)	1 (12.5)	3 (37.5)	4 (0.5)	4 (50.0)	3 (37.5)	1 (12.5)	4 (50.0)
28	**Compositae mix II**[e,Mix6]	**5.0**	**6 (0.8; 0.3–1.7)**	**3 (0.4)**	**4 (66.7)**	**1 (16.7)**	**1 (16.7)**	**1 (0.1)**	**2 (33.3)**	**1 (16.7)**	**2 (33.3)**	**3 (50.0)**
29	*N*-isopropyl-*N*′-phenyl-p-phenylenediamine (IPPD)[e]	0.1†‡	6 (0.8; 0.3–1.7)	8 (1.1)	4 (66.7)	0	2 (33.3)	2 (0.3)	1 (16.7)	2 (33.3)	2 (33.3)	2 (33.3)
30	Sesquiterpene lactone mix[e,Mix7]	0.1†‡	6 (0.8; 0.3–1.7)	1 (0.1)	5 (83.3)	1 (16.7)	0	0	2 (33.3)	3 (50.0)	1 (16.7)	2 (33.3)
31	4-*tert*-Butylphenol formaldehyde resin[e]	1.0†‡	5 (0.7; 0.2–1.5)	4 (0.5)	1 (20.0)	3 (60.0)	1 (20.0)	1 (0.1)	2 (40.0)	1 (20.0)	1 (20.0)	3 (60.0)
32	**Diazolidinyl urea**[e,g]	**2.0**	**5 (0.7; 0.2–1.5)**	**3 (0.4)**	**4 (80.0)**	**1 (20.0)**	**0**	**1 (0.1)**	**3 (60.0)**	**1 (20.0)**	**0**	**4 (80.0)**
33	**Dexamethasone-21-phosphate**[e]	**1.0**	**5 (0.7; 0.2–1.5)**	**8 (1.1)**	**3 (60.0)**	**2 (40.0)**	**0**	**0**	**1 (20.0)**	**3 (60.0)**	**0**	**2 (40.0)**
34	Quaternium-15[f,g]	1.0†‡	4 (0.5; 0.2–1.4)	2 (0.3)	3 (72.0)	1 (25.0)	0	0	0	4 (100)	0	0
35	Mercaptobenzothiazole[f]	2.0†‡	4 (0.5; 0.2–1.4)	5 (0.7)	2 (50.0)	2 (50.0)	0	1 (0.1)	2 (50.0)	2 (50.0)	0	2 (50.0)
36	**Imidazolidinyl urea**[e,g]	**2.0**	**3 (0.4; 0.1–1.2)**	**0**	**2 (66.7)**	**1 (33.3)**	**0**	**2 (0.3)**	**2 (66.7)**	**0**	**0**	**3 (100)**
37	Mercapto mix[e,Mix8]	2.0†‡	2 (0.3; 0.0–1.0)	4 (0.5)	2 (100)	0	0	1 (0.1)	1 (50.0)	1 (50.0)	0	1 (50.0)
38	Tixocortol pivalate[f]	0.1†‡	2 (0.3; 0.0–1.0)	6 (0.8)	2 (100)	0	0	2 (0.3)	0	1 (50.0)	1 (50.0)	0
39	Paraben mix[f,Mix9]	16.0†‡	1 (0.1; 0.0–0.7)	3 (0.4)	0	1 (100)	0		0	0	1 (100)	0
40	**Cetearyl alcohol**[e]	**20.0**	**1 (0.1; 0.0–0.7)**	**0**	**1 (100)**	**0**	**0**		**0**	**1 (100)**	**0**	**0**

Note: Substance with the highest frequency of positive reactions is shown first. Vehicle was petrolatum unless indicated otherwise. Reading criteria of the ICDRG were used: ?+ doubtful; + weak positive; ++ strong positive; +++ extreme positive.[2] Ten allergens regionally added to the EBS are marked in bold (i.e., they were not listed in †2015 EBS of 30 allergens[2] and ‡2019 EBS of 30 allergens[6]). Mix1: contains cinnamyl alcohol 1.0%, cinnamal 1.0%, hydroxycitronellal 1.0%, amyl cinnamal 1.0%, geraniol 1.0%, eugenol 1.0%, isoeugenol 1.0% and oakmoss absolute 1.0%. Mix2: contains disperse blue 35 1.0%, disperse yellow 3 1.0%, disperse orange 1 1.0%, disperse orange 3 1.0%, disperse red 1 1.0%, disperse red 17 1.0%, disperse blue 106 0.3% and disperse blue 124 0.3%. Mix3: contains hydroxyisohexyl 3-cyclohexene carboxaldehyde 2.5%, citral 1.0%, farnesol 2.5%, coumarin 2.5%, citronellol 0.5% and hexyl cinnamal 5.0%. Mix4, contains disperse blue 106 0.5% and disperse blue 124 0.5%. Mix5: contains TMTM (tetramethylthiuram monosulfide) 0.25%, TMTD (tetramethylthiuram disulphide) 0.25%, TETD (tetraethylthiuram disulphide) 0.25% and PTD (dipentamethylenethiuram disulphide) 0.25%. Mix6: contains *Anthemis nobilis* 1.2%, *Chamomilla recutita* 1.2%, *Achillea millefolium* 1.0%, *Tanacetum vulgare* 1.0%, *Arnica montana* 0.5% and parthenolide 0.1%. Mix7, contains alantolactone 0.033%, dehydrocostus lactone 0.033% and costunolide 0.033%. Mix8: contains N-cyclohexylbenzothiazyl sulfenamide 0.5%, mercaptobenzothiazole 0.5%, dibenzothiazyl disulphide 0.5% and morpholinylmercaptobenzothiazole 0.5%. Mix9: contains methylparaben 4.0%, ethylparaben 4.0%, propylparaben 4.0% and butylparaben 4.0%.

Abbreviations: aq., water; conc., concentration; D3, Day 3; D6/7, Day 6 or 7; Doubt., doubtful; ICDRG, International Contact Dermatitis Research Group; neg., negative; pos., positive.

[a]Positive (+/++/+++) on D3 and/or D6/7.

[b]The strongest reaction on D3 and D6/7.

[c]Negative (−) or doubtful (?+) on D3, but positive (+/++/+++) on D6/7.

[d]Evolution of positive reactions from D3 to D6/7 was classified as: 'decrescendo' (decrease in ICDRG criteria: +++, ++, +, ?+ or 0), 'plateau' (unaltered: +++, +++; ++, ++; +, +) or 'crescendo' (increase in ICDRG criteria: 0 or ?+, +, ++, +++).

[e]Allergens were provided by Chemotechnique.

[f]Allergens were provided by allergEAZE (SmartPractice).

[g]Formaldehyde releaser.[1]

TABLE 2 Formaldehyde and four formaldehyde releasers' co-reactivity

Allergen 1 (*n*, % of tested)	Allergen 2				
	Formaldehyde 2% aq. (% of allergen 1 positive [*n*/*n*])	2-bromo-2-nitropropane-1,3-diol 0.5% pet. (% of allergen 1 positive [*n*/*n*])	Diazolidinyl urea 2% pet. (% of allergen 1 positive [*n*/*n*])	Quaternium-15 1% pet. (% of allergen 1 positive [*n*/*n*])	Imidazolidinyl urea 2% pet. (% of allergen 1 positive [*n*/*n*])
Formaldehyde 2% aq.; 42, 5.6% (2.0%)[a]	100%	11.9% (5/42)	4.8% (2/42)	9.5% (4/42)	2.4% (1/42)
2-bromo-2-nitropropane-1,3-diol 0.5% pet.; 20, 2.7% (0.5%)[a]	25.0% (5/20)	100%	5.0% (1/20)	0% (0/20)	0% (0/20)
Diazolidinyl urea 2% pet.; 5, 0.7% (0.6%)[a]	40.0% (2/5)	20.0% (1/5)	100%	20.0% (1/5)	40.0% (2/5)
Quaternium-15 1% pet.; 4, 0.5% (0.7%)[a]	100% (4/4)	0% (0/4)	25.0% (1/4)	100%	0% (0/4)
Imidazolidinyl urea 2% pet.; 3, 0.4% (0.4%)[a]	33.3% (1/3)	0% (0/3)	66.7% (2/3)	0% (0/3)	100%

Note: Data are shown analogously to De Groot et al.[18] This table may be read as follows: horizontally: patients sensitized to allergen 1 co-react with allergen 2 in x% of cases. Vertically: patients sensitized to allergen 2 are also sensitized to allergen 1 in *x*% of cases.
[a]Sensitization rates in a study by Whitehouse et al.[1]

(given a non-normal distribution of age). p Value <0.05 was considered statistically significant.

3 | RESULTS

All tested allergens yielded positive patch test reactions. At least one positive reaction was found in 55.9% ($n = 418$) of patients. Frequencies of positive reactions are given in Table 1. The highest prevalence of patch test reactivity was found for nickel sulphate (19.0%), followed by potassium dichromate (9.6%), methyldibromo glutaronitrile (9.2%), *Myroxylon pereirae* (8.2%) and fragrance mix I (7.1%). A proportion of positive reactions ≥0.5% was found for 35 allergens. If a D6/7 reading had not taken place, 16.6% ($n = 167$) of positive reactions would have been missed (Table 1). Patterns of simultaneous positive (+, ++ or +++) patch test reactions (co-reactivity) for 10 additional allergens are given in Table S1, and patterns of simultaneous strong (++) or extreme (+++) positive reactions in Table S2.

No statistically significant differences in the frequency of patch test reactivity based on gender were found for 10 additional allergens, but patients with positive reactions to thiomersal ($n = 27$) were more often aged <40 years than ≥40 years (59.3% [16/27] vs. 40.7% [11/27], $p = 0.027$). Additionally, when patients with positive and negative reactions to thiomersal were compared, the former were younger ($p = 0.005$). No such statistically significant age differences were found for other nine additional allergens.

A proportion of positive reactions ≥0.5% was found for eight additional allergens (Table 1). Testing with *cocamidopropyl betaine* and *thiomersal* yielded 6.7% and 3.6% of positive reactions, respectively. Ninety-six percent of positive reactions to cocamidopropyl betaine were weak (+) positive and 4% were strong (++) positive. The majority (i.e., 92%) of positive reactions to this allergen were 'decrescendo'. More than 40% of reactions to thiomersal were strong positive and 63.0% 'crescendo' (Table 1). A high percentage of positive reactions (33.3%) to *disperse blue mix 106/124* would have been missed if late reading had not taken place (Table 1). There was a statistically significant co-reactivity between disperse blue mix 106/124 and textile dye mix ($p < 0.001$). Of 27 patients with patch test reactivity to disperse blue mix 106/124, 59.3% ($n = 16$) co-reacted to textile dye mix. (Table S1). Conversely, of 43 patients with positive reactions to textile dye mix, 37.2% ($n = 16$) co-reacted to disperse blue mix 106/124. There was also a statistically significant co-reactivity between disperse blue mix 106/124 and p-Phenylenediamine ($p = 0.010$) (Table S1).

Reactions to *formaldehyde* in our study were +, ++ and +++ positive in 61.9%, 26.2% and 11.9% of tested patients, respectively (Table 1). Data on formaldehyde and FRs' co-reactivity are presented in Tables 2, S1 and S2. Only 11.9%, 4.8%, 9.5% and 2.4% of patients with patch test reactivity to formaldehyde co-reacted with *2-bromo-2-nitropropane-1,3-diol*, *diazolidinyl urea*, *Quaternium-15* and *imidazolidinyl urea*, respectively (Table 2, horizontal). Co-reactivity with formaldehyde was found in 25.0%, 40.0%, 100% and 33.3% of patients with positive reactions to 2-bromo-2-nitropropane-1,3-diol, diazolidinyl urea, Quaternium-15 and imidazolidinyl urea, respectively (Table 2, vertical).

Propylene glycol is known to have some irritant potential.[2] The majority of patch test reactions to propylene glycol in our study were weak (+) positive and decrescendo, but 15.8% and 10.5% of reactions were strong positive and crescendo, respectively. Testing with *Compositae mix II* yielded 0.8% of positive reactions and 33.3% of them were late positive. Testing with sesquiterpene lactone mix also showed a low frequency of positive reactions (i.e., 0.8%). There was a statistically significant co-reactivity between *Compositae mix II* and sesquiterpene lactone mix (Table S1), but the absolute numbers of positive cases were small. Only five patients tested positive to *dexamethasone-21-phosphate* and tests with *cetearyl alcohol* resulted in <0.5% prevalence rate of positive reactions (Table 1).

4 | DISCUSSION

Cocamidopropyl betaine belongs to top 40 NACDG allergens with a reported sensitization prevalence of 1.6% (89/5592).[14] Positive and

doubtful or irritant reactions to this allergen were found in 2.3% and 3.5% of 17 324 patch-tested patients in Europe, respectively.[19] Our study showed higher percentages (i.e., 6.7% positive, 5.2% doubtful), but: (a) positive reactions were almost exclusively weak positive and (b) the amount of positive and doubtful reactions was nearly the same. Hence, assessment of clinical relevance is crucial for the evaluation of this allergen, but this was not done. Cocamidopropyl betaine has an irritant potential.[2] Our study may support this since a decrescendo pattern in 92.0% of positive tests may indicate resolution after removal of the irritant. Nevertheless, a high percentage of tested patients had palpable reactions. Technical errors for our surprising high prevalence can probably be excluded since readings were done by experienced physicians according to the ICDRG recommendations.

Positive results to *thiomersal* 0.1% pet. were found in 18.5% of 135 young adults patch tested in Poland in 2005,[20] 4.7% of 1141 adults tested in Germany in 2001[21] and 16% of 722 mixed adult/paediatric patients tested in Austria in 1991.[11] Furthermore, analysis of the NACDG showed that thiomersal 0.1% pet. induced positive reactions in 10.9% of 4087 patients, but these were considered clinically relevant in only 16.8% of sensitized patients, ranking thiomersal last in the relevance among the 50 allergens tested by the NACDG.[22] Our study showed a 3.6% frequency of positive reactions to thiomersal in adults and although its presence in the EBS does not seem clinically indicated, it remains intriguing for research. It is surprising that our patients with patch test reactivity to thiomersal were significantly more often younger adults.

Disperse blue mix 106/124 was tested in a few studies so far and reported frequencies of positive reactions ranged from 0.7% to 4.7%.[14,19,23–26] We performed parallel patch testing with disperse blue mix 106/124 and textile dye mix composed of eight dyes (including the former two). There was a statistically significant co-reactivity between these two test substances, but only 59.3% of patients with positive reactions to disperse blue mix 106/124 co-reacted to textile dye mix. This may also support the inclusion of both substances in the baseline series. We also found significant co-reactivity between disperse blue mix 106/124 and p-phenylenediamine, which has been described to be rare.[27]

The reported incidence of sensitization to *formaldehyde* in Europe is 1%–3%.[1,18] Positive reactions to formaldehyde were found in 5.6% of patients enrolled in our study. Formaldehyde is known to cause potential irritant reactions and there is a possibility that some of our weak (+) positives could have been irritant,[1] but 26.2% and 11.9% of patients who tested positive to formaldehyde had strong (++) or extreme (+++) positive reactions, respectively. It was reported that formaldehyde 2% aq. is not a useful means of detecting allergy to FRs[1,5,18,28,29] and that in the majority of instances a reaction to an FR indicates an allergy to the FR and not a cross reaction to formaldehyde.[5] Our results support this since 75.0%, 60.0% and 66.7% of patients in our study with positive reactions to *2-bromo-2-nitropropane-1,3-diol*, *diazolidinyl urea* and *imidazolidinyl urea* did not show positive test reactions when tested with formaldehyde, respectively. Based on the aforementioned findings and ≥0.5% prevalence rate, our study implies the diagnostic value of patch testing with two additional FRs (i.e., 2-bromo-2-nitropropane-1,3-diol and diazolidinyl urea). Imidazolidinyl urea turned out to be a rare allergen and quaternium-15 is known to often cross react with formaldehyde.[5] The same recommendation to include 2-bromo-2-nitropropane-1,3-diol and diazolidinyl urea in the EBS was made by the European Baseline Series Taskforce of the ESCD in 2021.[5]

The ESSCA reported 2.7% positive reactions to *Compositae mix* in 3622 tested patients.[23] Compositae mix is considered to be a more sensitive test for Compositae sensitizations than the sesquiterpene lactone mix.[23] According to the literature, sesquiterpene lactone mix used in the EBS detects 35%–65% of Compositae-sensitized patients.[16] Significant co-reactivity between these two allergens in our study should be interpreted with caution in the presence of a small number of positive cases.

Positive and doubtful or irritant reactions to *propylene glycol 20% aq.* were found in 2.4% and 2.2% of 16 832 patch-tested patients in Europe, respectively.[19] The NACDG reported 2.6% of positive reactions in 4232 tested patients.[30] Our study showed similar results (i.e., 2.5% positive, 2.5% doubtful) to propylene glycol 30% aq. *Dexamethasone-21-phosphate* has not been included in the standard series so far and our study revealed a low patch test reactivity rate (i.e., 0.7%). Additionally, we did not find statistically significant co-reactivity between dexamethasone-21-phosphate and other markers of contact allergy to corticosteroids (i.e., budesonide and tixocortol pivalate). Our study did not reveal an added value of testing with dexamethasone. We also found a low prevalence of positive reactions to *cetearyl alcohol* (i.e., 0.1%), which has been reported to result in 0.4%–0.9% positive reactions.[17,23]

An important limitation of our study arises from the fact that the degree of exposure to tested allergens and clinical relevance of positive reactions were not assessed. Nevertheless, our single-centre study provides a comprehensive profile of allergens possibly responsible for allergic contact dermatitis in Slovenia. Another limitation is the removal of the patches which was done by the patients themselves. The high percentage of patch test reactions to formaldehyde at 2% aq. may be due to the lack of standardization of the amount of allergen placed in the test chambers (i.e., pipettes were not used to apply predetermined amounts of this allergen).

To the best of our knowledge, patterns of significant simultaneous patch test reactions for 10 additional allergens have not been reported before. Further studies are needed to assess the possibility of their cross-reactivity (i.e., considering the chemical structure of one molecule and comparing it with that of another).[7]

Our data on patch testing results in consecutive patients with substances not included in the EBS may provide a ground for further investigations to determine whether five allergens (i.e., cocamidopropyl betaine, disperse blue mix 106/124, 2-bromo-2-nitropropane-1,3-diol, diazolidinyl urea and Compositae mix II) need to be added to the EBS. It was decided that these five allergens will remain a part of our baseline series.

AUTHOR CONTRIBUTIONS

Mojca Bizjak: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; visualization;

writing – original draft; writing – review and editing; supervision; validation. **Katja Adamič:** Investigation; project administration; writing – review and editing. **Nissera Bajrovič:** Investigation; project administration; writing – review and editing. **Renato Eržen:** Investigation; project administration; writing – review and editing. **Maja Jošt:** Resources; writing – review and editing. **Peter Kopač:** Investigation; project administration; writing – review and editing. **Mitja Košnik:** Investigation; project administration; writing – review and editing. **Nika Lalek:** Investigation; project administration; writing – review and editing. **Mihaela Zidarn:** Investigation; project administration; writing – review and editing. **Dejan Dinevski:** Formal analysis; writing – review and editing.

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CONFLICTS OF INTEREST

Mojca Bizjak has been a speaker and served on advisory boards for Novartis, outside the submitted work. Mihaela Zidarn has been a speaker for Takeda, outside the submitted work. The remaining authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Whitehouse H, Uter W, Geier J, et al. Formaldehyde 2% is not a useful means of detecting allergy to formaldehyde releasers - results of the ESSCA network, 2015-2018. *Contact Dermatitis*. 2021;84(2):95-102.
2. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis*. 2015;73(4):195-221.
3. Bruynzeel DP, Andersen KE, Camarasa JG, Lachapelle JM, Menne T, White IR. The European standard series. European Environmental and Contact Dermatitis Research Group (EECDRG). *Contact Dermatitis*. 1995;33(3):145-148.
4. Bruze M, Conde-Salazar L, Goossens A, Kanerva L, White IR. Thoughts on sensitizers in a standard patch test series. The European Society of Contact Dermatitis. *Contact Dermatitis*. 1999;41(5):241-250.
5. Wilkinson SM, Badulici S, Gimenez-Arnau A, et al. The European baseline series: criteria for allergen inclusion (with reference to formaldehyde releasers). *Contact Dermatitis*. 2021;85:125-128.
6. Wilkinson M, Goncalo M, Aerts O, et al. The European baseline series and recommended additions: 2019. *Contact Dermatitis*. 2019;80(1):1-4.
7. Lepoittevin JP, LeCoz CJ. *Dictionary of Contact Allergens*. Springer; 2007:286.
8. Van't Veen AJ. Vaccines without thiomersal: why so necessary, why so long coming? *Drugs*. 2001;61(5):565-572.
9. Moller H. All these positive tests to thimerosal. *Contact Dermatitis*. 1994;31(4):209-213.
10. Spiewak R. Patch testing for contact allergy and allergic contact dermatitis. *Open Allergy J*. 2008;I:42-52.
11. Aberer W. Vaccination despite thimerosal sensitivity. *Contact Dermatitis*. 1991;24(1):6-10.
12. Uter W, Schwitulla J, Thyssen JP, Frosch PJ, Statham B, Schnuch A. The 'overall yield' with the baseline series - a useful addition to the array of MOAHLFA factors describing departmental characteristics of patch tested patients. *Contact Dermatitis*. 2011;65(6):322-328.
13. Bourke J, Coulson I, English J. Guidelines for the management of contact dermatitis: an update. *Br J Dermatol*. 2009;160(5):946-954.
14. DeKoven JG, Warshaw EM, Zug KA, et al. North American Contact Dermatitis Group patch test results: 2015-2016. *Dermatitis*. 2018;29(6):297-309.
15. Bauer A, Geier J, Schreiber S, Schubert S, IVDK. Contact sensitization to plants of the Compositae family: data of the Information Network of Departments of Dermatology (IVDK) from 2007 to 2016. *Contact Dermatitis*. 2019;80(4):222-227.
16. Paulsen E, Andersen KE. Screening for Compositae contact sensitization with sesquiterpene lactones and Compositae mix 2.5% pet. *Contact Dermatitis*. 2019;81(5):368-373.
17. Uter W, Aberer W, Armario-Hita JC, et al. Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007-2008. *Contact Dermatitis*. 2012;67(1):9-19.
18. De Groot AC, Flyvholm MA, Lensen G, Menne T, Coenraads PJ. Formaldehyde-releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. *Contact Dermatitis*. 2009;61(2):63-85.
19. Uter W, Geier J, Hausen BM, IVDK; Germa Contact Dermatitis Research Group. Contact allergy to Disperse Blue 106/124 mix in consecutive German, Austrian and Swiss patients. *Contact Dermatitis*. 2003;48(5):286-287.
20. Spiewak R. Atopy and contact hypersensitivity: a reassessment of the relationship using objective measures. *Ann Allergy Asthma Immunol*. 2005;95(1):61-65.
21. Schafer T, Bohler E, Ruhdorfer S, et al. Epidemiology of contact allergy in adults. *Allergy*. 2001;56(12):1192-1196.
22. Belsito DV. Thimerosal: contact (non)allergen of the year. *Am J Contact Dermat*. 2002;13(1):1-2.
23. Writing ESSCA Group. The European Surveillance System of Contact Allergies (ESSCA): results of patch testing the standard series, 2004. *J Eur Acad Dermatol Venereol*. 2008;22(2):174-181.
24. Uter W, Geier J, Lessmann H, Hausen BM, IVDK and the German Contact Dermatitis Research Group. Information Network of Departments of Dermatology. Contact allergy to disperse blue 106 and disperse blue 124 in German and Austrian patients, 1995 to 1999. *Contact Dermatitis*. 2001;44(3):173-177.
25. Uter W, Hildebrandt S, Geier J, Schnuch A, Lessmann H. Current patch test results in consecutive patients with, and chemical analysis of, disperse blue (DB) 106, DB 124, and the mix of DB 106 and 124. *Contact Dermatitis*. 2007;57(4):230-234.
26. Slodownik D, Williams J, Tate B, et al. Textile allergy - the Melbourne experience. *Contact Dermatitis*. 2011;65(1):38-42.
27. Schnuch A, Lessmann H, Frosch PJ, Uter W. Para-phenylenediamine: the profile of an important allergen. Results of the IVDK. *Br J Dermatol*. 2008;159(2):379-386.

28. Latorre N, Borrego L, Fernandez-Redondo V, et al. Patch testing with formaldehyde and formaldehyde-releasers: multicentre study in Spain (2005-2009). *Contact Dermatitis*. 2011;65(5):286-292.
29. De Groot A, White IR, Flyvholm MA, Lensen G, Coenraads PJ. Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. Part 2. Patch test relationship to formaldehyde contact allergy, experimental provocation tests, amount of formaldehyde released, and assessment of risk to consumers allergic to formaldehyde. *Contact Dermatitis*. 2010;62(1):18-31.
30. Warshaw EM, Maibach HI, Taylor JS, et al. North American contact dermatitis group patch test results: 2011-2012. *Dermatitis*. 2015;26(1):49-59.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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